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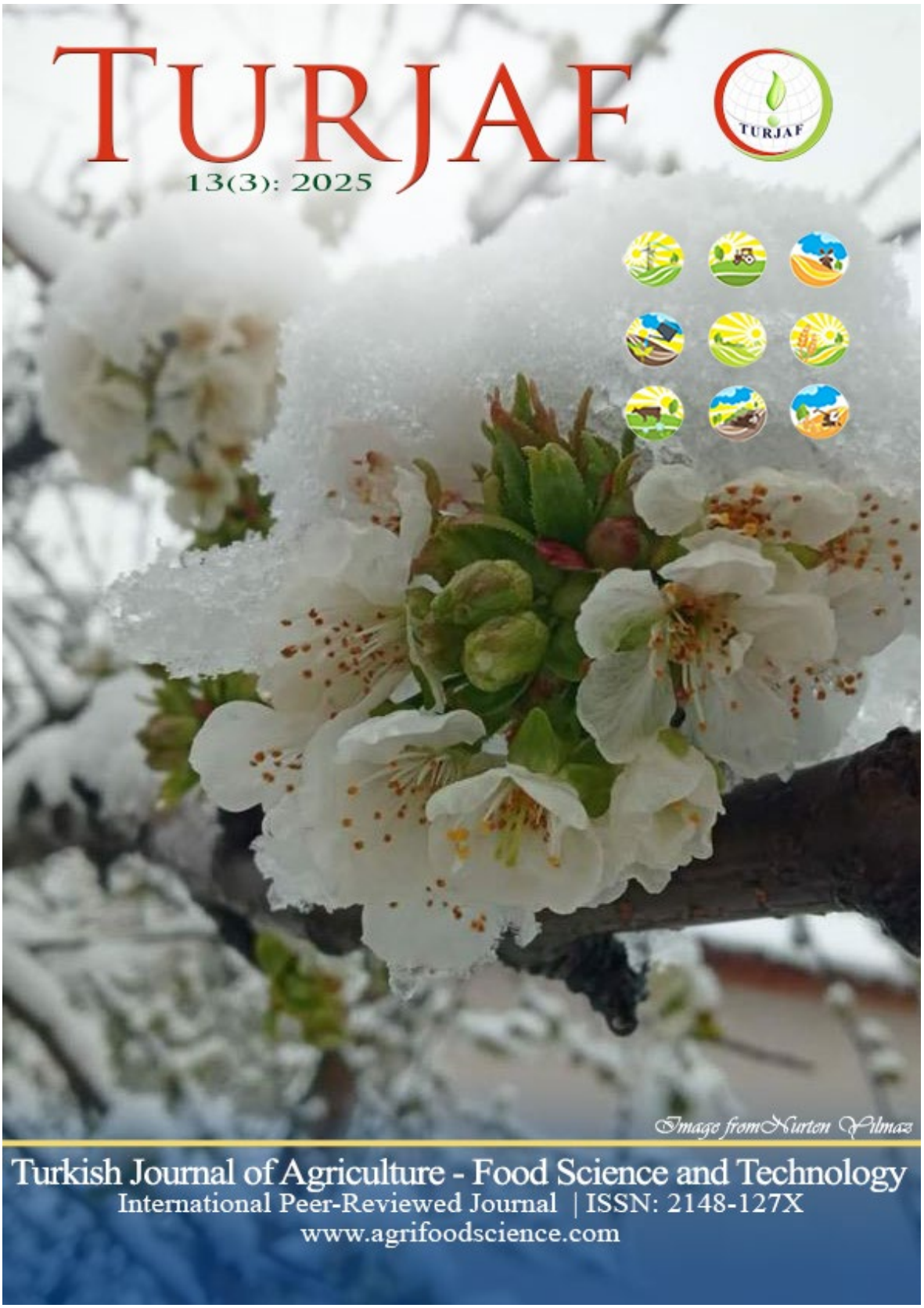

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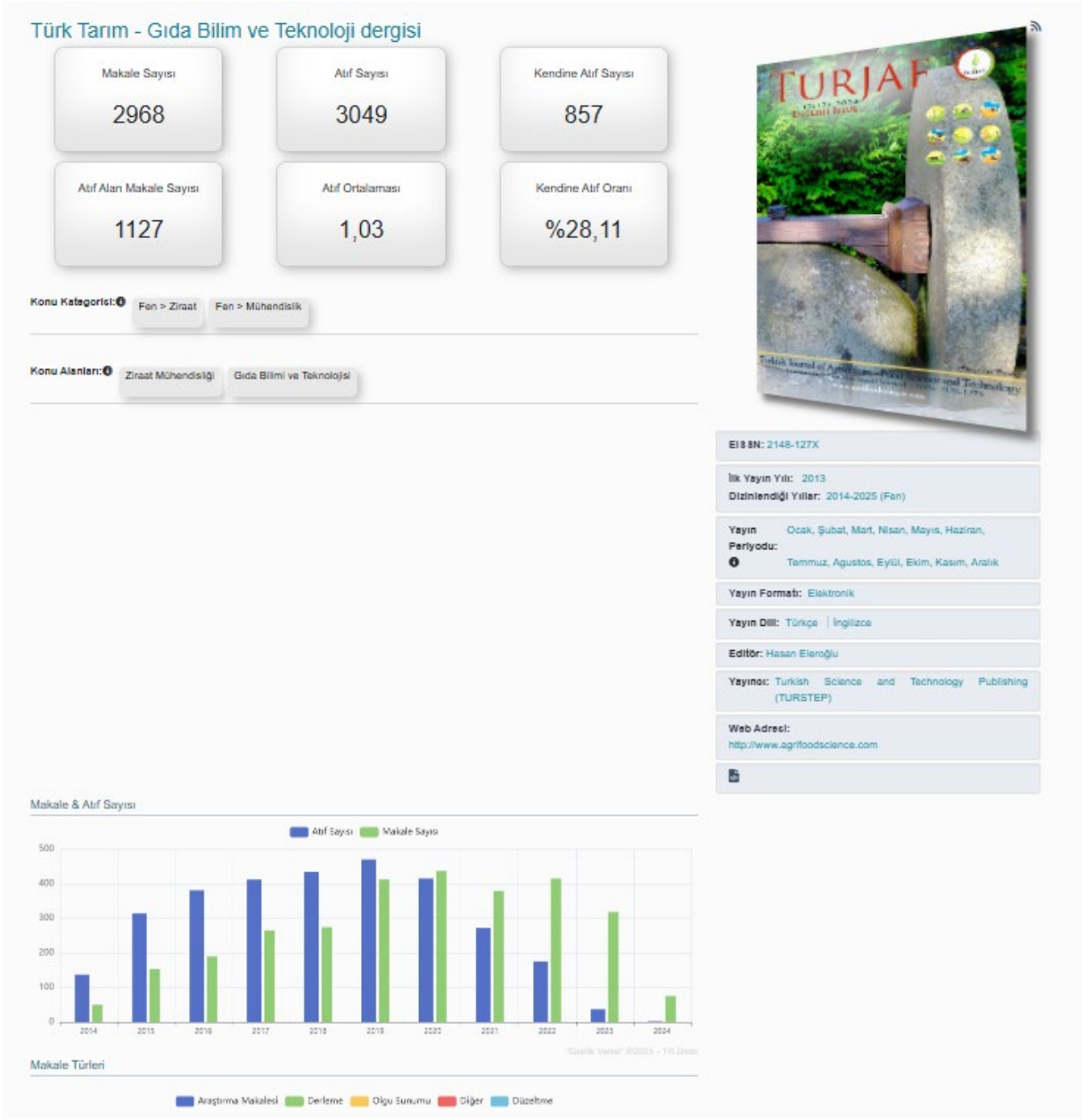

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# A Study on Consumers' Knowledge of Distinguishing Natural and Organic Honey

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ABSTRACT

In today's marketing processes, knowing what consumers want plays an active role in shaping the products businesses offer and gaining a competitive advantage. This study aims to obtain a guiding result for companies in this sector by questioning the knowledge levels of consumers regarding honey products. The study aims to reveal the uncertainties in consumer perception between natural and organic honey and examine this distinction's impact on purchasing decisions. The research seeks to create more informed consumer preferences in the honey market and to show the contributions of positioning the product correctly in the minds of consumers. In this study, content analysis was conducted to determine the responses of 117 participants to the statement, "There is a difference between natural honey and organic honey, and I have information about what this difference is." After the analysis, the study is shaped by dividing consumers into four categories according to their level of knowledge about honey. The qualitative research aims to obtain comprehensive information on the participants' awareness of natural and organic honey. The study results show that the concepts of natural and organic honey can be confused by consumers, and the difference between these two products cannot be fully conveyed. This result shows that organic honey businesses and regulatory organizations should inform consumers more accurately and clearly through marketing communication efforts in marketing their products. In the study, recommendations are presented to consumers and businesses based on the results of the qualitative analysis, and the methods that companies should apply to overcome the deficiencies in consumer perception of the distinction between natural and organic honey are included.

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## Introduction

The distinction between natural and organic honey is increasingly important in consumer markets, reflecting broader trends in health awareness and environmental sustainability. As consumers become more aware of the health benefits of honey, their preferences are shifting towards products perceived as more beneficial. Research shows that consumers generally associate organic honey with higher quality and health benefits, which may influence their purchasing decisions (Cosmina et al., 2016; Testa et al., 2019; Zanchini et al., 2022). The organic label is a quality indicator that aligns with consumers' interest in sustainable and responsible food sources (Sparacino et al., 2022). Studies show that consumers are willing to pay more for organic honey and view it as a healthier alternative to conventional options (Mi et al., 2023; Vapa-Tankosić et al., 2020). This trend is particularly evident among demographic groups prioritizing health and well-being, such as older adults and those with greater awareness of nutrition (Sparacino et al., 2022).

The perception of honey as a natural remedy drives demand for organic varieties, with consumers increasingly seeking products free of synthetic additives and pesticides (Escuredo & Seijo, 2019). Despite the growing interest in organic honey, there is a significant gap in consumers' knowledge of the differences between natural and organic classifications. Many consumers may not fully understand the implications of organic labels, which can lead to confusion and incorrect purchasing decisions by misinformed consumers (Šedík et al., 2022; Elsadibah, 2023). This lack of awareness highlights the need for many initiatives to inform consumers about natural and organic honey's unique properties and benefits, enabling them to make more informed choices (Šedík et al., 2021). As demand for honey grows, producers and marketers need to understand consumers' knowledge and attitudes toward these products. This study was undertaken to provide insight into the complexity of consumer preferences, health perceptions, and market dynamics in the current landscape of honey consumption. It also aims to determine consumers' level of knowledge about the differences between natural and organic honey.

This research is essential as it addresses a significant gap in understanding how consumers differentiate between natural and organic honey, which can influence purchasing decisions and overall market dynamics. Consumers' knowledge of honey types is influenced by factors such as local production preferences and the perceived quality of honey. Studies show that consumers prefer locally produced honey and associate it with higher quality and better taste (Šedík et al., 2022; Kallas et al., 2019). The preference for local honey is further complicated by the growing awareness of the health benefits attributed to honey, which consumers increasingly recognize as a natural alternative to refined sugars (Testa et al., 2019). Moreover, willingness to pay for honey is significantly influenced by its attributes, such as whether it is organic or locally sourced. This is because it shows that consumers are interested in production methods as well as the origin of the product (Vapa-Tankosić et al., 2020; Cosmina et al., 2016).

Consumer perception must clarify the distinction between natural and organic honey (Bilici, 2024). Research shows that while consumers prioritize organic attributes, they value honey's overall quality and health benefits. Consumers may be confused about the definitions of terms (Cosmina et al., 2016; Závodná & Pospíšil, 2016). The therapeutic properties of honey, which vary according to botanical origin, make consumer understanding and preferences even more challenging to choose (Šedík et al., 2019). This highlights the need for more transparent labeling and consumer education to increase knowledge about the differences between natural and organic honey. Demographic factors such as age and socioeconomic status also shape consumers' attitudes towards honey. Younger consumers may have different preferences compared to older generations, as indicated by studies examining intergenerational differences in honey consumption (Šedík et al., 2023; Šedík et al., 2018). Understanding the importance of demographic influences at this point can raise critical awareness about targeted marketing strategies and educational campaigns aimed at improving consumer knowledge.

Natural honey is a product rich in nutrients and bioactive compounds produced by honey bees from the nectar of flowers (Ajibola et al., 2012; Aljohar et al., 2018; Bilici, 2024). This natural product, which contains sugars, proteins, organic acids, vitamins, minerals, flavonoids, and enzymes, has potent antioxidant, antimicrobial, and anti-inflammatory properties (Bilici, 2024; Khan et al., 2018; Lu et al., 2013; Mandal & Mandal, 2011). The composition of natural honey may vary according to the flower sources, geographical factors, and beekeeping methods, leading to differences in flavor, color, and nutritional values (Bilici, 2024; Chitarrini et al., 2020; Molanaei et al., 2020).

Organic honey, on the other hand, is produced through sustainable practices that ensure that chemicals and pesticides are not used in beekeeping and that bees have access only to organic plant sources (Bilici, 2024; Julia et al., 2023). The main difference between natural honey and organic honey lies in the methods used in production processes and the nature of plant resources. Organic honey is produced under certified organic farming practices, which assures consumers it is free of chemical residues. This honey is certified by organizations authorized by the country's Ministry of Food and Agriculture and is approved as organic through strict inspection and laboratory analysis (Bilici, 2024).

Natural honey is pure honey produced by the bees through natural processes without any additives. While flower honey is obtained from the nectar of plants, glandular honey is obtained from the secretions of the living parts of plants or from the secretions of insects that suck plant sap. In organic honey, however, the bees feed only on organically grown plants, and the hives are managed using natural methods (Julia et al., 2023).

As a result, both types of honey are natural. However, organic honey differs from natural honey because it complies with certified organic farming standards. Both natural and organic honey offer significant health benefits and are valued for their natural origin.

Natural honey is first produced by bees from the nectar of flowers and then processed through enzymatic action and evaporation to create the final product. During this process, the quality and naturalness of honey may vary depending on the available floral resources, environmental conditions, beekeeping practices used, and the intention of the producer (Bilici, 2024; Rababah et al., 2013; Datti et al., 2020). In contrast, organic honey production adheres to stricter regulations prohibiting synthetic pesticides and fertilizers in the surrounding environment and ensuring that bees forage in a chemical-free area. This distinction is crucial as it affects not only the quality and safety of honey but also its marketability and consumer perception (Cucu et al., 2021; Pocol et al., 2022).

Organic honey production often requires a certification process that involves rigorous inspections and compliance with specific agricultural practices. For example, organic beekeepers must ensure that their hives are located in areas free from chemical contamination and that bees are not fed sugar syrups or other non-organic substances (Hawari et al., 2021; Toledo et al., 2022). The fact that such regulations may not be strictly enforced and could lead to potential counterfeiting or contamination from nearby agricultural activities does not apply to natural honey (Warui et al., 2019). Studies have shown that organic honey tends to have higher levels of certain beneficial compounds, such as antioxidants and phenolic compounds, attributed to the diverse and uncontaminated floral sources bees have access to (Pavlešić et al., 2022; Spirić, 2023).

Processing methods of honey can also differ significantly between natural and organic varieties. While natural honey can undergo minimal processing, such as essential filtration to remove impurities, organic honey is generally subjected to much stricter processing and inspection standards to preserve its natural properties while ensuring safety (Chen et al., 2012; Sereia et al., 2017). For example, organic honey must be processed without high heat, which can degrade its nutritional and therapeutic properties (Irish et al., 2011). This careful processing is essential as it helps preserve honey's bioactive components, which are crucial for its health benefits (Cucu et al., 2021; Majtán, 2014). Regarding authenticity and quality assessment, both natural and organic honey face challenges related to counterfeiting and mislabeling.

Contaminants and ensuring traceability from hive to consumer (Pocol et al., 2022; Escuredo & Seijo, 2019). Natural honey may not always be subject to the same level of scrutiny, which can raise potential issues related to quality and authenticity (Hawari et al., 2021; Toledo et al., 2022). Advanced analytical techniques such as stable

isotope analysis have been used to distinguish between natural honey and counterfeit products, providing a scientific basis for quality assurance in both categories (Kamdee et al., 2023).

In the context of consumer behavior, consumers evaluate honey based on various qualitative attributes such as taste, aroma, and physical condition. For example, Šedík et al. suggest that urban consumers in Slovakia evaluate honey quality through sensory attributes (Šedík et al., 2022). This preference is further reinforced by the fact that consumers are increasingly aware of the unique properties of natural honey, which increases its attractiveness as a sought-after product (Šedík et al., 2021). Testa et al. found that Italian consumers are willing to pay extra for organic honey, suggesting a strong correlation between organic certification and consumer demand (Testa et al., 2019). This trend is also supported by Vapa-Tankosić et al., who identified food safety and local community support as important factors influencing consumers' willingness to pay more for organic honey in Serbia (Vapa-Tankosić et al., 2020). Bilici (2024) also found that consumers' attitudes are influenced by health awareness, perceived quality, the color of honey, perceived nutritional value, and perceived price, and that attitude towards natural honey strongly influences attitude towards more honey and willingness to pay more. Such results from the literature suggest that consumers are motivated not only by taste and quality but also by ethical concerns and health awareness surrounding production practices.

Health benefits associated with honey consumption also play an essential role in shaping consumer preferences. Research by Ritten et al. shows that consumers are increasingly attracted to honey due to its medicinal properties, which are often emphasized in marketing strategies (Ritten et al., 2019). This result is confirmed by studies showing that consumers perceive honey as a healthy alternative to sugar, especially among older demographic groups that may have historical links to honey consumption instead of sugar (Kowalczuk et al., 2017). Neto et al. (2020) found that health consciousness significantly influences honey purchasing patterns as consumers seek products that align with their nutritional goals.

Demographic factors affecting honey consumption also need to be considered. Younger consumers may exhibit different preferences compared to older consumers. Studies show that older individuals consume honey more frequently (Šedík et al., 2023). Income and brand awareness also significantly influence consumer choices, as in the study conducted in Indonesia, where health benefits and taste are most important (Melina et al., 2023).

## Materials and Methods

In this study, content analysis was conducted to determine the responses of 117 participants to the statement, “There is a difference between natural honey and organic honey, and I have information about what this difference is.” Content analysis is a systematic method used in qualitative research that enables researchers to interpret and analyze textual, visual, and conceptual data. It is mainly used to extract meaning from various forms of communication, such as interviews, articles, and social media content. This method facilitates a deeper understanding of the topic under investigation by identifying patterns, themes, and categories in qualitative data (Agarwal, 2023; Suryaningrum et al., 2019). Content analysis in qualitative research can be handled inductively or deductively. An inductive approach involves deriving categories and themes directly from the data without pre-acquired concepts, which allows realizations to emerge more naturally (Mazaheri et al., 2014; Pagnaer et al., 2021). In contrast, a deductive approach starts with predefined categories based on existing theories or frameworks that researchers then apply to the data (Naal et al., 2022; Abdullahzadeh, 2023). This flexibility makes content analysis versatile in various disciplines, including sociology, psychology, and communication studies (Türk et al., 2022; Mustapha & Ebomoyi, 2019).

The content analysis process includes data collection, coding, categorization, and interpretation. Initially, researchers collect qualitative data through interviews, focus groups, or document reviews. The data is then coded, where text sections are labeled with codes representing specific themes or concepts. These codes are then analyzed into categories to draw conclusions and inferences about the data (Azizan et al., 2016; Taufik & Sila, 2023; Horúcková & Berthet, 2017). This systematic approach increases the reliability and validity of the findings as it allows for cross-validation of the data (Mindarti et al., 2021; Dumay & Cai, 2015). This study involved 117 participants who classified and summarized responses through content analysis. This method comprehensively overviews participants' thoughts and perceptions (Serafini & Reid, 2019; Ristovska, 2019).

## Results

The research was conducted in a face-to-face interview, and the participants' demographic data was collected first. Then, one-question closed-ended and one-question open-ended questions were asked to determine whether consumers know the distinction between natural honey and organic honey.

When the demographic data in Table 1 are analyzed, it is seen that women (64.10%) are more represented than men (35.90%) in the gender distribution of the participants. This suggests that women are more interested in natural and organic products or are more involved in such research. Regarding marital status, 51.28% of the participants were married, and 48.72% were single. These rates suggest that married and single individuals were represented in the survey in a balanced way. However, married individuals may be more conscious about family health and safe food preferences.

When the distribution according to age groups is analyzed, respondents aged between 31-35 constitute the largest group with a rate of 21.37%. In comparison, individuals aged 45 years and over represent a significant segment with a rate of 18.80%. The density in the young and middle age groups may indicate that these age groups are more interested in organic and natural products. When the level of education is analyzed, it is seen that 36.75% of the participants have a bachelor's degree, and 30.77% have a postgraduate degree. These results suggest that educated individuals are more willing to respond to organic and natural products.

Table 1. Demographic Characteristics of the Participants

| Demographic Characteristics | | N | % |
|---|---|---|---|
| Gender | Male | 42 | 35.90% |
| | Female | 75 | 64.10% |
| Marital status | Married | 60 | 51.28% |
| | Single | 57 | 48.72% |
| Age | 25 ≤ | 20 | 17.09% |
| | 26-30 | 16 | 13.68% |
| | 31-35 | 25 | 21.37% |
| | 36-40 | 19 | 16.24% |
| | 41-45 | 15 | 12.82% |
| | 45 ≥ | 22 | 18.80% |
| Education Level | High School and Below | 19 | 16.24% |
| | Associate degree | 19 | 16.24% |
| | Undergraduate | 43 | 36.75% |
| | Graduate | 36 | 30.77% |
| Income Level (Turkish Lira / TRY) | 15.000 ≤ | 18 | 15.38% |
| | 15.001 – 30.000 | 43 | 36.75% |
| | 30.001 – 45.000 | 25 | 21.37% |
| | 45.001 – 60.000 | 23 | 19.66% |
| | 60.001 ≥ | 8 | 6.84% |
| Total | | 117 | 100.00% |

When the income levels are analyzed, 36.75% of the respondents are in the income range of 15.001-30.000 TL, which shows that most are at low-income levels. This suggests that the interest in organic and natural honey products may be concentrated mainly in the low-income group. Price and quality perception may play an essential role in the preference for these products. In general, the participant profile of the research is primarily female, belonging to the middle age group, educated and middle-income individuals.

Participants were asked, "There is no difference between natural honey and organic honey. They both refer to the same product." it is seen that the majority of the participants (64.84%) believe that there is a difference between natural honey and organic honey. This shows that consumers know these two products and perceive the differences. On the other hand, only 35.16% believe these two products are the same, indicating a lack of knowledge about natural and organic honey. The results reveal the need for awareness raising on natural and organic honey and show that consumers are willing to learn more about it. Consumer awareness-raising efforts can support a better understanding of these differences and increase the market share of organic products.

The responses to the open-ended question "There is a difference between natural honey and organic honey, and I have information about this difference. (If you think there is a difference between the two types of honey, you can write 'yes' and if you want, you can write what the difference is.)" were examined and classified one by one, collected below under headings, and divided into four categories:

Providers of Accurate Information: Those who correctly explain the difference between natural and organic honey.

Misinformers: Those who give incorrect, inaccurate, or incomplete information.

Those Who Say There is No Difference: Those who state that there is no difference between the two honeys.

Those who do not know: Those who stated that there was a difference but did not.

Some prominent responses and percentages were calculated according to these categories. The distribution of categories according to consumers' responses is as follows:

*1. Those who provided accurate information (35.90%)*

- "The two are different. Organic honey requires a certificate."
- "Organic honey is a honey produced from bees raised in places where controlled and certified agricultural production is carried out from production to consumption without chemical inputs that do not harm human health and the environment. On the other hand, natural honey is produced by feeding entirely from nature without external additives for the bee's nutrition in any environment."
- "Organic food refers to food produced with organic methods and controlled at every stage from the soil where it is produced to its consumption. Natural food: foods found in nature and grown naturally are in this group."
- "Natural honey is honey produced by bees in a natural environment with components such as pollen, etc., taken from the source without any control. Organic honey, on the other hand, is honey produced by bees with pollen, etc., taken from a designated (organic) source."
- "Organic honey is a honey produced in locations that meet organic production conditions."
- "Natural honey is a honey produced spontaneously by bees in the natural environment, without controls. Organic honey is a honey produced according to a standard and certified."

*2. Those Providing False or Incomplete Information (32.48%)*

- "There is a difference in smell and taste. Organic honey is candied."

- "If bees produce organic and natural honey, there is no difference."
- "Organic honey is produced without any non-organic production materials. On the other hand, natural honey does not comply with organic rules but uses natural production materials for the bee."

*3. Those who said there is no difference (9.40%)*

- "Natural honey can also crystallize over time to protect itself."
- "I do not think organic honey has to be natural. It can be produced in a production facility. I think natural honey is a product derived from its ecosystem."

*4. Those who do not know (22.22%)*

- "I think there is a difference, but I cannot explain it scientifically."
- "According to an experiment on a bear in a bee field, the bear recognized the natural honey every time and ate it."
- "I do not know the difference; I do not know to make a distinction."
- "There is a difference, but I do not know what it is."

In addition, when the responses of the participants were grouped under headings, other responses were categorized as follows:

*Certification of Organic Honey and Rules:* According to some respondents, compliance with specific standards and certification is essential in producing organic honey. The idea that organic honey is certified and subject to various controls and rules during the production process is shared among the respondents. According to some respondents, the fact that organic honey is subject to the certification process and compliance with specific rules is emphasized. Some respondents know that organic honey has to meet particular standards. It is commonly stated that organic honey is a product that is produced according to organic production standards approved by the Ministry of Agriculture and Forestry, and its compliance with these standards is based on specific criteria. Among the requirements that organic honey must meet are that it must be at least 1 km away from areas where chemicals or products are used (mines, factories, etc.), that the certifying organization must accept medicines and bee foods according to specific standards, and that no chemical additives or external factors are used in the production process.

Production by Bees and Definition of Natural Honey: According to some respondents, honey produced in their natural environment is often called "natural honey." However, there are many opinions on this definition. While some users stated that honey produced by bees is automatically called "natural," others noted that this definition is limited.

Bear Experiment and Preference for Honey: Some participants mentioned an experiment conducted in a bee field. According to this experiment, it is claimed that bears prefer natural honey. However, no clear information was given about the scientific reliability of this experiment.

Flavor and Other Characteristics: Survey respondents indicated differences between organic and natural honey regarding smell, taste, color, and sweetness. There are opinions that organic honey generally tends to sweeten.

Environment and Health-Friendly Organic Honey: Participants emphasized that the production conditions of organic honey are environmentally friendly and suitable for human health. Factors such as the absence of chemical inputs and the lack of diesel-powered vehicles were mentioned by the participants as factors that determine the compliance of organic honey with health standards.

Production Process of Natural Honey: According to some participants, natural honey is produced by bees in their natural environment under minimal human intervention.

## Discussion

This study aimed to examine how consumers perceive the difference between natural and organic honey and the level of knowledge they have about these two types of honey. The study results reveal that consumers' knowledge about natural and organic honey varies and that they have a certain level of awareness. Most participants stated that natural and organic honey differ and that organic honey is produced within specific rules. This result shows that organic honey is associated with particular standards in the minds of consumers and that consumers have developed a sense of trust towards these products. 64.84% of consumers believe there is a difference between natural and organic honey. However, the respondents have insufficient knowledge of how to define this difference. In particular, those who have correct information about the distinction between organic honey and natural honey are represented by 35.90%, and those who have incorrect or incomplete information are represented by 32.48%. The closeness of these percentages reveals a significant confusion and lack of knowledge regarding consumer awareness.

When consumers' responses are categorized, the idea that organic honey should be certified and subject to specific rules comes first. Some consumers correctly stated that organic honey is produced without chemicals and should be approved. On the other hand, some have incorrect or incomplete information. Generally, superficial factors such as taste, smell, or sugar level incorrectly explain the differences between organic and natural honey. These consumers' answers reveal that they do not have in-depth knowledge about organic products and that education and awareness-raising activities are essential in promoting these products.

9.40% of the participants verbally stated that there was no difference between natural honey and organic honey, while 22.22% thought there was a difference but had difficulty in explaining this difference. These answers show the imbalance in consumer knowledge level and the lack of clear information about organic honey. The answers given by the participants in the "Do not Know" group especially reveal that social awareness of organic products should be increased. A better understanding of organic products and a more precise positioning in the minds of consumers are essential for the growth of this market.

## Conclusion

The research results show that the concepts of natural and organic honey can be confused by consumers, and the difference between these two products is not fully

conveyed. This result indicates that organic honey businesses and regulatory organizations need to inform consumers more accurately and clearly through marketing communication efforts in marketing their products. In particular, it is necessary to explain better the production processes of organic honey, explain the certification process transparently, and organize informative marketing communication campaigns about the environmental and health criteria that these products meet. The idea that organic products are environmentally friendly, sustainable, and more suitable for human health is widespread among consumers. However, it is essential to convey this idea to a broader audience.

The limitations of the research should also be taken into consideration. The sample size and demographic structure of the study have certain limitations. The research results may not be generalizable to a broader population because the majority of the participants in the sample are middle-aged, female, and highly educated. A study with participants from different age groups and spread over a wider geographical area may reveal more clearly how the awareness of natural and organic honey is shaped throughout society. In addition, this study could not fully address the impact of differences in income levels on consumer preferences.

A second limitation is related to the data collection method. The study evaluates the participants' level of knowledge about natural and organic honey only based on self-reported information. However, these statements may not always be accurate and reliable. In particular, respondents who provided inaccurate or incomplete information may have based their responses on subjective experiences or perceptions, which may raise some concerns about the accuracy of the results.

Finally, the study's cross-sectional nature does not capture changes over time. Organic and natural honey awareness may change with marketing communication campaigns and awareness-raising efforts. Therefore, conducting longer-term and follow-up research on this issue may support examining the problem more clearly.

Based on the results of the research, the following recommendations can be made for businesses and consumers:

- Consumer Education and Awareness Raising: Businesses should organize information campaigns to explain the difference between organic and natural honey more clearly. In particular, consumers should be given more information about the certification process, production standards, and environmental sensitivity of organic honey.
- Labeling and Certification: Providing detailed information about the certification process of organic honey on product labels will enable consumers to make an informed choice. In addition, emphasizing the assurance provided by certification will likely increase the preference for organic honey.
- Price and Quality Balance: Especially for low-income consumers, the price-quality balance of organic honey should be emphasized. Presenting the advantages of organic honey in a way suitable for a broad audience, rather than appealing only to high-income consumers, will enable this product to spread to a wider consumer group.
- Environment and Health-Focused Messages: Emphasizing the environmentally friendly production processes of organic honey and its benefits for human health will likely increase the preference rate of these products. Consumers should understand that organic honey is not just a food product and supports a sustainable production model.

The following suggestions can be made for future research in this field:

- Use a Larger Sample: Larger-scale studies can be conducted to include participants from different geographical regions and various demographic groups. This research could provide a clearer picture of consumers' perception of the difference between natural and organic honey.
- Monitoring Changes over Time: Long-term monitoring studies should be conducted to understand how awareness and consumer preferences for organic products change over time. These studies will help evaluate the effectiveness of marketing strategies.
- Comparative Studies with Different Product Categories: It would be helpful to examine the difference between organic and natural products in the context of food products other than honey. Such comparative studies can provide a more detailed and broad perspective on general consumer awareness and attitudes towards organic products.

This research has taken an essential step towards understanding consumers' knowledge and perceptions of natural and organic honey. However, future studies can contribute to the literature in this field by providing broader and more in-depth information**.**

## Declarations

### *Ethical Approval Certificate*

The experimental procedures of this study were approved by the Local Animal Care and Ethics Committee of Bursa Uludag University University, 26 January 2024 approval date, and number: 2024-01

### *Author Contribution Statement*

Please indicate how each author contributed to this work and at what stage. For example:

Fatih Bilici: Data collection, investigation, formal analysis, methodology, and writing the original draft

Nebi Seren: Project administration, methodology, supervision, review and editing

### *Conflict of Interest*

The authors declare no conflict of interest.

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# Spent Mushroom Substate (SMS) Usability as Casing Material in *Agaricus bisporus* Cultivation

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ABSTRACT

In this research, the usability of spent mushroom compost/substrate (SMC/SMS) as casing material was investigated. For this purpose, different volumes of peat and spent mushroom substrate (peat, peat + SMS (1/1), peat + SMS (1/2), peat + SMS (2/1), SMS) were used as casing material. The effects of spent mushroom substrate waste used as casing material and different volumes of peat applications on cap length, cap diameter, stipe length, stipe diameter, hardness, number of mushrooms, average mushroom weight, yield of cultivated mushrooms were determined. Different casing material applications affected stipe length, hardness, number of mushrooms, average mushroom weight and yield. Although the highest yield was obtained from peat application (59.86 kg 100 $kg^{-1}$ compost), it has been shown that spent mushroom substrate waste can be used in casing material mixtures in mushroom cultivation.

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## Introduction

Cultivated mushroom production in the world is increasing regularly, and the annual increase rate is distributed between 6-7% on average (Abak, 2024). According to FAO 2022 records, the total world mushroom and truffle production amount is 48 million tons, and in our country, it is around 65 thousand tons (FAO, 2024). The unique taste, aroma, and nutritional value of mushrooms increase the demand for them in the world (Sun et al., 2020). Mushrooms contain biological active substances (e.g., antioxidant, antitumor, antimicrobial, anti-inflammatory) that are beneficial for human health (Baktemur et al., 2022).

The compost resulting after production in mushroom cultivation is called spent mushroom compost (SMC) or spent mushroom substrate (SMS) (Phan & Sabaratnam, 2012; Pekşen & Yamaç, 2016; Jasińska, 2018). It is reported that 5 kg of SMS are released for each kg of mushrooms produced, regardless of the mushroom species (Semple et al., 2001; Williams et al., 2001; Lau et al., 2003; Ma et al., 2014; Jasińska, 2018). The amount of SMS released as a result of mushroom production is estimated to be around 240 million tons/year in the world and 325 thousand tons/year in Turkey.

The increase in cultivated mushroom production in the world and in Turkey every year also causes an increase in the amount of compost that becomes waste after harvest. The SMS released in large quantities is removed from the enterprises by burning, throwing it in the trash, or mixing it with the soil in agricultural areas. These practices are either uneconomic for businesses or create a significant environmental pollution problem. However, SMS is a material that can be used in many different areas and brought into the economy (Pekşen & Yamaç, 2016).

It is possible to utilize used mushroom compost in different ways. Some of these are; source of organic matter for plants grown in greenhouses (Çelikel & Çağlar 1999; Çelikel, 1999a; Polat et al., 2009; Peker, 2018) and in open fields (Courtney, & Mullen, 2008; Aydın, 2009; Sagar, et al. 2009; Uğur, 2019), in seedling production (Medina et al. 2009; Kwack et al. 2012), ornamental plant cultivation (Birben 1998; Çiçek, 2004; Sönmez, 2009), growing media in soilless culture (Çelikel, 1999b), compost in the production of different mushroom species (Gimenez, 2008; Mamiro & Royse 2008; Cunha Zied et al. 2020), casing material mushroom cultivation (Pardo Giménez & Pardo-González 2008; Pardo-Giménez et al. 2010; Pardo-Giménez et al. 2011), animal feed (Ayala et al., 2011; Kim

et al., 2011), vermicompost production material (Pekşen & Yamaç, 2016), bioremediation agent (Lau et al., 2003; Phan, and Sabaratnam 2012; Zhou et al., 2014; Rinker, 2017), plant disease and pest management (Gent et al., 1999; Litterick et al., 2004; Gea et al., 2014), enzyme production (Phan, & Sabaratnam 2012; Kökcü, 2020), fuel and biogas production (Zhu et al., 2013; Kapu et al., 2012; Pérez-Chávez et al., 2019) and particleboard manufacturing (Yağlıca, 2019).

The fact that SMS is low-cost, available in large quantities and rich in organic matter makes its use in agricultural production attractive (Pekşen & Yamaç, 2016; Shimira et al., 2022). However, the instability of SMS content, low water retention capacity, high ammonia content and high soluble salt level are the most important factors that may limit its use in plant production (Holozlu, 2013; Sütçü, 2018; Wever et al., 2005; Jasińska, 2018). Before evaluating the SMS, it is mandatory to reduce the EC value to the desired levels. Many researchers recommend that, due to the high salt content it contains, it should be made into piles in the field and left to decompose for 1-2 years (Birben, 1998; Guo et al., 2001; Polat et al., 2004;), and subjected to a certain washing process (Wever et al., 2005; Polat et al., 2009; Holozlu, 2013; Sütçü, 2018) or additions such as soil and peat it is recommended to use suitable mixtures prepared by making plants in plant cultivation (Birben, 1998; Çaycı et al., 1998; Kütük, 2000; Eren & Boztok, 2013).

Casing material provides the transition of *Agaricus bisporus* mycelia from the vegetative to the generative cycle (Özşimşir & Arın, 1996). In addition to encouraging fungus formation, casing material also supports the adhesion of cultivated fungus carpophores and meets the water requirement of the fungus (Çetin et al., 2016). The physical, chemical properties and biological structure of the casing material directly affect the yield and quality of the mushroom produced. For this reason, the most commonly preferred material in commercial mushroom production is peat (Erkel, 1992; Çolak, 2004; Eren, 2008; Pardo-Giménez, et al., 2017). However, the availability of peat, the depletion of reserves, and the instability of its structure due to changes in ecosystems have led to the search for alternative materials (Pardo-Giménez et al., 2017). Although many natural inorganic and organic materials, as well as peat moss, are used as casing material, very few of them have physical and chemical properties that can be used instead of peat (Pardo et al., 1999).

In this study, the possibility of using spent mushroom compost/substates kept in open areas for a year as a casing material, as an alternative to peat, which is widely used in *Agaricus bisporus* cultivation, was investigated.

## Materials and Methods

The research was conducted at Selçuk University, Faculty of Agriculture, Department of Horticulture, between November and January 2019-2020. In the study, 60 bags containing 10 kg of compost planted with mycelia of the Sylvan A15 variety of *Agaricus bisporus* species, obtained from Mega Tesnim A.Ş., were used. The used mushroom composts resulting from mushroom production a year ago were stacked in an open area and then kept as a casing material material.

***Preparation of the Growing Room***

The growth room was sprayed with 3% formaldehyde and the next day, after the room was ventilated, the bags containing compost were placed on the shelves in the growth room. The tools used in the room were also treated with 2% formaldehyde. After the bags placed on the shelves were opened, they were covered with paper. (Figure 1).

Figure 1. Covering compost with paper

***I. Mycelial Development Period***

During the mycelial development phase, the room temperature was kept at 21-22°C for 2 weeks. Compost temperature is 2-4°C higher than room temperature. In this regard, the compost temperature was kept between 25-26 $^0$C. A thermometer was placed in the room to control the temperature. During the micelle wrapping phase, the indoor humidity was tried to be kept at 90-95%. The papers on the compost were checked and moistened every day. Humidity and temperature control were monitored regularly until the mycelia surrounded the compost.

***Casing Material Saying, Establishment of the Experiment and II. Mycelial Development Period***

After the mycelia completely surrounded the compost, the papers on the compost were removed and a 4 cm thick casing material was laid. (Figure 2). Different volumes of peat and spent mushroom substrate (peat, peat + SMS (1/1), peat + SMS (1/2), peat + SMS (2/1), SMS) were used as casing material. For each application, 4 replicates and 3 bags were used in each repetition. The casing material was irrigated with irrigation water after laying. After all the procedures were completed, the room was sprayed with 1% formaldehyde. After the casing material was laid, the temperature was not changed for 3-4 days, allowing the mycelia to jump into the casing material. Then, the temperature was gradually reduced to 16 $^0$C and an attempt was made to keep it constant at this temperature. After the emergence of mycelia on the upper surfaces of the casing material was observed, the part of the casing material equal to the compost was mixed. With this process, which we call scratching, the mycelia were distributed evenly throughout the casing material.

***Harvest Period***

Harvest started 20 days after the casing material was laid. The mushroom cap diameter was harvested when it was 4.5-5 cm (Figure 3). The dirty bottom part of the harvested mushrooms was removed with a sharp knife, the top was cleaned, then they were weighed, measured and packaged.

Figure 2. Laying casing material on compost

Figure 3. View of mushrooms that have reached harvest size

Table 1. The effect of SMC used as casing material on the yield and quality of cultivated mushrooms.

| Treatments | CL | CD | SL | SD | F | NM | AMW | Y |
|---|---|---|---|---|---|---|---|---|
| Peat | 21.36a | 39.90a | 20.48ab | 18.64a | 2.36b | 366.67a | 15.76a | 59.87a |
| Peat + SMS (1/1) | 21.31a | 39.20a | 21.76a | 17.63a | 3.11a | 306.40b | 14.57bc | 45.62b |
| Peat + SMS (1/2) | 21.25a | 38.73a | 19.87ab | 17.28a | 2.47b | 295.53bc | 14.87b | 41.39bc |
| Peat + SMS (2/1) | 21.06a | 38.67a | 20.58ab | 18.81a | 2.36b | 315.53ab | 14.85b | 49.43b |
| SMC | 21.33a | 39.48a | 18.75b | 18.59a | 2.39b | 249.17c | 13.95c | 34.77c |

C: Cap length (mm); CD: Cap diameter (mm); SL: Stipe length (mm); SD: Stipe diameter (mm); F: Firmness (kg/cm$^2$); NM: Number of mushrooms (fruits/bag); AMW: Average mushroom weight (g); Y: Yield (kg 100 kg$^{-1}$ compost); *Means with different letters in the same column were significantly different ($p<0.05$). SMC: Spent mushroom substrate

***Analysis and Measurements***

In the study, in all applications, mushrooms obtained from each harvest were measured separately and at the end of the 45-day harvest period, yield (kg 100 kg$^{-1}$ compost), number of mushrooms (fruits/bag), and average mushroom weight (g) were determined. Cap diameter (mm), cap length (mm), stipe diameter (mm), stipe length (mm), hardness (kg/cm$^2$) measurements were determined by measurements made on 10 mushroom samples randomly selected from all repetitions of each application. Hardness measurements were made with the help of a penetrometer. Hat diameter, hat length, hat diameter and hat length were measured using a digital caliper with an accuracy of +0.01 mm. The experiment was set up with 4 replications according to the random parcel design and 3 bags were used in each replication. The obtained data were grouped using the Duncan multiple comparison test by performing variance analysis with the SPSS statistical program. In their evaluation, the significance between the differences (5%) was determined.

**Results and Discussion**

In the study, the effects of spent mushroom substrate and peat and their mixtures in different volumes as casing material on the cap height, cap diameter, stipe length, stipe diameter, hardness, number of mushrooms, average mushroom weight and yield of cultivated mushrooms were statistically investigated. As a result, while the effect of the media used as casing material on stipe length, hardness, number of mushrooms, average mushroom weight and total yield was found to be statistically significant, its effect on cap height, cap diameter and stipe diameter was insignificant (Table 1).

The effect of spent mushroom substrate (SMC), peat and their mixtures in different volumes on the stem length of cultivated mushrooms was found to be statistically significant. The highest stipe length value was obtained from the peat + SMC (1/1) application, 21.76 mm, and the lowest value, 18.75 mm, was obtained from the SMC application (Table 1). According to the results of cover soil trials in *Agaricus bisporus* cultivation, it is reported that the stem length values of mushrooms vary between 15.8-30.6 mm (Çetin & Eren, 2017; Önel, 2020; Duran et al., 2023).

The effect of different casing material used on mushroom firmness was found to be statistically significant. The highest value in hardness measurements was obtained from peat + SMC (1/1) application (3.11 kg/cm$^2$) (Table 1). It is compatible with the hardness values (1.42-3.87 kg/cm$^2$) determined by Duran et al., (2023).

The effect of different casing material on the number of mushrooms was found to be statistically significant. The highest number of mushrooms was obtained from the peat application (366,67 fruits/bag) and the lowest was obtained from the SMC application (249.17 fruits/bag) (Table 1). In the study of vermicompost, rose oil processing waste compost, and spent coconut fiber as casing material in button mushroom cultivation, they obtained a mushroom count of 4.88-184.83 fruits/bag (Duran et al., 2023). In his study conducted by Yücel, 2024, they obtained the number of mushrooms between 295.5-337.8 fruits/bag. It is seen that the number of mushrooms obtained decreases depending on the SMC ratio used as casing material (Table 1). It can be said that this is due to the increasing EC value in the casing material.

The effect of different casing material mixtures on the average mushroom weight was found to be statistically significant. While the highest mushroom weight was obtained from peat application with 15.76 g, the lowest value was obtained from SMC application with 13.95 g. In different studies on casing material, average mushroom weights varied between 12.8-23.8 g (Çetin & Eren , 2017; Duran et al., 2023; Yücel, 2024). Similar to the number of mushrooms, the mushroom weights obtained decreased due to the increasing SMC rate (Table 1).

The effect of spent mushroom substrate and peat and their mixtures in different volumes on the yield of cultivated mushrooms was found to be statistically significant. The highest yield value (59.86 kg 100 $kg^{-1}$ compost) was obtained from peat application, and the lowest yield value was obtained from SMC application (34.77 kg 100 $kg^{-1}$ compost) (Table 1). In the study investigating the possibility of using peat mixtures of seagrass, tea waste, sugar beet lime and spent mushroom substrate as casing material in mushroom cultivation, a yield of 3.55-21.96 kg per 100$kg^{-1}$ compost was obtained (Eren & Boztok, 2013). Polat & Önel, 2021, in their study where they used perlite and vermicompost as casing material, they achieved a yield of 22.73 - 35.69 kg per 100 $kg^{-1}$. In the experiment, taking the product in 4 flash periods for 45 days increased the yield values.

## Conclusion

Peat is an ideal casing material for cultivated mushroom (*Agaricus bisporus*) (Erkel, 1992; Çolak, 2004; Eren, 2008; Pardo-Giménez, et al. 2017). However, peat is also widely used in the seedling sector and soilless agriculture. It is not a sustainable resource in the future due to the limited resources of peat in the world and the damage it causes to the environment during its extraction. Just as other natural resources will run out in the near future, peat resources will also run out. As long as human beings exist, they will be fed, and it is inevitable for agricultural activities to be sustainable in order to be fed. For this reason, some new materials that can replace peat in cultivated mushroom cultivation have been emphasized and various studies have been conducted on this subject (Gülser & Pekşen, 2003; Çolak, 2004; Taşkın et al., 2008; Çetin & Eren, 2017). It has been reported that materials such as composted pine tree bark, wood sawdust, tree bark, spent mushroom substrate, coconut fiber, sugar beet lime, seagrass, tea waste and waste paper can be among the alternative casing material (Gülser & Pekşen, 2003; Pardo-Giménez & Pardo- González, 2008; Pardo-Giménez et al., 2017; Boztok, 2013).

SMC is a potential material that can be used as casing material. As can be understood from the efficiency elements of the experiment, SMC can be an alternative material to peat, provided that the salinity problem is completely eliminated by practices such as washing, keeping it in open areas or mixing it with low conductivity materials.

## Declarations

### *Conflicts of Interest*

The author declare no conflict of interest.

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# Evaluation of Antioxidant and Anti-inflammatory Potential of *Alpinia officinarum* with Different Ionic Solutions

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ABSTRACT

*Alpinia officinarum*, which attracts attention with its antioxidant and anti-inflammatory properties, is used in traditional medicine, especially to relieve stomach and digestive system disorders. Although many studies have revealed the various pharmacological effects of *Alpinia officinarum*, the effect of different ionic solvents on its biological activities has yet to be investigated. In this study, the effects of homogenization of *Alpinia officinarum* roots with potassium chloride (KCl), sodium chloride (NaCl), and phosphate (PBS) buffer solutions on the antioxidant and anti-inflammatory properties of the plant were investigated. *Alpinia officinarum* plant was collected from the Adana region during the season, and fresh root parts were separated and analyzed. Superoxide dismutase (SOD), catalase (CAT), myeloperoxidase (MPO) enzyme activities, and malondialdehyde (MDA) levels of plant homogenates prepared with KCl, NaCl, and PBS were determined by spectrophotometric analysis. The highest MPO and CAT enzyme activities were observed in the KCl solution, while lower levels were observed in NaCl and PBS solutions, respectively. The highest MDA level was observed in the PBS solution. Moreover, SOD enzyme activity showed a decreasing trend in NaCl, KCl, and PBS solutions, respectively. These findings suggest that the biological activity of plant extracts may vary depending on the solvent used. Determination of the conditions under which the antioxidant and anti-inflammatory effects of *Alpinia officinarum* in different ionic solvents are the highest supports increasing the bioavailability of the plant.

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## Introduction

*Alpinia officinarum* is mainly grown for medicinal purposes in Türkiye since it prefers tropical climates (Abd Rahman et al., 2024; Ozkan et al., 2016). It is widely used against stomach and intestinal diseases with its digestive system supportive feature. *Alpinia officinarum*, a member of the ginger family (Zingiberaceae), shows similar biological properties to ginger (Lei et al., 2024)(Herbarium code: LINN-HS 6.3). This plant has attracted attention due to its antioxidant, anti-inflammatory, and anticancer properties due to its high flavonoid and phenolic compound content, and it has been included in scientific research (Lin et al., 2020).

Previous in vitro and in vivo studies have demonstrated the effects of *Alpinia officinarum* in alleviating oxidative stress and inflammation by changes in superoxide dismutase (SOD), catalase (CAT) , malondialdehyde (MDA), glutathione, myeloperoxidase (MPO), interleukin, and nuclear factor kappa B (NF-κB) levels (Ashtari et al., 2023; Lin et al., 2023; Rajendiran et al., 2018; Suja & Chinnaswamy, 2008; Xin, 2011). It was reported that *Alpinia officinarum* extract caused a decrease in oxidative stress markers by modulating SOD and CAT enzyme activities in various organs in a hypertensive rat model (Javaid et al., 2021). Similarly, it was reported that hydro-alcoholic extract of *Alpinia officinarum* suppressed cisplatin-induced testicular toxicity by decreasing MDA levels and increasing SOD activity. It was emphasized that the antioxidant effects of the plant extract may be supportive in increasing the safety of cancer treatments (Ashtari et al., 2023). In addition, other in vivo studies on the antioxidant effects of *Alpinia officinarum* show that the plant extract reduces oxidative stress markers, alleviates lung damage by increasing SOD activity, and prevents diseases associated with the digestive system (Lei et al., 2024; Xin, 2011; Zhao et al., 2019). In vitro studies investigating the chemical and biological effects of *Alpinia*

*officinarum* extract have generally focused on the cytotoxicity of the plant in various cancer cell lines (F et al., 2019). It was reported that the ethanolic extract of the plant suppressed cell proliferation by causing DNA damage in cell lines derived from prostate adenocarcinomas (Suja & Chinnaswamy, 2008) It was also reported that the plant extract exhibited antiproliferative effects by activating the caspase-dependent cell death pathway in breast cancer cells (Ghil, 2013).

*Alpinia officinarum* extracts contain various bioactive flavonoid components such as quercetin, galangin, kaempferol, and curcumin, strengthening the plant's anti-inflammatory and antioxidant activities. The anti-inflammatory effects of *Alpinia officinarum* rich in flavonoids were tested in the RAW264.7 cell model in which inflammation was induced by lipopolysaccharide (LPS). This study reported that *Alpinia officinarum* and its bioactive compounds suppressed the inflammatory response by inhibiting COX-2, IL-1β, IL-6, NF-κB, NO, and TNF-α production (Li et al., 2021). In addition, in vivo anti-inflammatory and COX-2 inhibitory effects of *Alpinia officinarum*, which may be related to the presence of phenolic content, were investigated, and the relationship between these effects and in vitro antioxidant activities was revealed. It has been reported that the phenolic content of the plant extract with anti-inflammatory and antioxidant activities may provide supportive effects in the treatment of inflammatory diseases (Honmore et al., 2016)

Previous studies have addressed the broad pharmacological profile of *Alpinia officinarum*, particularly its antioxidant, anti-inflammatory, and anticancer activities. However, these studies usually utilize extractions with solvents such as ethanol or methanol, with no specific evaluation of the effects of KCl, NaCl, or PBS solutions on biological activity. This study aimed to investigate the effects of antioxidant and anti-inflammatory activities of *Alpinia officinarum* roots using solutions with different ionic environments. Evaluation of antioxidant/oxidant parameters such as CAT, SOD, MPO, and MDA in various solutions will contribute to the enhancement of the pharmacological efficacy of the plant and potential health applications.

## Materials and Methods

The root of the naturally growing *Alpinia officinarum* (galangal) plant used in this study was collected from the borders of Adana province in the Mediterranean Region. Plant roots, flowers, stems, and leaves were removed and used fresh for the analyses. Plant description was carried out by researchers at the Faculty of Agriculture of our university.

### *Biochemical Analyses*

*Plant preparation for biochemical analysis*

The preparation of plant samples was carried out in the Medical Biochemistry Research Laboratory of KSU Faculty of Medicine. Fresh plant roots brought to the research laboratory were cut into small pieces using a sterile scalpel. Plant homogenate was prepared with 1.15% KCl, 0.9% NaCl, and 0.1 M PBS solutions at indicated concentrations. The solutions were added 1/5 to the plant root pieces and homogenized (lavion mechanical disintegrator). The plant homogenate was filtered and centrifuged (Hettich 420 R) at 5000 rpm for 5 min, and the supernatant was used for analysis. Analyses were carried out in five replicates for fresh plant roots.

*Determination of SOD Activity*

SOD enzyme activity in plant extracts was measured using the assay method described in detail previously (Fridovich, 1995). The method is based on the interaction of superoxide radicals produced by xanthine and xanthine oxidase with NBT to form a purple formazan dye. The intensity (OD) of formazan dye was measured in a spectrophotometer at a wavelength of 505 nm. A low absorbance value indicates high SOD enzyme activity.

*Determination of CAT activity*

CAT activity was determined by spectrophotometric measurement of H2O2 concentration at 230 nm wavelength in the reaction in which hydrogen peroxide was the substrate (Beutler, 1984). CAT enzyme catalyzes the conversion of hydrogen peroxide to water and oxygen. CAT activity was expressed as U/mg protein. The rate of decrease in absorbance value (ΔA/min) is considered an indicator of catalase activity.

*Determination of MDA level*

It is based on the principle that MDA, which is the secondary product of lipid peroxidation formed as a result of incubation of the sample at 90-95 C$^{o}$ with thiobarbituric acid (TBA) at pH 3.40 under aerobic conditions, forms a pink complex with TBA. This color intensity is directly proportional to the MDA concentration in the medium and is evaluated spectrophotometrically at 532 nm (Ohkawa et al., 1979).

*Determination of Protein level*

Both protein and polyphenol compounds can be determined by the Folin Cioacalteu method. The Folin technique is based on the absorbance measurement at 750 nm by the spectrophotometric method of the color reaction of tyrosine and tryptophan residues contained in proteins in the extract with phosphotungstic-phosphomolybdic acid (Lowry et al., 1951). Bovine serum albumin was used as a standard.

### *Statistical Analyses*

GraphPad Prism 10 software was used to analyze the data obtained in this study. Normality tests were applied to determine whether the data fit normal distribution. An unpaired t-test for independent groups was used to evaluate whether the differences between the groups were statistically significant. The significance level was accepted as $p<0.05$. The results obtained are presented as mean ± standard error.

## Results and Discussion

This study showed that the enzyme activities and antioxidant profile of the *Alpinia officinarum* plant may vary depending on the solvent medium. Although there are many studies in the literature demonstrating the antioxidant and anti-inflammatory properties of *Alpinia officinarum* in various disease models (Ashtari et al., 2023; Rajendiran et al., 2018; Xin, 2011), there is no study investigating the effects of specific ionic and buffer solutions such as KCl, NaCl, and PBS on antioxidant and anti-inflammatory enzyme activities and oxidant markers of the plant.

Table 1. Effects of different ionic and buffer solutions on antioxidant enzyme activities and oxidative stress markers of *Alpinia officinarum* Extract

| Measurements | KCl | PBS | NaCl |
|---|---|---|---|
| CAT (U/mg protein) | 0.470±0.122[a] | 0.0940±0.0270[b] | 0.418±0.115[a] |
| SOD (U/mg protein) | 1.05±0.187[b] | 0.282±0.0259[c] | 1.36±0.153[a] |
| MDA (nmol/mg protein) | 1.48±0.195[b] | 2.62±0.392[a] | 1.09±0.0988[c] |
| MPO (U/mg protein) | 0.136±0.0168[a] | 0.0816±0.0109[b] | 0.113±0.0151[b] |

Data are presented as mean ± standard deviation (SD) (n=5). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test; Superscript letters (a, b, c) indicate significant differences among the solvents (KCl, PBS, and NaCl) within the same parameter. Groups sharing the same letter are not significantly different (p>0.05).

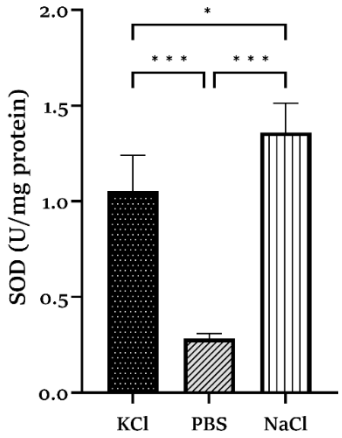


Figure 1. Comparison of CAT enzyme activity changes after Alpinia officinarum plant root homogenization in different solvents (KCl, NaCl, and PBS).

CAT (U/mg protein) levels were measured in root extracts. CAT activities of KCl and NaCl homogenate were significantly higher than PBS homogenate (***p<0.001).

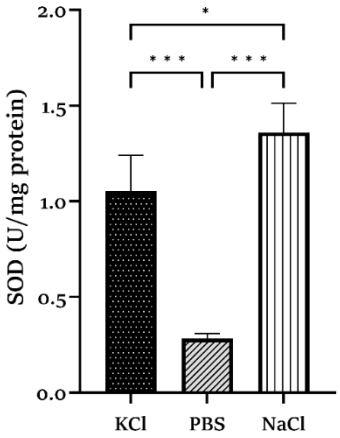


Figure 2. Variation of SOD activity of Alpinia officinarum plant root depending on different solvent media (KCl, NaCl, and PBS)

SOD (U/mg protein) levels were measured in root extracts. SOD activities of KCl and NaCl homogenate were significantly higher than PBS homogenate (***p<0.001). The SOD activity of the NaCl group was significantly higher than that of the KCl group (*p<0.05).

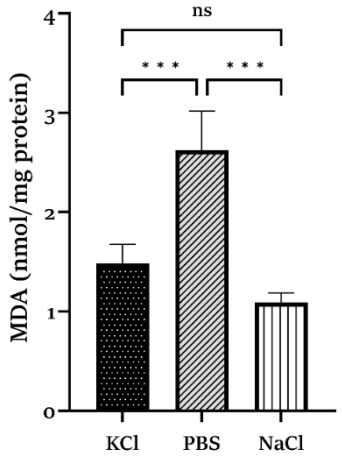


Figure 3. Changes in MDA levels after homogenization of Alpinia officinarum plant root in different solvents (KCl, NaCl, and PBS).

MDA (nmol/mg protein) levels were measured in root extracts. MDA levels of PBS homogenate. PBS homogenate was significantly higher than KCl and NaCl homogenate (***p<0.001). There was no statistically significant difference between the MDA levels of KCl and NaCl homogenates.

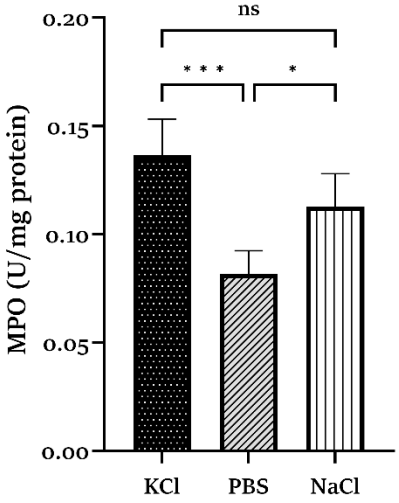


Figure 4. Variation of MPO activity of Alpinia officinarum plant root depending on different solvent media (KCl, NaCl, and PBS)

MPO (U/mg protein) levels were measured in root extracts. MPO activities of KCl and NaCl homogenate were higher than PBS homogenate (***p<0.001 and *p<0.05).

In this context, our study shows that the solvent medium used plays an important role in maintaining the antioxidant and anti-inflammatory enzyme stability of *Alpinia officinarum*. The findings revealed that the activities of plant-derived antioxidant and anti-inflammatory modulators may vary depending on the solution conditions. Changes in CAT, SOD, MPO enzyme activities, and MDA levels after homogenization of galangal root in different solvents (KCl, NaCl, and PBS) are shown in Table 1 and Figure 1, 2 and 3. The results

showed that KCl and NaCl significantly increased CAT enzyme activity. In particular, it was observed that KCl and NaCl stabilized CAT enzyme activity more effectively than PBS (Figure 1). Similarly, SOD enzyme activity was significantly increased in the groups homogenized with KCl and NaCl solutions. The highest SOD enzyme activity was determined in the NaCl group (Figure 2). It is thought that the increasing effect of ionic solutions such as KCl and NaCl on CAT and SOD enzyme activities is due to the stabilization of the three-dimensional structure of the enzymes in the plant root by potassium and sodium ions. In addition, sodium and potassium ions may indirectly affect the function of enzymes by affecting the pH and ionic strength of the medium. High ionic strength can strengthen enzyme-substrate interactions. In addition, sodium or potassium ions are used as cofactors for some enzymes. This facilitates the substrate binding of enzymes (Page & Di Cera, 2006). In support of our study, other studies have examined the changes in antioxidant capacities of different plants after exposure to various ionic environments. In potato shoots exposed to specific concentrations of NaCl, CAT enzyme activity and glutathione levels, which indicate antioxidant capacity, were found to increase (Aghaei et al., 2009). Similarly, the effects of different ionic media such as seawater, KCl, NaCl, CaCl2, and MgCl2 on quinoa seedling emergence and antioxidant properties were investigated. It was reported that CAT, APX, POX, and SOD enzyme activities varied in ionic media such as $Na^+$, $K^+$, $K^+$, $Ca^{2+}$ , and $Mg^{2+}$ and that CAT and SOD antioxidant enzyme activities increased in quinoa plants at specific ionic concentrations (Panuccio et al., 2014). This study analyzed MDA levels after homogenizing *Alpinia officinarum* roots in different solvents (KCl, NaCl, and PBS). MDA levels, an indicator of lipid peroxidation, were highest in the PBS solution and lowest in the NaCl solution. In statistical analyses, MDA levels in PBS solution were significantly higher than in KCl and NaCl solutions (Figure 3). However, no statistically significant difference was observed between KCl and NaCl solutions. The high MDA level observed in the PBS solution suggests that the plant homogenate promotes more lipid peroxidation in the PBS medium. Although PBS is known to increase the stability of the solution due to its buffering properties, it triggered lipid peroxidation by increasing the solubility of oxidation-sensitive compounds in the homogenate of *Alpinia officinarum* root. The observation of lower MDA levels in KCl and NaCl solutions suggests that these ionic environments contribute less to lipid peroxidation and may have a more protective effect against oxidation. In the studies, three different pomegranate varieties were exposed to increasing concentrations of NaCl, KCl, and K2SO4 solutions, and their biochemical responses were evaluated. It was reported that high concentrations of NaCl, KCl, and $K_2SO_4$ solutions caused an increase in MDA levels, whereas low concentrations of solutions were associated with low lipid peroxidation (Dichala et al., 2022). Similarly, a dose-dependent increase in MDA levels due to salt tolerance was expressed in tobacco seedlings exposed to eight different NaCl concentrations ranging from 0-350 mM (Çelik & Atak, 2012). In addition, oxidative and antioxidative responses of NaCl and KCl solutions on Chenopodium album were examined by superoxide, hydrogen peroxide, MDA concentrations, and SOD, CAT, and POX levels (Yao et al., 2010). It was reported that oxidative stress levels were similar to the control group at 50 mM NaCl and KCl solution concentrations. However, a significant dose-dependent increase in SOD, CAT, and POX activities was observed at 50 and 300 mM concentrations; it was reported that growth parameters were positively affected in plants exposed to 50 mM concentration (Yao et al., 2010). However, these studies focused on the physiological and molecular effects of salt stress on plants rather than the effects of ionic solvents on the antioxidant-oxidant balance of plants. Therefore, in our study, the focus of plant homogenization using ionic solutions and PBS on the plant's enzyme activities and antioxidant defense mechanisms will provide important contributions to the literature in this field.

This study investigated the effects of different solutions on the antioxidant content of *Alpinia officinarum* and its anti-inflammatory content. The findings revealed that MPO activity in the plant was higher in KCl and NaCl solutions compared to PBS solution **(Figure 4**). This finding indicates the potential of intracellular potassium and sodium levels to modulate MPO activity in inflammatory processes. Moreover, the increase in MPO activity in the KCl solution was statistically more significant than in the NaCl solution. Although the activity in the NaCl solution was lower than in KCl, it was higher than in the PBS solution, indicating that $Na^+$ ions also support MPO activity at a certain level. These results suggest that the anti-inflammatory properties of *Alpinia officinarum* roots may vary depending on the solvent medium. Considering that MPO is an enzyme activated during inflammation (Frangie & Daher, 2022), these results suggest that ionic solutions such as KCl and NaCl may provide a suitable medium to evaluate the anti-inflammatory potential of this plant. .In the literature, the anti-inflammatory and antioxidant properties of Alpinia officinarum extracted in different solvents have been evaluated together. In studies, it was reported that hexane extract of Alpinia officinarum showed prophylactic effects in acute and chronic experimental colitis models, and these effects were associated with decreased levels of inflammatory markers and lipid peroxidation (Rajendiran et al., 2018). It has also been reported that Alpinia officinarum exhibits anti-inflammatory and antioxidative effects in gastrointestinal diseases through its flavonoid content, and regulatory mechanisms on MPO, SOD, GSH, and MDA levels mediate these effects (Lin et al., 2023).

In conclusion, this study revealed the varying antioxidant and anti-inflammatory enzyme activities of Alpinia officinarum in different ionic and buffer solution media such as PBS, KCl, and NaCl. The findings indicate that plant sources' antioxidant and anti-inflammatory enzyme activities may vary depending on the solution conditions. Therefore, selecting the appropriate solution for plant extraction or homogenization is important in preserving the plant's antioxidant and anti-inflammatory properties and increasing its effectiveness. This will contribute to optimizing the efficacy of bioactive plants such as Alpinia officinarum and increasing their potential for clinical use, especially in herbal treatment approaches.

## Declarations

### *Ethical Approval Certificate*

This research does not involve any data or materials related to humans, animals, or other living organisms. As a result, ethical committee approval and informed consent is not required for this study.

### *Author Contribution Statement*

*Nuray Üremiş:* Data analysis, formal analysis, and writing the original draft

*Figen Güzelgül:* Data collection, conceptualization, methodology, and review.

*Ergül Belge Kurutaş:* Data collection, conceptualization, methodology, and review.

### *Fund Statement*

Not applicable

### *Conflict of Interest*

The authors declare no conflict of interest.

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# Investigation of Antioxidant Properties of *Spartium junceum* L.: Effect of Plant Parts and Storage Conditions

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ABSTRACT

*Spartium junceum* L. is a plant traditionally used for different medicinal purposes. While limited research data explicates its antioxidizing ability, interest in this plant is induced mainly due to its possible role, especially against stress-causing oxidative effects. The objectives of this study were to compare antioxidant activity in flowers and leaves of *Spartium junceum* L., as well as time under different storage conditions implemented for antioxidative mechanisms. *Spartium junceum* L. plants were obtained from the Kahramanmaras, Turkey region; subsequently, the flowering and leaf parts of the plant were separated and analyzed. Plant homogenates were prepared, and the activities of SOD and CAT enzymes, as well as MDA levels, were determined using spectrophotometric methods. Enzyme activity upon storage at +4°C, -20°C, and -70° temperature enzyme samples were carried out separately and operated for less than one month in our laboratory. Flowers exhibited higher SOD and CAT activities than leaves. Flowers also showed higher levels of MDA. It may be due to the structural and biochemical differences, where flowers experience extra oxidative stress. The optimal enzyme retention under storage conditions was at -70°C, and a decrease in temperature increased the stability of this biocatalyst. In contrast, MDA levels increased at low temperatures at total capacity. The antioxidant properties of the flower extract had stronger antioxidant potential than those of the leaf part, which also means that chemically active substances show much higher concentrations in this plant section. Storage temperature significantly affects the stability of enzymes, and it was stated that low temperatures mainly maintain antioxidant activity. The results obtained from this study recommend *Spartium junceum* L. as a valuable antioxidant food resource.

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## Introduction

*Spartium junceum* L. is a perennial herb that grows in the Mediterranean region. It's been used for thousands of years in traditional medicine and is highly considered an antioxidant. *Spartium junceum* L., for instance, has been reported to have a range of pharmacological properties, including anti-inflammatory and cytotoxic activities, based on its biological activity studies. The aromatic juice of the plant has been reported to have a cytotoxic effect (Cerchiara et al., 2012; Teresa Cerchiara et al., 2013), and its flower extracts were studied for their anti-inflammatory and analgesic activities (Menghini et al., 2006; Zengin et al., 2019). It has been reputedly described as a sedative and diuretic for the flowers (Menghini et al., 2006). Studies *in vivo* have shown that *Spartium junceum* L. has antiulcerogenic effects on gastric ulcers, highlighting the possible role of this plant for therapeutic use in stomach ulcers (Yesilada, Takaishi, et al., 2000). Also, extracts from the plant were revealed as very toxic against some cancer cell lines and might potentially be a natural anti-tumor agent (Cerchiara et al., 2012).

This antioxidant capacity of plants is important as it can protect cells from the harmful effects of oxidative stress. Oxidative stress leads to free radical-induced damage of cells and is a causal agent in the pathogenesis of aging and chronic diseases (e.g. diabetes, cardiovascular disease) / inflammation Antioxidants prevent harmful free radicals from interfering with cell integrity and lower your risk of chronic diseases including heart disease, diabetes, and even cancer (Aune, 2019; Çelik & Pepe, 2024; Jena et al., 2023; Karaman & Türkay, 2023). Various studies on the antioxidant action of *Spartium junceum* L. have drawn attention to its phenolic compounds and free radical scavenging activity (Eruygur et al., 2022; Habibatni et al., 2016). Its extracts have been shown to scavenge free radicals and relieve oxidative stress (Yesilada, Tsuchiya, et al., 2000). The plant is known for its antioxidant properties, with flavonoids playing an important role. Flavonoids are reputed to be free radical scavengers and can protect cells from toxic injury due to survival antioxidant properties. Many studies showed that *Spartium junceum* L. contains

flavonoid glycosides with free radical scavenging activity (Roy et al., 2022). It may be helpful in its antioxidant effect on parameters measured in this study.

Moreover, *Spartium junceum* L. extracts have been found to exhibit superoxide dismutase (SOD) activity and might act like this enzyme in converting toxic superoxides into less harmful forms (Yesilada, Tsuchiya, et al., 2000). These results suggest that the plant could be used to prevent oxidative stress-associated cellular damage. Given the high content of flavonoids and other bioactive components in this plant, we may assume it has significant antioxidant activity. This property might generate protective effects against oxidative stress (Nanni et al., 2018).

Nevertheless, the detailed antioxidant action of each organ in plant parts such as leaves and flowers from *Spartium junceum* L. and its differences among different conditions that might occur during storage still need to be studied to a great extent. However, the information regarding biochemical stability and activity of antioxidant compounds at various temperatures (both higher (+4 ºC) and lower than this range (-20º, -70º C)) during their storage for different periods like 1 to up to a maximum of 30 days is less represented in published literature. The storage conditions of plants directly affect the antioxidant activities and stabilization of biochemical components, which is why studies in this field are insufficient.

This study was intended to analyze the antioxidative ability of *Spartium junceum* L., and it included measurement of SOD, catalase (CAT), and malondialdehyde (MDA) content in leaves as well as flowers. Also, to evaluate how they are impacted by the storage conditions (at three different temperatures: +4ºC, -20ºC, and-70ºC) at specific periods (1, 3, 5, 15 and 30 days). The focus of this research is to provide a more detailed source of information on the particular antioxidant activity (in terms of leaves and flowers) and explore the various health benefits by which relatively fewer scientific studies with respect to *Spartium junceum* L remain.

## Materials and Methods

The naturally growing *Spartium junceum L.* plant used in this study was collected from the borders of Kahramanmaraş province in the Mediterranean Region. Plant description was carried out by researchers at the Faculty of Agriculture of our university. Plant samples were sterilized, sectioned, separated into flowers and leaves, and prepared for analysis.

### *Biochemical Analysis*

*Plant preparation for biochemical analysis*

Plant sampling is processed in the Kahramanmaraş Sütçü İmam University Faculty of Medicine, Medical Biochemistry Research Laboratory. Fresh plant samples delivered to the laboratory were mixed and separated from flowers and leaves with a sterile scalpel and milled using a mechanical cutter Lavion company Brand. Plant homogenate ground with 1/10 KCl was rended and filtered. The filtrate was centrifuged (Hettich 420 R) at 5000 rpm for five minutes, and the supernatant was used in the analysis. Analyses were carried out in five replicates for fresh plant leaves and flowers and single measurements for storage conditions.

### *Determination of SOD Activity*

The enzymic activity was assayed by following Fridovich method (1995). The process depends on the formation of red formazan dye (Fridovich, 1995) due to an interaction of superoxide radicals produced by xanthine and additionally by xanthine oxidase with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl tetrazolium chloride which is called as pyodonitrotetrazolium violet (NBT). The spectrophotometer readings are made by determining the optical density (OD) of formazan that has been formed, which is read at a wavelength of 505 nm. The intensity of color decreases under SOD action, which in turn is known to indicate the amount of activity.

*Determination of CAT activity*

CAT activity was determined by measuring the decreased hydrogen peroxide concentration at 230 nm (Beutler, 1984). CAT activity was expressed as U/mg protein. CAT catalyzes the degradation of hydrogen peroxide. The hydrogen peroxide degradation rate by CAT was measured spectrophotometrically using the light absorption of hydrogen peroxide at 230 nm.

*Determination of MDA level*

It is based on the principle that MDA, which is the secondary product of lipid peroxidation formed as a result of incubation of the sample at 90-95 Cº with thiobarbituric acid (TBA) at pH 3.40 under aerobic conditions, forms a pink complex with TBA. This color intensity is directly proportional to the MDA concentration in the medium and is evaluated spectrophotometrically at 532 nm (Ohkawa et al., 1979).

*Protein level*

The levels of protein and polyphenol compounds can be determined by the Folin Cioacalteu method. The Folin technique is based on the absorbance measurement at 750 nm by the spectrophotometric method of the color reaction of tyrosine and tryptophan residues contained in proteins with phosphotungstic-phosphomolybdic acid (Lowry et al., 1951). Bovine serum albumin was used as standard. SOD, CAT, and MDA levels were calculated and then divided by the measured protein levels, and the results were given as SOD, CAT activity, and MDA level per mg protein.

### *Statistical Analyses*

GraphPad Prism 10 software was used to analyze the data obtained in this study. Normality tests were applied to determine whether the data fit normal distribution. An unpaired t-test for independent groups was used to evaluate whether the differences between the groups were statistically significant. The significance level was accepted as $p<0.05$. The results obtained are presented as mean ± standard error.

## Results and Discussion

This study demonstrated that the flowers of *Spartium junceum* L. exhibit an enhanced antioxidant potential starting from extracts than leaves. Results showed that the floral tissues possessed higher activities of enzymatic antioxidants and malondialdehyde (MDA) levels, which indicate an enhanced action for antioxidative defense. Then, this result associated with the phenol-richness of flowers would have promising implications for preventing oxidative stress.

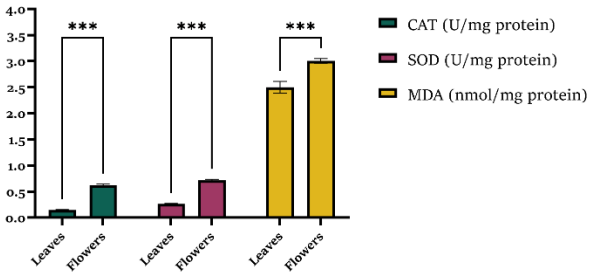


Figure 1. Comparison of catalase (CAT), superoxide dismutase (SOD) activities, and malondialdehyde (MDA) levels in *Spartium junceum L.* flowers and leaves.

CAT (U/mg protein), SOD (U/mg protein), and MDA (nmol/mg protein) levels were measured in flower and leaf extracts. CAT, SOD activities, and MDA levels were significantly higher in flowers than in leaves (***p<0.001).

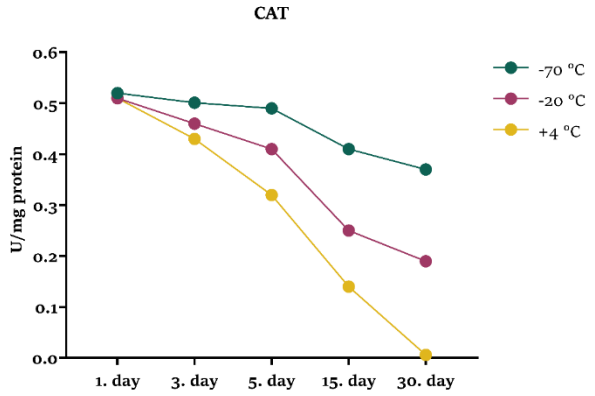


Figure 2. Time-dependent catalase (CAT) activity variation in *Spartium junceum L.* samples at different storage temperatures.

CAT activity measured in the plant's flower and leaf parts (pool) was monitored at +4°C, -20°C, and -70°C for periods of 1, 3, 5, 15, and 30 days. While the maximum decrease was observed at +4°C, CAT activity was maintained at the highest levels in samples stored at -70°C.

CAT enzyme activity was determined according to 0.626 U/mg protein in flower extracts and 0.146 U/mg protein of leaf extract, respectively (Figure 1). By scavenging hydrogen peroxide into water and oxygen, the CAT enzyme protects cells against reactive oxygen species (ROS), which cause cellular damage (Nandi et al., 2019). The result shows that the antioxidative defense system in flowers is more sensitive than in leaves. This high CAT activity accords with a compensatory phenolic system in response to oxidative stress, given that the concentration of these compounds and such enzymatic capacity would provide a greater degree of detoxification. These findings conform to the literature where plant antioxidants are frequently reported as positive protectants against ROS (Çelik, 2023; Eruygur et al., 2022; Habibatni et al., 2016). In addition, various enzymes that include CAT are critical in protecting from oxidative stress-associated diseases (Menghini et al., 2006). Hence, there is an interest in *Spartium junceum* L. to prevent damage induced by oxidative stress (Cerchiara et al., 2012; Nanni et al., 2018).

The highest activity of superoxide dismutase (SOD) was detected in flowers, and its specific activity was 0.718 U/mg protein also. In leaves, this value was 0.262 U/mg protein (Figure 1). The SOD enzyme serves in the antioxidant defense, removing superoxide radicals to hydrogen peroxide (Younus, 2018). The high SOD activity in flowers may reflect the effect of flavonoids and other polyphenols to increase the activity of these enzymes (Beslo et al., 2023). The scant information found in the literature regarding SOD activity of *Spartium junceum* L. points to this species being a good contender for oxidative stress (Eruygur et al., 2022). Consumption of food with high activities of SOD can be essential to prevent cardiovascular and neurodegenerative diseases (Cerchiara et al., 2012; Teresa Cerchiara et al., 2013).

MDA levels (an index of lipid peroxidation) were reported at 3.006 nmol/mg protein in flowers and 2.5 nmol/mg protein in leaves (Figure 1). The flowers exhibited the highest MDA contents in this experiment, indicating increased oxidative processes and metabolic rates. This agrees with the prior study: flowers undergo higher oxidative stress than fruits and leaves, retaining their structural conformationality involving biochemical pathways.

The ABTS radical scavenging activity of *Spartium junceum* L. methanol extracts was reported in the literature as indicating a potent radical-scavenging ability against ABTS. The fact that this effect was especially evident in the flowers supports that this part may be a richer source of antioxidant compounds. In superoxide radical scavenging tests, the plant extracts also showed good potency. Flower extracts were found to be the most active, and this activity was also notably higher when the compounds were redissolved in methanol (Zengin et al., 2019). This could indicate that the biological properties of *Spartium junceum* L. might be due to its antioxidant properties as well.

*Spartium junceum* L. has been studied for its anti-herpesviruses activity, and the aqueous extracts exhibited potent activities against herpes simplex virus type 1 (HSV-1) *in vitro* experiments (Duman et al., 2019). This provides the basis that the antiviral activity is probably related to the high antioxidant content of plants' properties. Furthermore, with the cytotoxic effect it has against glioblastoma cell lines (Abusamra et al., 2015), this plant could be suitable for cancer treatment. These plants' antioxidant activities and anticancer properties have been associated with their high radical scavenging effects, which point to structural features responsible for each activity that will lead to future research.

We also studied how different storage temperatures and durations impacted the stability of antioxidant enzymes along with oxidative markers. Therefore, this work is significant in optimizing preservation methodologies so that a return to plants retains bioactivity. The temperatures used for the assay of enzyme activities between 30 days showed that high CAT and SOD activities were maintained at -70°C up to 30 days: CAT 0.37 U/mg protein and SOD 0.501 U/mg protein (Figure 2 and 3). With increasing temperatures, CAT 0.19 U/mg protein and SOD 0.39 U/mg protein were measured at -20°C, while at +4°C CAT 0.006 U/mg protein and SOD 0.11 U/mg protein were associated with decreasing enzymatic activities. This supports the effect of temperature on slowing enzymatic degradation and metabolism and maintaining enzyme activity over time (Stajner et al., 2010).

When the enzyme activities were analyzed over time, CAT and SOD showed a significant reduction in activities with increasing storage period at -70°C, beginning on day 1 (0.52 U/mg protein for CAT, 0.68 U/mg protein for SOD)

to day 30 (0.37 U/mg protein for CAT, 0.501 U/mg protein for SOD) (Figure 2 and 3). This could be due to the apparent preservation of enzyme structure in low temperatures stimulating short-term activity, which is lost during long-term storage. Factors such as prolonged freeze-thaw cycles may lead to structural changes in enzyme molecules and affect their activity (Teresa Cerchiara et al., 2013).

When the MDA levels were analyzed according to storage conditions, paradoxically, it was observed that MDA levels increased with decreasing temperatures, and the highest level at day 30 was 2.35 nmol/mg protein at -20°C (Figure 4). Furthermore, MDA levels increased from day 1 to day 30 at all temperatures. These findings suggest that lipid peroxidation may increase under cold storage while enzyme activity is maintained, possibly due to membrane phase changes that trigger oxidative stress. This highlights a critical balancing act required under storage conditions where maintenance of enzymatic activity may inadvertently increase oxidative damage (Teresa Cerchiara et al., 2013).

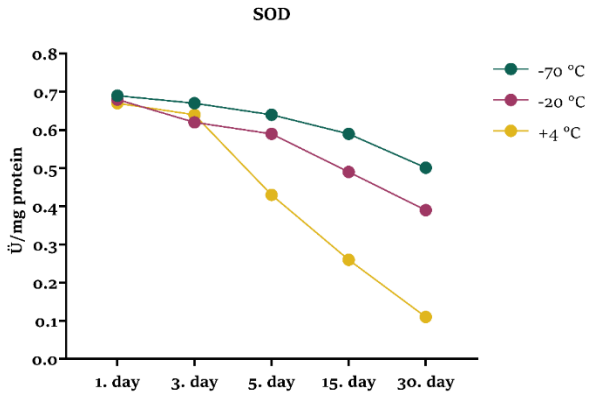


Figure 3. Time-dependent variation of superoxide dismutase (SOD) activity in *Spartium junceum L.* samples at different storage temperatures.

SOD activity measured in both flower and leaf parts (pool) of the plant was monitored at +4°C, -20°C, and -70°C for periods of 1, 3, 5, 15, and 30 days. While the maximum decrease was observed at +4°C, CAT activity was maintained at the highest levels in samples stored at -70°C.

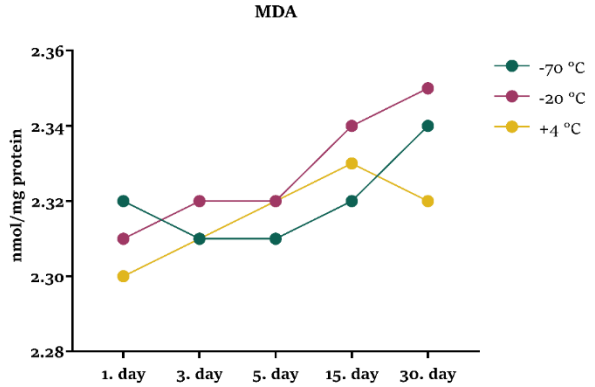


Figure 4. Time-dependent malondialdehyde (MDA) level variation in *Spartium junceum L.* samples at different storage temperatures.

MDA analyses of the plant's flower and leaf parts (pool) were monitored at +4°C, -20°C, and -70°C for periods of 1, 3, 5, 15, and 30 days. The results showed that MDA levels, an indicator of oxidative stress, increased significantly over time, especially in samples stored at -20°C. The increase in MDA levels was more limited in samples stored at -70°C.

## Conclusion

In conclusion, this study compared the antioxidant enzyme activities between the flowers and leaves of *Spartium junceum L.* at different storage temperatures. Flowers demonstrated higher antioxidant enzyme activity and lipid peroxidation than leaves, indicating their more significant antioxidative potential. Storage conditions significantly influenced enzyme stability, with -70°C identified as the optimal temperature for preserving the functional stability of antioxidant enzymes such as SOD and CAT over time. These findings highlight the importance of low-temperature storage to maintain the antioxidant properties of plant-based extracts. Furthermore, the higher antioxidant activity observed in the flowers of *Spartium junceum L.* underscores their potential as a natural source of antioxidants, warranting further evaluation for nutraceutical or therapeutic applications.

## Declarations

### *Ethical Approval Certificate*

This research does not involve any data or materials related to humans, animals, or other living organisms. As a result, ethical committee approval and informed consent is not required for this study.

### *Author Contribution Statement*

Muhammed Mehdi Üremiş: Data analysis, formal analysis, and writing the original draft

Ergül Belge Kurutaş: Data collection, conceptualization, methodology, and review.

### *Fund Statement*

Not applicable

### *Conflict of Interest*

The authors declare no conflict of interest.

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# GIS-Based Analysis of Agricultural Land Use Changes in Socio-Economically Less Developed Rural Settlements: The Case of Saray, Şarköy, and Hayrabolu Districts (Tekirdağ/Türkiye)

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ABSTRACT

Agricultural land cover has changed over time, and monitoring these changes has become an effective tool in development processes by linking them to ecological and socio-economic issues. In this context, the main hypothesis of the study is that "determining land use changes spatially and temporally using CORINE Land Cover data is crucial in development-oriented planning processes." The study analyzes changes in agricultural land use based on CORINE land cover classes between 1990 and 2018 in the districts of Saray, Şarköy, and Hayrabolu in Tekirdağ Province, which have low levels of socio-economic development. The research aims to answer the following questions: during which periods did significant changes occur in agricultural areas in Saray, Şarköy, and Hayrabolu? What are the total rates of increase or decrease in these areas? Into what types of land have agricultural are+as significantly transformed? The methodology was developed using Geographic Information Systems (GIS)-based maps, graphs, and tables. The findings reveal the spatial and temporal dimensions of changes in agricultural land use and land cover between 1990 and 2018. The data obtained are anticipated to provide a concrete foundation for developing rural development policies and ensuring the sustainable use of natural resources.

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## Introduction

Land use morphology represents a general pattern of land use types shaped by natural and socio-economic factors, which can change over time (Grainger, 1995). Land use and land cover emerge as a result of the interaction between natural and socio-economic elements across spatial and temporal dimensions (Rawat & Kumar, 2015). In regions significantly influenced by climate change and population growth, changes in land use have become increasingly critical for environmental management (Zoungrana et al., 2015; Niculae et al., 2023). On both local and global scales, these changes are fundamental to understanding ecological, biophysical, social, and climatic impacts (Turner, 2002; Foley et al., 2005; Mamun et al., 2022).

Many studies have highlighted land use changes as a core component of sustainability research (Turner et al., 2007; Bakker & van Doorn, 2009), often associating them with global climate change and socio-economic challenges (Long & Li, 2002; Kalnay & Cai, 2003; Long et al., 2007; Lambin & Meyfroidt, 2011). Kaya et al. (2020) emphasized that land use changes serve as a basis for studies in economic and socio-cultural domains. Similarly, Yohannes et al. (2018) pointed out that these studies should be considered in environmental protection strategies and sustainable resource management.

Grainger (1995) stressed the direct relationship between land use changes and socio-economic conditions. Similarly, Olğaç and Doğan (2020), along with Milentijević et al. (2024), underlined that identifying temporal changes in land use is a crucial tool for the effective and accurate management of natural resources. Pukowiec-Kurda and Vavrouchová (2020) noted the scientific importance of land use changes in rural settlements and identified a research gap in the literature due to their dynamic and ongoing nature.

In this context, the CORINE (Coordination of Information on the Environment) system, initiated by the European Commission in 1985, has been a fundamental data source enabling the monitoring of spatial and temporal changes in land use through satellite imagery and Geographic Information Systems (GIS) (EEA, 2007). CORINE has been widely used in research to examine rural development, urbanization, and agricultural transformation processes (Feranec et al., 2007; Türker, 2021).

In Türkiye, studies utilizing CORINE Land Cover (CLU/CLC) data have revealed that agricultural lands are increasingly being converted into urban areas, providing critical insights for sustainable planning (Sarı & Özşahin, 2016; Kurtar, 2021). Both nationally and internationally, numerous studies have focused on identifying land cover and land use changes and examining temporal shifts using CLU/CLC data (Keleş & Durduran, 2019; Kaya et al., 2020; Üyük et al., 2020; Pazúr et al., 2024). Feranec et al. (2007) analyzed changes in grassland areas in Slovakia between 1990 and 2000 using CLU/CLC data and emphasized that these changes are significant indicators for understanding trends in agricultural landscape development. Bayar (2018) highlighted that identifying changes in agricultural areas is effective for developing planning decisions and taking preventive measures. Sarı and Özşahin (2016) examined land use and land cover (LULC) changes in Tekirdağ Province based on the CLU/CLC system over a 15-year period from 2000 to 2015. Kurtar (2021) investigated land cover changes in Tekirdağ between 1990 and 2018 using CLU/CLC data, finding that artificial areas had expanded while agricultural lands had decreased. Üyük et al. (2020) analyzed temporal changes in land use in Denizli Province between 1990 and 2018 using CLU/CLC data, revealing an increase in agricultural areas, a decrease in grasslands, and an expansion of urban areas. Türker (2021) studied land use changes in Uşak Province over 28 years (1990-2018) and found that unirrigated arable lands were the most common type of use, while the proportional increase in urban structures was linked to urbanization and economic factors.

These studies demonstrate that CLU/CLC data is an effective tool for examining spatial and temporal land use and land cover changes, serving as a vital component in regional development policies and planning processes (Irwin & Geoghegan, 2001; Bürgi et al., 2004; Grassi et al., 2017; IPCC, 2019).

The primary hypothesis of this study is that "determining spatial and temporal land use changes with CLU/CLC data is essential in development-oriented planning processes." Based on this premise, the study analyzed agricultural land use changes between 1990 and 2018 in the districts of Saray, Şarköy, and Hayrabolu in Tekirdağ Province, which are characterized by low levels of socio-economic development, using CLU/CLC classes. The study aims to answer the following questions:

During which periods did significant changes occur in agricultural lands in Saray, Şarköy, and Hayrabolu?

What are the total rates of increase or decrease in agricultural areas in these districts?

Into which land use types have agricultural lands been transformed?

Within this framework, agricultural land use and land cover changes in the high-agricultural-potential yet socio-economically underdeveloped districts of Şarköy, Saray, and Hayrabolu in Tekirdağ Province were analyzed using CLU/CLC data for the 1990-2018 period, employing GIS techniques. The findings of the study are expected to provide a solid foundation for rural development policies and the sustainable use of natural resources.

## Materials and Methodology

The methodology of this study comprises five main stages:

### *Stage 1: Selection of the Study Area*

The study focuses on the districts of Hayrabolu, Şarköy, and Saray, located in the north, south, and east of Tekirdağ Province, as shown in Figure 1. These districts have high agricultural potential but rank in the 3rd and 4th levels in socio-economic development within Tekirdağ. The selection of the study areas was based on data from the Socio-Economic Development Index (SEGE) (2022) and the Tekirdağ Agricultural Report (2022). Criteria such as location, socio-economic development level, and agricultural potential were prioritized.

According to SEGE 2022, Saray ranks 295th, Şarköy 303rd, and Hayrabolu 420th among districts nationwide. Analysis of the distribution of cultivated agricultural lands in Tekirdağ indicates that 19.40% of cultivated areas are in Hayrabolu, 8.15% in Saray, and 3.94% in Şarköy (Tekirdağ Agricultural Report, 2022). Vision plans for the districts identify agriculture as a core opportunity. In Saray's Vision Plan, the broad and fertile agricultural lands are recognized as a key advantage, with a focus on enhancing agricultural potential (Saray District Vision Plan, 2012). In Şarköy's Vision Plan, the strong agricultural sector and its diversification are highlighted, envisioning the integration of tourism and agriculture (Şarköy District Vision Plan, 2011). Hayrabolu's Vision Plan identifies its strength in agricultural production as a core opportunity but also notes the lack of economic transformation of agricultural outputs as a challenge. The plan aims to develop Hayrabolu as a rural center distinguished by its agricultural production (Hayrabolu District Vision Plan, 2012).

The population figures for the districts are presented in Table 1. When analyzed based on the years corresponding to the CORINE land cover classifications, an increase in population is observed in Şarköy and Saray between 1990 and 2018, whereas a decrease is noted in Hayrabolu.

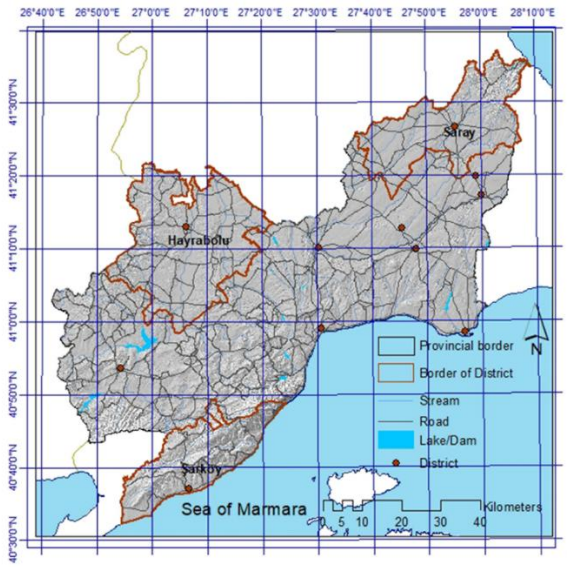



Figure 1. Study area: Hayrabolu, Şarköy, and Saray districts

Table 1. Population of Şarköy, Saray, and Hayrabolu districts (1990-2018) (TÜİK, 2022).

| Districts | 1990 | 2000 | 2007 | 2012 | 2018 |
|---|---|---|---|---|---|
| Şarköy | 28.480 | 32.660 | 29.395 | 29.991 | 32.267 |
| Saray | 33.716 | 41.217 | 44.540 | 47.522 | 49.605 |
| Hayrabolu | 45.640 | 40.130 | 36.942 | 33.488 | 32.268 |

Table 2. CLU/CLC classification used in the study (Ministry of Agriculture and Forestry, 2024).

| Level 1 | Level 2 | Level 3 |
|---|---|---|
| 1. Artificial Surfaces | 1.1 Urban fabric | 111 Continuous urban fabric<br>112 Discontinuous urban fabric |
| 2. Agricultural Areas | 2.1 Arable Land | 211 Non-irrigated arable land<br>212 Permanently irrigated land<br>213 Rice fields |
| | 2.2 Parmenant Crops | 221 Vineyards<br>222 Fruit trees and berry plantations<br>223 Olive groves |
| | 2.3 Pastures | 231 Pastures |
| | 2.4 Heterogeneous Agricultural Areas | 242 Complex cultivation patterns<br>243 Land principally occupied by agriculture, with significant areas of natural vegetation |
| 3. Forest and seminatural areas | 3.1 Forest | 311 Broad-leaved forest<br>312 Coniferous forest<br>313 Mixed forest |
| 4.Wetlands | 4.1 Inland Wetlands | 4.1.1 Inland marshes |
| 5. Water | 5.1 Inland waters<br>5.2 Marine waters | 5.1.1 Water courses<br>5.1.2 Water bodies<br>5.2.3 Sea and ocean |

***Stage 2: Defining the objective***

The purpose of this study is to examine the spatial and temporal changes in agricultural lands using CORINE land cover classes and data in rural settlements with high agricultural potential but low socio-economic development levels. Accordingly, the changes in agricultural lands within Hayrabolu, Şarköy, and Saray districts between 1990 and 2018 were analyzed.

***Stage 3: Land use classification***

In this study, the classification of agricultural land use for the years 1990–2018 was performed using GIS based on CORINE land cover classes for each district. Agricultural lands include areas harvested annually, fallow lands, and crops requiring intensive water usage, such as rice (Ministry of Agriculture and Forestry, 2024). The study utilized third-level agricultural land classes from CORINE land cover data shown in Table 2.

***Stage 4: Temporal and spatial change analysis***

Using the CORINE Land Cover classes from 1990 to 2018, agricultural land use maps for the years 1990, 2000, 2006, 2012, and 2018 were generated with ArcGIS 10.8 software. In this process, agricultural land use classes were calculated at the district level within the total agricultural land use area, and percentage change rates were determined using Microsoft Excel 2024. The sizes and percentage ratios of agricultural land use classes for each district were visualized through tables and graphs. Additionally, the percentage ratios of forests, settlements, wetlands, and water features were evaluated based on total area size, and land use changes were analyzed in the results section. The representations of maps and graphs illustrating agricultural land use conditions between 1990 and 2018 were prepared using the codes from the CORINE Land Cover classification. In the numerical expressions within the graphs, decimal values were rounded by considering only the first decimal place.

***Stage 5: Results and recommendations***

The spatial change rates of agricultural lands were compared across different years, general evaluations were made, and recommendations were developed.

## Results

In this study, the spatial and temporal changes in agricultural areas in the districts of Hayrabolu, Saray, and Şarköy between 1990 and 2018 were analyzed based on CORINE land cover data. The maps presented in Figures 1, 2, and 3 show the thematic maps of CORINE land cover classes for Hayrabolu, Saray, and Şarköy for the years 1990, 2000, 2006, 2012, and 2018, respectively. In each map, spatial changes in land use types across these three districts are visualized using different colors.

***Hayrabolu, Saray, and Şarköy Districts: Temporal and Spatial Changes (1990-2018)***

*Hayrabolu district:* A significant transformation in agricultural lands was observed in Hayrabolu district between 1990 and 2018 (see Figure 2). The "Non-irrigated arable land (211)" category showed an increase of 28.1%, while the "Permanently irrigated land (212)" category experienced a decrease of 22.6%. This indicates that a significant portion of irrigated agricultural land has been converted to dryland farming. Unlike the other districts, the "Rice fields (213)" category stands out in Hayrabolu. In 1990, this category accounted for 6.36% of the total area, but by 2018, it had decreased to 5%, marking a 1.36% reduction (see Figure 3).

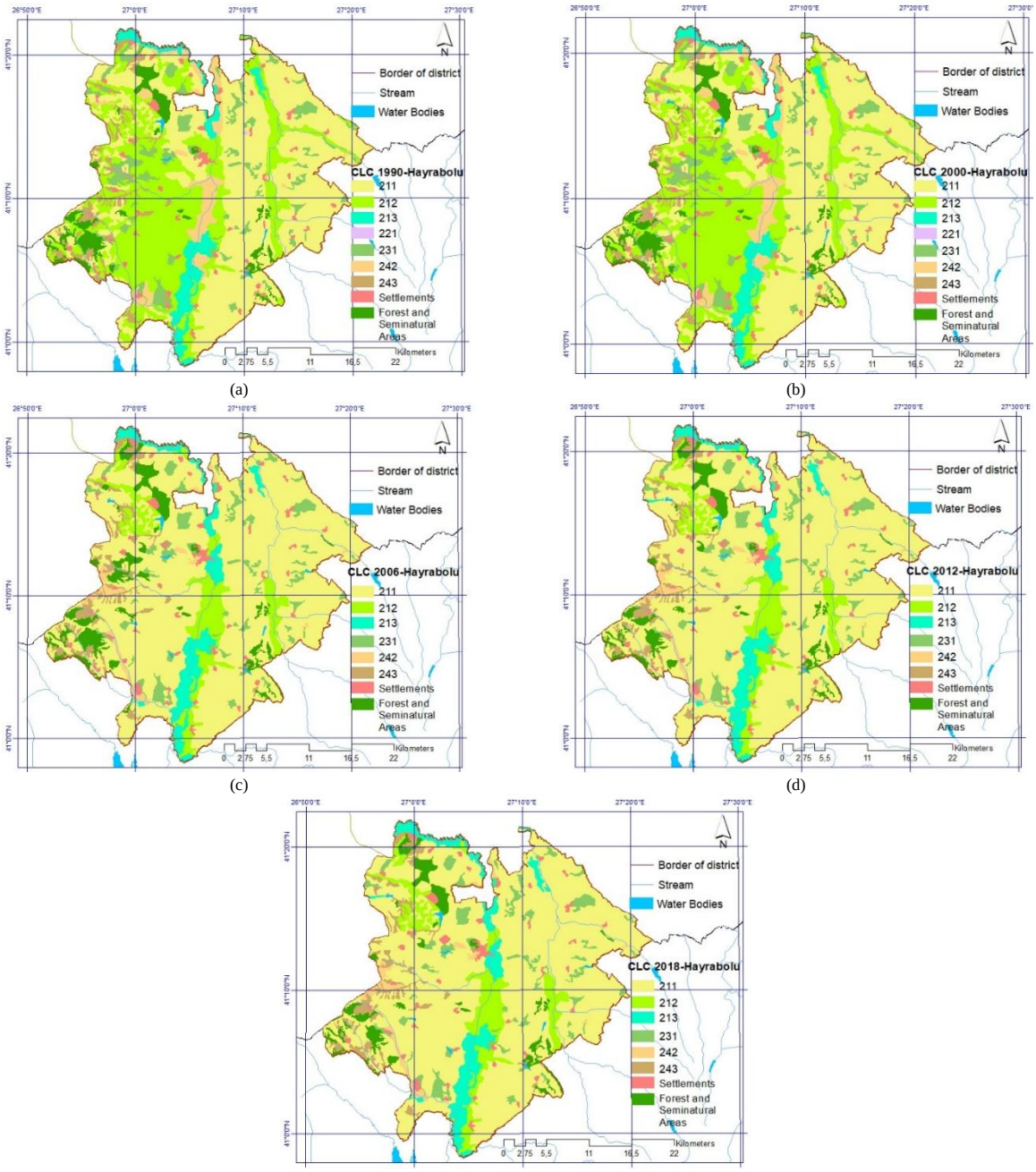
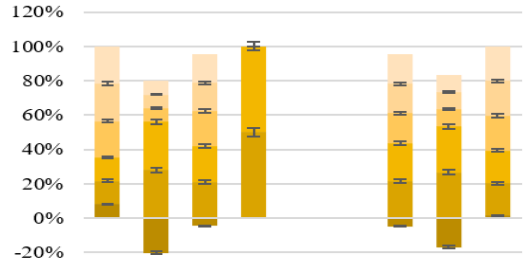



Figure 2. CLU/CLC maps of Hayrabolu district: (a) 1990, (b) 2000, (c) 2006, (d) 2012, and (e) 2018




| | 211 | 212 | 213 | 221 | 222 | 223 | 231 | 242 | 243 |
|---|---|---|---|---|---|---|---|---|---|
| 2018 | 74.9 | 8.8 | 5 | 0 | 0 | 0 | 5.4 | 1.7 | 4.2 |
| 2012 | 74.9 | 8.8 | 5 | 0 | 0 | 0 | 5.5 | 1.7 | 4.2 |
| 2006 | 73.5 | 9 | 6.1 | 0 | 0 | 0 | 5.5 | 1.7 | 4.2 |
| 2000 | 46.8 | 31.5 | 6.3 | 0.03 | 0 | 0 | 7 | 4.6 | 4 |
| 1990 | 46.8 | 31.4 | 6.4 | 0.03 | 0 | 0 | 6.9 | 4.6 | 3.9 |
| Difference (1990-2018) | 28.1 | -22.6 | -1.4 | 0 | 0 | 0 | -1.5 | -2.9 | 0.3 |

Figure 3. Changes in the spatial size of agricultural areas in Hayrabolu district according to CLU/CLC Level 3 classification over the years (1990-2018)

It was found that the decreased areas were largely converted into "Non-irrigated arable land (211)". It was noted that there were no "Vineyards (221)" or "Olive groves (223)" categories in the district. The "Fruit trees and berry plantations (222)" category was present only in 1990 and 2000, with a minimal 0.03% presence, and disappeared in subsequent years. The "Pastures (231)" category saw a 1.5% reduction, with most of this decrease being converted into "Non-irrigated arable land (211)". Similarly, the "Complex cultivation patterns (242)" category experienced a 2.9% decline, with some of these areas being converted into "Non-irrigated arable land (211)" or "Permanently irrigated land (212)".

Additionally, no significant changes were observed in the category "Land principally occupied by agriculture, with significant areas of natural vegetation (243)".

*Saray district:* In Saray district, significant changes in land use were observed between 1990 and 2018 (Figure 4).

During this period, while there was a decrease in other agricultural areas, the "Non-irrigated arable land (211)" category saw an increase of 6.8%. This increase indicates that agricultural activities have become concentrated in certain areas, which have retained their importance. The "Permanently irrigated land (212)" category experienced a decrease of 0.8%. This reduction is understood to be linked to the conversion of these areas to "Non-irrigated arable land (211)" (Figure 5).

It was found that the district did not include "Vineyards (221)" or "Olive groves (223)" categories. However, the "Fruit trees and berry plantations (222)" category began to form at 0.1% in 2012 and maintained this percentage through 2018. A 1.6% reduction in the "Pastures (231)" category was observed, indicating that pasture areas have been converted to agricultural or settlement areas. Similarly, the "Complex cultivation patterns (242)" category experienced a 1.7% decrease, with these areas largely being transformed into "Non-irrigated arable land (211)". A 2.9% reduction occurred in the "Land principally occupied by agriculture, with significant areas of natural vegetation (243)" category, and this loss was largely converted into "Non-irrigated arable land (211)" or settlement areas.

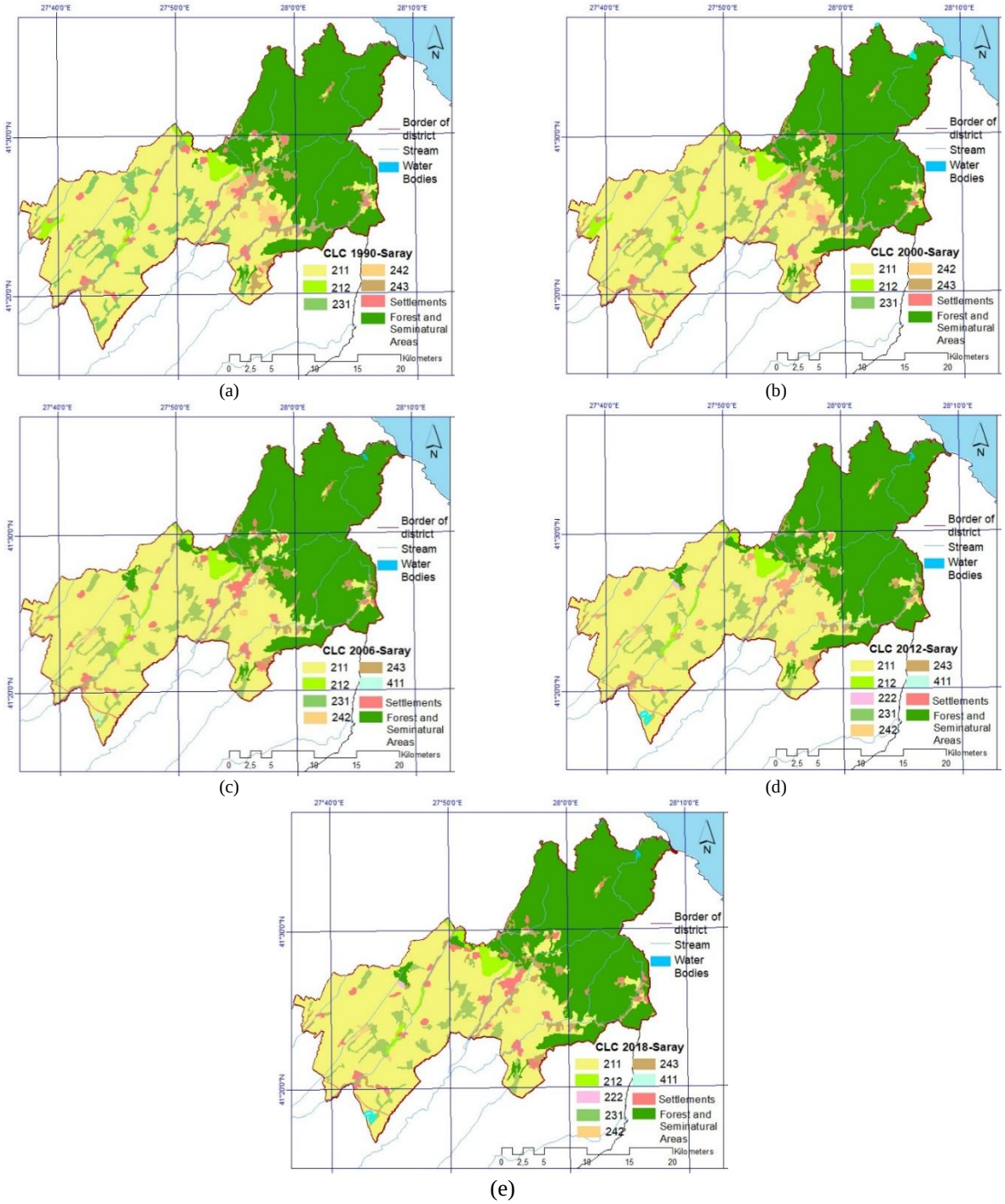
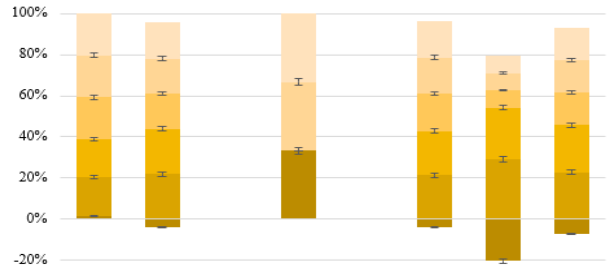



Figure 4. CLU/CLC maps of Saray district: (a) 1990, (b) 2000, (c) 2006, (d) 2012, and (e) 2018.




| | 211 | 212 | 221 | 222 | 223 | 231 | 242 | 243 |
|---|---|---|---|---|---|---|---|---|
| 2018 | 81.8 | 3.4 | 0 | 0.1 | 0 | 7.6 | 0.7 | 6.3 |
| 2012 | 81.9 | 3.3 | 0 | 0.1 | 0 | 7.6 | 0.7 | 6.4 |
| 2006 | 81.7 | 3.3 | 0 | 0 | 0 | 7.9 | 0.7 | 6.4 |
| 2000 | 75 | 4.2 | 0 | 0 | 0 | 9.3 | 2.1 | 9.3 |
| 1990 | 75 | 4.2 | 0 | 0 | 0 | 9.2 | 2.4 | 9.2 |
| Difference (1990-2018) | 6.8 | -0.8 | 0 | 0.1 | 0 | -1.6 | -1.7 | -2.9 |

Figure 5. Changes in the spatial size of agricultural areas in Saray district according to CLU/CLC level 3 classification over the years (1990-2018).

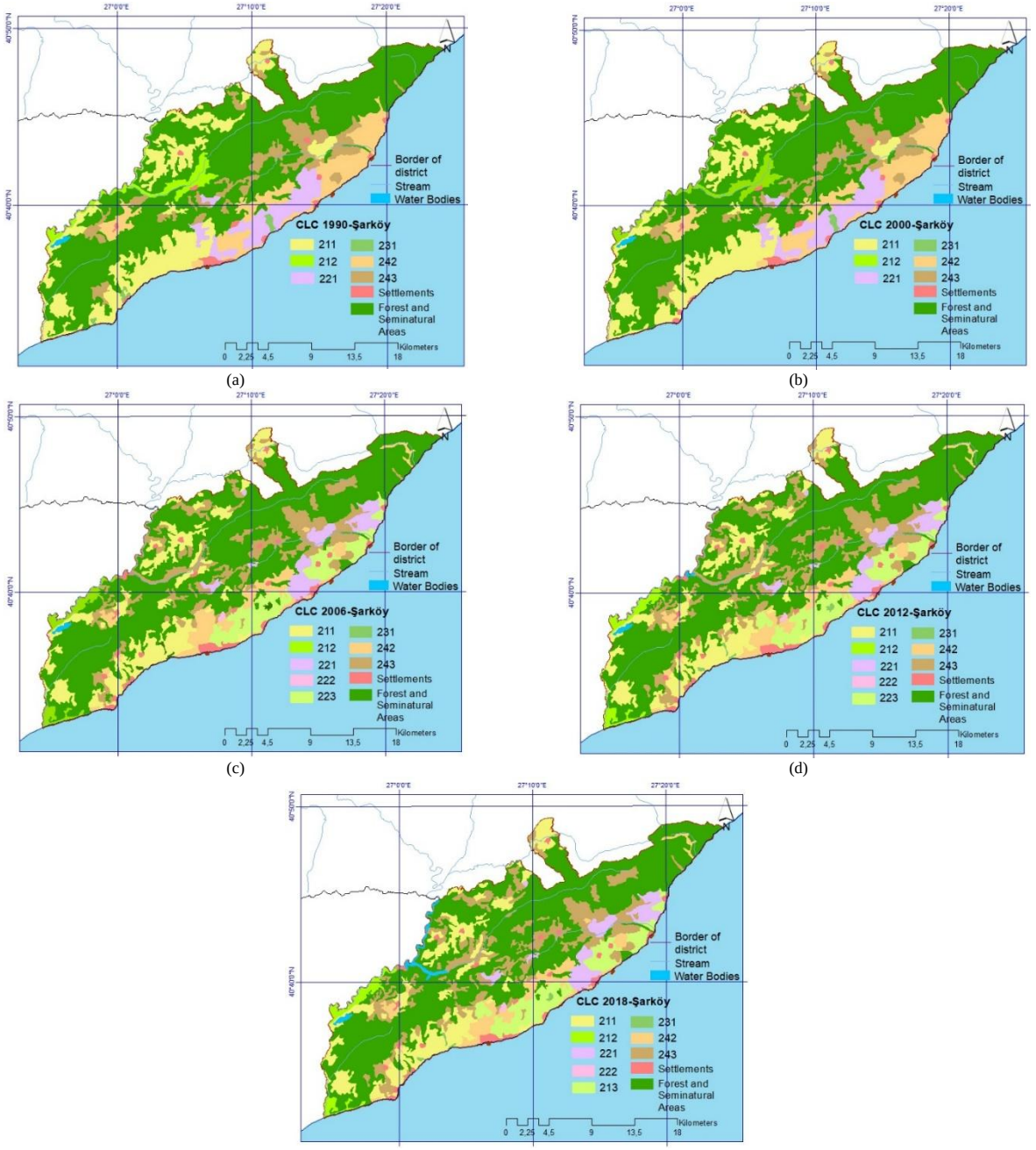
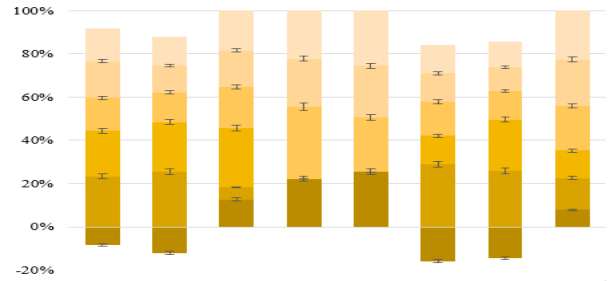



Figure 6. CLU/CLC maps of Şarköy district: (a) 1990, (b) 2000, (c) 2006, (d) 2012, and (e) 2018

*Şarköy district:* Significant temporal and spatial changes in land use and land cover were observed in Şarköy district between 1990 and 2018 (Figure 6).

During this period, the "Non-irrigated arable land (211)" category experienced a notable decrease of 15.7%, while the "Complex cultivation patterns (242)" category decreased by 13.1%. Additionally, declines were observed in the "Permanently irrigated land (212)" and "Pastures (231)" categories. These reductions indicate that these areas were largely transformed into forested areas, other agricultural categories such as "Land principally occupied by agriculture, with significant areas of natural vegetation (243), or settlement areas (Figure 7).




| | 211 | 212 | 221 | 222 | 223 | 231 | 242 | 243 |
|---|---|---|---|---|---|---|---|---|
| 2018 | 28 | 3.8 | 8.8 | 0.2 | 14.5 | 0.5 | 10.5 | 33.5 |
| 2012 | 31.9 | 3.5 | 8.1 | 0.2 | 13.6 | 0.5 | 10.1 | 31.9 |
| 2006 | 28.8 | 3.9 | 9.1 | 0.3 | 14.4 | 0.6 | 12 | 30.9 |
| 2000 | 39.6 | 6.5 | 13.2 | 0 | 0 | 0.5 | 21.3 | 18.9 |
| 1990 | 43.7 | 7.2 | 2.7 | 0 | 0 | 1.1 | 23.6 | 21.7 |
| Difference (1990–2018) | -15.7 | -3.4 | 6.1 | 0.2 | 14.5 | -0.6 | -13.1 | 11.8 |

Figure 7. Changes in the spatial size of agricultural areas in Şarköy district according to CLU/CLC Level 3 classification over the years (1990-2018)

Table 3. Spatial changes in CLU/CLC (1990-2018) in selected districts: agricultural areas, forests, and settlements

| CLU/CLC Level 3 | CLU/CLC data years | Hayrabolu | Saray | Şarköy |
|---|---|---|---|---|
| | 1990 | 46.8 | 75 | 43.7 |
| | 2000 | 46.8 | 75 | 39.6 |
| 211 | 2006 | 73.5 | 81.7 | 28.8 |
| | 2012 | 74.9 | 81.9 | 31.9 |
| | 2018 | 74.9 | 81.8 | 28 |
| Percentage Difference | 1990–2018 | +28.1 | +6.8 | +15.7 |
| | 1990 | 31.4 | 4.2 | 7.2 |
| | 2000 | 31.5 | 4.2 | 6.5 |
| 212 | 2006 | 9 | 3.3 | 3.9 |
| | 2012 | 8.8 | 3.3 | 3.5 |
| | 2018 | 8.8 | 3.4 | 3.8 |
| Percentage Difference | 1990–2018 | -22.6 | -0.8 | -3.4 |
| | 1990 | 6.36 | 0 | 0 |
| | 2000 | 6.25 | 0 | 0 |
| 213 | 2006 | 6.1 | 0 | 0 |
| | 2012 | 5 | 0 | 0 |
| | 2018 | 5 | 0 | 0 |
| Percentage Difference | 1990–2018 | -1.36 | 0 | 0 |
| | 1990 | 0.03 | 0 | 2.7 |
| | 2000 | 0.03 | 0 | 13.2 |
| 221 | 2006 | 0 | 0 | 9.1 |
| | 2012 | 0 | 0 | 8.1 |
| | 2018 | 0 | 0 | 8.8 |
| Percentage Difference | 1990–2018 | -0.03 | 0 | +6.1 |
| | 1990 | 0 | 0 | 0 |
| | 2000 | 0 | 0 | 0 |
| 222 | 2006 | 0 | 0 | 0.3 |
| | 2012 | 0 | 0.1 | 0.2 |
| | 2018 | 0 | 0.1 | 0.2 |
| Percentage Difference | 1990–2018 | 0 | +0.1 | +0.2 |
| | 1990 | 0 | 0 | 0 |
| | 2000 | 0 | 0 | 0 |
| 223 | 2006 | 0 | 0 | 14.4 |
| | 2012 | 0 | 0 | 13.6 |
| | 2018 | 0 | 0 | 14.5 |
| Percentage Difference | 1990–2018 | 0 | 0 | +14.5 |
| | 1990 | 6.9 | 9.2 | 1.1 |
| | 2000 | 7 | 9.3 | 0.5 |
| 231 | 2006 | 5.5 | 7.9 | 0.6 |
| | 2012 | 5.5 | 7.6 | 0.5 |
| | 2018 | 5.4 | 7.6 | 0.5 |
| Percentage Difference | 1990–2018 | -1.5 | -1.6 | -0.6 |
| | 1990 | 4.6 | 2.4 | 23.6 |
| | 2000 | 4.6 | 2.1 | 21.3 |
| 242 | 2006 | 1.7 | 0.7 | 12 |
| | 2012 | 1.7 | 0.7 | 10.1 |
| | 2018 | 1.7 | 0.7 | 10.5 |
| Percentage Difference | 1990–2018 | -2.9 | -1.7 | -13.1 |
| | 1990 | 3.9 | 9.2 | 21.7 |
| | 2000 | 4 | 9.3 | 18.9 |
| 243 | 2006 | 4.2 | 6.4 | 30.9 |
| | 2012 | 4.2 | 6.4 | 31.9 |
| | 2018 | 4.2 | 6.3 | 33.5 |
| Percentage Difference | 1990–2018 | +0.3 | -2.9 | +11.8 |

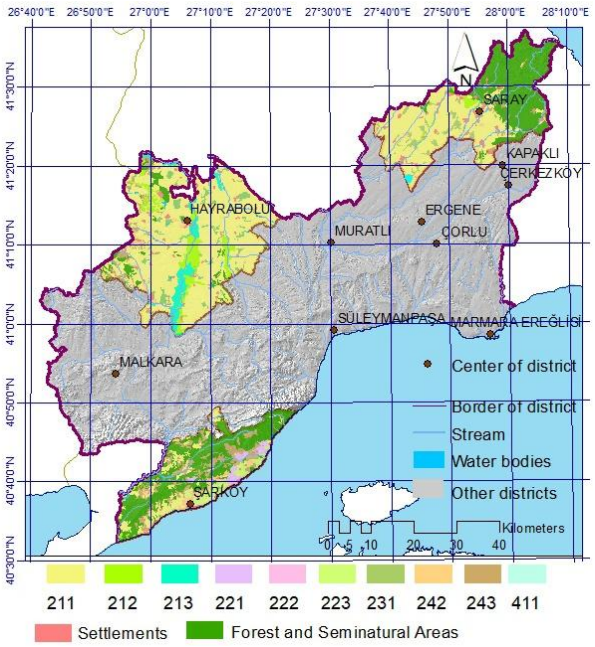



Figure 8. Agricultural land use map of Saray, Şarköy, and Hayrabolu districts in 2018

However, significant increases were also observed in certain land use categories. For instance, the "Vineyards (221)" category saw an increase in area share from 2.7% in 1990 to 8.8% in 2018. This increase demonstrates the continued importance and growth of viticulture in the region. The "Fruit trees and berry plantations (222)" category, which was not present in 1990, was recorded at 0.3% in 2006, and this proportion remained relatively stable in subsequent years. Furthermore, the "Olive groves (223)" category, which did not exist in 1990, began to emerge in 2006 and reached 14.5% by 2018. This indicates that olive cultivation has become a new and significant agricultural activity in the region. The 0.6% decrease in the "Pastures (231)" category suggests that pasture areas have been converted to agricultural or settlement areas. Similarly, the 13.1% reduction in the "Complex cultivation patterns (242)" category points to the transformation of these areas, particularly into "Olive groves (223)" and other agricultural categories. Another notable increase occurred in the "Land principally occupied by agriculture, with significant areas of natural vegetation (243)" category, which saw an 11.8% increase between 1990 and 2018. This increase appears to be linked to the conversion of "Non-irrigated arable land (211)" into this category.

## Discussion

This study examines the temporal and spatial changes in agricultural land use in Hayrabolu, Saray, and Şarköy districts in Tekirdağ province, which have high agricultural potential but low socio-economic development levels, based on CORINE land cover data. The map illustrating the agricultural land use of the districts covered in the study for the year 2018 is presented in Figure 8.

In the three selected districts, significant changes in agricultural areas were most notably observed in 2006. The change rates between 1990 and 2018 are presented in Table 3, and the changes within agricultural areas were evaluated internally. Transitions between sub-classes are detailed in the findings section. Accordingly, a shift to dry farming was observed in the districts of Hayrabolu, Saray, and Şarköy. In addition, increasing trends in "Olive groves (223)" and "Vineyards (221)" were identified in Şarköy as of 2006.

Furthermore, considering the overall spatial distribution rates, it was determined that agricultural areas were converted into settlement areas in Hayrabolu and Saray (Figure 9).

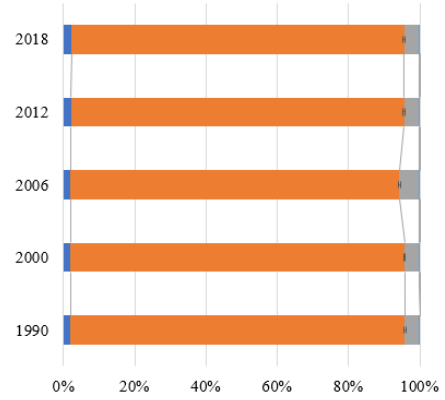
According to the Tekirdağ Agricultural Report (2018), the total agricultural areas in Hayrabolu, Saray, and Şarköy are reported as 805.66 km², 338.45 km², and 163.66 km² (Tekirdağ Agricultural Report, 2018), respectively. However, according to CORINE land cover data, these values are 933.69 km² for Hayrabolu, 391.98 km² for Saray, and 237.40 km² for Şarköy. This discrepancy arises from differences in the calculation methods between the CORINE system and official agricultural reports. This highlights the differences between the data provided by the CORINE land cover dataset and the "Agricultural Report" data.

In the study, agricultural land use changes from 1990, 2000, 2006, 2012, and 2018 were assessed in 9 different classes at Level-3 scale, based on CORINE land cover data, and land use classes for each district were analyzed. Table 3 shows the spatial changes in agricultural areas, forests, and settlements across the selected districts.

Kurtar (2021) examined land cover changes in Tekirdağ province using the CORINE land cover database, finding a 1.96% decrease in agricultural land between 1990 and 2018. In the three districts studied, the total agricultural land area decreased by approximately 0.34% over the same period. This change can be associated with socioeconomic factors. The study conducted by the Trakya Development Agency, which surveyed 910 producers to identify agricultural issues in Tekirdağ, supports this finding. The study highlighted key issues such as economic problems (81%), agricultural debt (43%), and migration (34%) (Trakya Development Agency, 2013). These findings suggest that land use changes are closely linked to socioeconomic issues, supporting numerous studies (Long & Li, 2002; Kalnay & Cai, 2003; Long et al., 2007; Lambin & Meyfroidt, 2011).

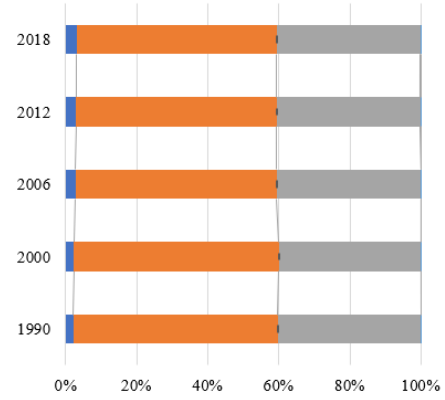
The presence of "Rice fields (213)" in Hayrabolu and "Vineyards (221)" and "Olive groves (223)" in Şarköy reveal the agricultural differences in these districts. The most common land use across the selected districts from 1990 to 2018 was in the "Non-irrigated arable land (211)" category. Similarly, in a study by Türker (2021) on Uşak province, it was found that the most common land use was "Non-irrigated arable land (211)".

According to 2018 CLC data, the highest proportional distribution in the "Non-irrigated arable land (211)" category was found in Saray district at 81.8%, while the highest increase in this category was observed in Hayrabolu at 28.1%. In contrast, the "Permanently irrigated land (212)" category showed a decrease of 22.6%, with Hayrabolu being notably affected. The "Pastures (231)" category showed decreases ranging from 0.6% to 1.6% across the districts, indicating that the category was generally maintained. Furthermore, the "Fruit trees and berry plantations (222)" category was not significantly present in any of the three districts.



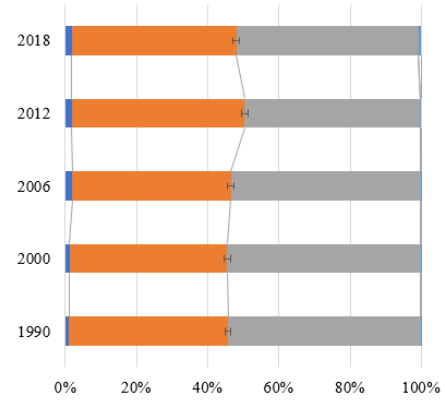

| | 1990 | 2000 | 2006 | 2012 | 2018 |
|---|---|---|---|---|---|
| Settlements | 1.97 | 2.03 | 2.03 | 2.06 | 2.11 |
| Agricultural areas | 93.86 | 93.66 | 92.16 | 93.46 | 93.51 |
| Forest and seminatural areas | 4.11 | 4.1 | 5.52 | 4.19 | 4.09 |
| Wetlands | 0 | 0 | 0 | 0 | 0 |
| Water bodies | 0.18 | 0.21 | 0.28 | 0.28 | 0.29 |

(a)




| | 1990 | 2000 | 2006 | 2012 | 2018 |
|---|---|---|---|---|---|
| Settlements | 2.17 | 2.38 | 2.81 | 2.87 | 3.05 |
| Agricultural areas | 57.58 | 57.59 | 56.46 | 56.57 | 56.3 |
| Forest and seminatural areas | 40.25 | 40 | 40.57 | 40.38 | 40.46 |
| Wetlands | 0 | 0 | 0 | 0.09 | 0.09 |
| Water bodies | 0.0046 | 0.04 | 0.07 | 0.08 | 0.09 |

(b)




| | 1990 | 2000 | 2006 | 2012 | 2018 |
|---|---|---|---|---|---|
| Settlements | 1.08 | 1.2 | 2.02 | 1.95 | 1.75 |
| Agricultural areas | 44.63 | 44.23 | 44.57 | 49.89 | 46.28 |
| Forest and seminatural areas | 54.08 | 54.36 | 53.2 | 50.84 | 51.13 |
| Wetlands | 0 | 0 | 0 | 0 | 0 |
| Water bodies | 0.21 | 0.21 | 0.21 | 0.27 | 0.84 |

Figure 9. Percentage distribution of land use types in the selected districts: (a) Hayrabolu, (b) Saray, (c) Şarköy.

The percentage distribution of settlement areas, agricultural areas, forests and seminatural areas, wetlands, and water bodies based on the total area size in the selected districts is presented in Figure 9. Settlement areas in Saray and Şarköy increased by approximately 0.9% and 0.7%, respectively, a trend that supports the findings of Sarı and Özşahin (2016). This growth appears to be associated with population growth rates in both districts and indicates that the increase in settlement areas has had a limited yet noticeable impact of urban expansion on rural landscapes.

When examining the general distribution rates of land use, agricultural areas have the highest percentage value among the three selected districts, with Hayrabolu accounting for 94% according to CLC 2018. In the other two districts, the distribution rates of agricultural and forest areas range between 40% and 56%, showing relatively similar values. District vision plans also support this finding, emphasizing that the agricultural sector is the primary socio-economic strength in all three districts (Saray District Vision Plan, 2012; Şarköy District Vision Plan, 2011; Hayrabolu District Vision Plan, 2012). Grainger (1995) similarly highlighted the relationship between land use rates and socio-economic conditions in his study.

A detailed analysis of agricultural land distribution reveals a decrease of approximately 0.4% and 1.3% in Hayrabolu and Saray, respectively. The decline in Hayrabolu is likely associated with the reduction in “Rice fields (213)”, whereas in Saray, the changes can be linked to transitions between “Pastures (231)”, “Complex cultivation patterns (242)” and “Land principally occupied by agriculture, with significant areas of natural vegetation (243)” land cover classes. On the other hand, Şarköy experienced an increase of 1.7% in agricultural land, which can be attributed to the expansion trends in “Vineyards (221)” and “Olive groves (223)”.

Regarding forests and seminatural areas, no significant changes were observed in Hayrabolu and Saray districts, while Şarköy district experienced a notable decrease of approximately 3%. As for the distribution of water bodies, a slight increasing trend was observed across all three districts.

When evaluated by district:

- *Hayrabolu:* Among seven different agricultural land use classes, the “Non-irrigated arable land (211)” category showed a remarkable increase of 28.1%. In contrast, a decrease of 22.6% was recorded in the “Permanently irrigated land (212)” category, indicating a shift from irrigated to dryland farming. No significant changes were observed in other categories.
- *Saray:* Among six different agricultural land use classes, a 6.8% increase was found in the “Non-irrigated arable land (211)” category. No significant changes were recorded in other land use categories.
- *Şarköy:* Among eight different agricultural land use classes, increases were observed in the “Vineyards (221)”, “Olive groves (223)”, and “Land principally occupied by agriculture, with significant areas of natural vegetation (243)” categories. In particular, the “Vineyards (221)” category showed an increase compared to 1990, with this growth primarily concentrated in the eastern coastal regions where the

greatest reduction was seen in "Complex cultivation patterns (242)". This finding is similar to the increase observed in "Vineyards (221)" in a study by Üyük et al. (2020) in Denizli province. The "Olive groves (223)" category, which was absent in 1990, began to emerge in 2006 and reached a 14.5% share by 2018. This suggests that olive cultivation has become an increasingly important agricultural activity in the region.

## Conclusion

With globalization, factors such as technological advancements, population growth, climate change, and pandemics have driven significant changes and transformations in both urban and rural areas. This study highlights that these factors have had a notable impact on agricultural land use, particularly in rural areas, as observed in the study region. National-scale rural development plans, such as the Eleventh and Twelfth Development Plans, emphasize the preparation of agricultural land use plans to ensure the conservation, sustainable use, and effective management of agricultural lands (Presidency of the Republic of Türkiye, Strategy and Budget Department, 2019; Presidency of the Republic of Türkiye, Strategy and Budget Department, 2023).

The findings from this study underline the importance of developing strategies to conserve agricultural land cover. For example, the observed decrease in irrigated agricultural areas in Hayrabolu and the expansion of residential areas in Şarköy and Saray (Figure 9) suggest that population growth and land use changes are intricately linked. This aligns with existing literature, which associates land use changes with socio-economic conditions and underscores the critical role of detecting these changes for sustainable resource management (Long & Li, 2002; Kalnay & Cai, 2003; Long et al., 2007; Lambin & Meyfroidt, 2011; Zoungrana et al., 2015; Olğaç & Doğan, 2020; Kaya et al., 2020; Milentijević et al., 2024). Similarly, Gilbert and Shi (2023) emphasized that detecting and predicting land use/land cover (LULC) changes is a crucial tool for achieving sustainable development.

This study further suggests that integrating the dynamics of land use changes with future scenario development will be essential for effective rural development planning. For instance, the results point to the need for targeted conservation strategies in regions where agricultural land is under pressure from urban expansion. Future research should prioritize the use of high-resolution spatial data, combined with fieldwork validations, to refine monitoring systems and scenario-based planning. This approach will contribute to the sustainable use and management of agricultural lands, enhancing their role in national development goals.

## Evaluation of Study Limitations and the use of CORINE CLC Data

This study was conducted based on the detailed classification advantages provided by CORINE Level 3 data. Land cover classification systems such as CORINE offer a standardized approach to monitor and analyze land use changes over time, allowing for detailed classification of land cover types (Duymaz, 2024). CORINE Level 3 includes 44 distinct land use categories (semantic coverage), spans 39 European countries (spatial coverage), and provides temporal coverage from 1990 to 2018 (Falťan et al., 2020; García-Álvarez & Nanu, 2022). CORINE maps, based on 1:100,000 scale satellite imagery, feature a minimum mapping unit of 25 hectares and a resolution of 100 meters, making them a robust dataset for large-scale analyses (Özür & Ataol, 2018a; Aytop, 2024). However, the literature widely discusses certain limitations of these features concerning data accuracy.

Büttner (2014) and Altürk (2023) highlighted thematic accuracy issues when using CORINE data, particularly at local scales. Büttner et al. (2014) noted that the accuracy rate of CORINE data for 1990 fell below 86% for many countries. Furthermore, specific categories, such as mining sites, construction areas, and mixed agricultural lands, were reported to have significantly lower accuracy rates (Baudoux et al., 2021). However, the 2012 evaluation addressed some shortcomings by introducing 12 new subcategories at Level 4 for Türkiye (CORINE, 2018). As a result of technological advancements, the accuracy rates of CORINE data for 2012 and 2018 improved significantly, ranging between 86% and 98% (Moiret-Guigand et al., 2021). These advancements align with the observations of Özür and Ataol (2018b), who emphasized that future iterations of CORINE data are expected to overcome current limitations and provide more accurate and detailed information on land cover.

Our study utilized CORINE data as a "preliminary study and baseline," relying on existing literature findings that validate its applicability for independent analyses. However, we have explicitly acknowledged the limitations of CORINE data in this study and emphasize the need for future research to enhance accuracy through methods such as field validation and the integration of additional data sources.

## Declarations

### *Author Contribution Statement*

M.G.T.: Conceptualization, Investigation, Methodology, Data collection, Data curation, Software, Visualization, Writing – Original draft preparation.

T.K.: Conceptualization, Investigation, Methodology, Writing-Reviewing and Editing.

### *Conflict of Interest*

The authors declare no conflict of interest.

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# Determination of Energy Production Potential from Walnut (*Juglans regia* L.) Residues in Kırşehir Province

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ABSTRACT

The present study aims to evaluate the energy production potential from walnut (*Juglans regia* L.) residues, specifically pruning waste and shell biomass, in Kırşehir province, Türkiye. Data collected between 2019 and 2023 were analyzed to quantify the biomass availability and its corresponding energy potential across various districts in the region. The findings reveal that total biomass production from pruning residues increased from 1220.36 t in 2019 to 1322.69 t in 2023, resulting in an energy potential growth from 19,904.02 GJ to 21,573.11 GJ. A similar trend was observed in shell biomass, which rose from 483.11 t to 523.62 t, resulting in an energy potential increase from 9164.60 GJ to 9933.12 GJ. The Kaman district consistently dominated, accounting for over 55% of the total energy potential, while Boztepe and Çiçekdağı exhibited the lowest contributions. The total energy production potential from walnut residues in 2023 was estimated at 31,506.22 GJ (31.51 TJ), corresponding to an annual electricity generation capacity of approximately 8751.74 MWh. The study emphasizes the higher energy potential of pruning residues compared to shell biomass and highlights regional disparities in biomass availability, underscoring the need for targeted strategies to optimize resource utilization. The findings indicate that the utilization of walnut residues for bioenergy purposes has the potential to substantially mitigate fossil fuel dependency and to promote sustainable energy development in the region.

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## Introduction

The walnut (*Juglans regia* L.) is a hard-shelled fruit with high nutritional value, playing a significant role in both human nutrition and agricultural economics. The walnut fruit, which is abundant in both its shell and kernel, is widely utilized in the food, pharmaceutical and cosmetics industries (Yılmaz and Akça, 2017). However, the shells and other plant residues generated during walnut production are often regarded as waste and are not utilized efficiently.

In recent years, the utilization of agricultural waste as an energy source has gained increasing importance. The mounting global energy demand and the associated environmental concerns related to fossil fuel consumption have prompted a shift towards renewable energy sources (Degirmencioglu et al. 2019). Biomass waste derived from agricultural production presents a significant potential for sustainable energy production (Ertuğrul et al. 2024). In this context, walnut shells, due to their high energy content, constitute a valuable resource for biofuel production (Gupta et al., 2019).

Research studies have demonstrated that thermochemical conversion processes are effective methods of utilizing walnut shells and other agricultural residues. For instance, Shah et al. (2018) analyzed the thermal properties of walnut shells and found that their calorific values are comparable to those of wood waste and lignite coal. In a similar vein, Naderi and Vesali-Naseh (2019) demonstrated that hydrothermal carbonization of walnut shells results in the production of fuel products characterized by high carbon content, low ash content, and thermal stability. Moreover, Li et al. (2023) emphasized that the catalytic co-pyrolysis of walnut shells with oily sludge plays a substantial role in waste management and recycling. Jekayinfa and Omisakin (2005) investigated the potential use of agricultural waste as a local fuel source and concluded that biomass sources could contribute to energy savings and waste disposal.

Kırşehir province is a prominent agricultural production region, with a notable role in walnut cultivation. However, no comprehensive study has yet been conducted

to determine the energy potential of the shells and pruning residues generated from walnut production in this region. However, previous research has indicated that Boyacı et al. (2021) assessed the energy potential of plant waste from greenhouse tomato production in Kırşehir and demonstrated that utilizing the thermal energy potential of tomato waste is crucial for reducing reliance on imported energy. The findings also highlighted the environmental benefits of using these residues as bioenergy sources, such as reducing fossil fuel dependency and lowering $CO_2$ emissions. A similar approach to walnut waste would enable the transformation of these agricultural byproducts into energy, thereby contributing to sustainable energy production.

The study aims to quantity of biomass waste derived from walnut production in Kırşehir province, assessed its energy potential, and explored its utilization possibilities.

## Material and Method

### Material

*Location*

Kırşehir is located in the central region of Türkiye, with coordinates spanning from 39°41' to 39°48' North latitude and 33°25' to 34°43' East longitude. The province falls within the geographical confines of the Central Anatolia Region, with Kırıkkale to the north, Aksaray to the south, Nevşehir to the east, and Ankara to the west delineating its borders. The province's total area is approximately 6,570 km², and its continental climate is characterized by hot, dry summers and cold, snowy winters (Kırşehir Governorship [Kırşehir Valiliği], 2025).

The topography of the region is predominantly characterized by plateaus, low hills, and agricultural plains, with an average elevation of approximately 985 meters above sea level. The region is traversed by the Kızılırmak River, which is regarded as Türkiye's longest river, thereby contributing to the area's agricultural potential. The region's climate, classified as semi-arid, and the topography, predominantly comprising plateaus, low hills, and agricultural plains, at an average elevation of 985 meters above sea level, are conducive to walnut cultivation, in addition to other agricultural activities such as wheat and barley production.

The province's geographic and climatic conditions exert a substantial influence on walnut production, affecting parameters such as growth rate, yield, and nut quality. The province has seen a rise in interest in sustainable agricultural practices and the utilization of agricultural residues for energy purposes, making it a valuable case study for bioenergy potential assessments.

*Data*

The dataset encompasses the period from 2019 to 2023, incorporating annual walnut production volumes, as well as the number of fruit-bearing and non-bearing walnut trees in various towns within Kırşehir. A summary of these data can be found in Table 1, obtained from the Turkish Statistical Institute (TurkStat) (Turkish Statistical Institute, 2025).

### *Method*

To assess the biomass potential of walnut residues in Kırşehir province, walnut production areas and their respective output levels were systematically organized using Excel. The yield values were computed alongside the potential biomass production and the corresponding energy output derived from this production. Furthermore, an extensive literature review was conducted to evaluate the biomass and energy potential, and relevant formulas were analyzed based on previous studies. According to Bilandzija et al. (2018), the number of fruit-bearing walnut trees was considered, with an assumption that 0.485 t $ha^{-1}$ of biomass available for energy production after pruning with a potential of 16.31 MJ $kg^{-1}$ lower heating value (LHV). Bilandzija et al. (2018) investigated the biomass amount of continuous plantations. After pruning, weight data of the pruned biomass of each tree were determined and mean values of the plantation are calculated accordingly.

Considering total number of trees and mean value of pruned biomass of each tree, total annual pruned biomass quantity were determined by multiplying the values. Bilandzija et al. (2018) calculated the ratio of walnut shell by considering mean fruit yield of each tree, mean kernel percentage and number of tree data.

Table 1. Annual walnut production and tree statistics for Kırşehir (2019-2023).

| Fact | Year | Towns | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Akpınar | Akçakent | Boztepe | Kaman | Merkez | Mucur | Çiçekdağı |
| Number of fruit-bearing trees | 2019 | 4115 | 780 | 120 | 199207 | 27632 | 22320 | 7600 |
| | 2020 | 4180 | 780 | 130 | 199000 | 30270 | 22350 | 7600 |
| | 2021 | 28308 | 810 | 159 | 199000 | 30390 | 22350 | 7600 |
| | 2022 | 28415 | 810 | 167 | 206000 | 31030 | 21365 | 7600 |
| | 2023 | 28180 | 850 | 169 | 221000 | 32030 | 21365 | 7600 |
| Number of non-bearing trees | 2019 | 1910 | 2380 | 501 | 45460 | 31310 | 29012 | 14350 |
| | 2020 | 1940 | 2380 | 493 | 44000 | 33640 | 32015 | 14350 |
| | 2021 | 1965 | 2460 | 460 | 44000 | 33570 | 37015 | 14350 |
| | 2022 | 1973 | 2660 | 457 | 41000 | 33090 | 38018 | 14350 |
| | 2023 | 2020 | 2770 | 437 | 42000 | 32220 | 38018 | 12550 |
| Production (t) | 2019 | 221 | 10 | 1 | 1296 | 562 | 363 | 74 |
| | 2020 | 251 | 10 | 2 | 1791 | 605 | 447 | 76 |
| | 2021 | 1730 | 12 | 3 | 1781 | 608 | 447 | 91 |
| | 2022 | 1518 | 10 | 3 | 2130 | 642 | 368 | 98 |
| | 2023 | 1042 | 1 | 5 | 2452 | 593 | 395 | 35 |

The researchers determined the kernel percentage and yield data according to Cerović et al. (2010). The ratio of walnut shell residues was stated as 0.202 t ha$^{-1}$ and available shell residues for energy production were 0.192 t ha$^{-1}$ with a LHV of 18.97 MJ kg$^{-1}$. Considering production area (PA) of walnut cultivated in Kırşehir and the data gathered from Bilandzija et al. (2018) the equations 1 and 2 are derived for energy potential of walnut pruning residues ($EP_P$) and energy potential of walnut shell residues ($EP_S$). The total energy potential (EP) is calculated thorough Equation 3 (Ertuğrul et al. 2024).

$$EP_P\,(\mathrm{MJ}) = 0.485 \cdot PA \cdot LHV \quad (1)$$

$$EP_S\,(\mathrm{MJ}) = 0.192 \cdot PA \cdot LHV \quad (2)$$

$$EP\ (\mathrm{MJ}) = EP_P + EP_S \quad (3)$$

In the hypothetical scenario where all available waste is utilized for electricity generation, the electricity equivalent of potential energy output can be calculated using Equation 4.

$$EP_E\,(\mathrm{kWh}) = EP \cdot 0277778 \quad (4)$$

## Results and Discussion

From 2019 to 2023, assumption of total biomass production that can be obtained by pruning increased from 1220.36 t to 1322.69 t, reflecting an 8.4% growth. Kaman district consistently contributed the highest available pruning waste potential, accounting for approximately 55% of the total in 2023 with 727.50 t. In contrast, Boztepe and Çiçekdağı showed the lowest production levels, with Boztepe remaining stable around 3-3.5 t and Çiçekdağı decreasing from 40.74 t (2019-2022) to 31.96 t in 2023 (Figure 1).

Depending on the potential available pruning waste amounts, energy production potential from pruning increased from 19,904.02 GJ in 2019 to 21,573.11 GJ in 2023, marking an 8.4% rise. Kaman demonstrated the highest contribution with 11,865.53 GJ in 2023, surpassing half of the total potential (Figure 2).

Shell biomass production grew from 483.11 t in 2019 to 523.62 t in 2023, an 8.4% increase. Kaman led in shell biomass production with 288.00 t in 2023 (Figure 3).

Accordingly, energy potential from walnut shells increased from 9164.60 GJ in 2019 to 9933.12 GJ in 2023. Kaman remained the leading contributor, accounting for 5463.36 GJ in 2023 (Figure 4).

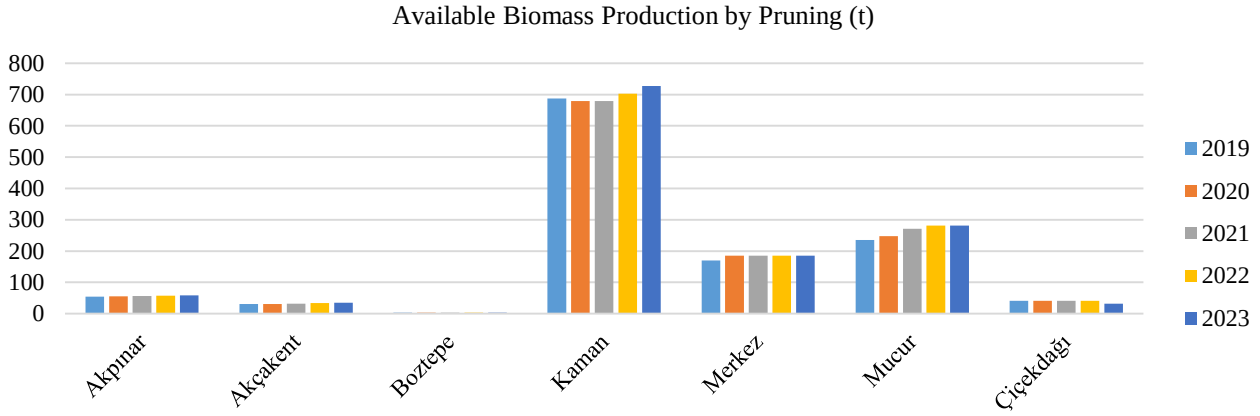


Figure 1. Assumption of available biomass production by pruning between 2019-2023

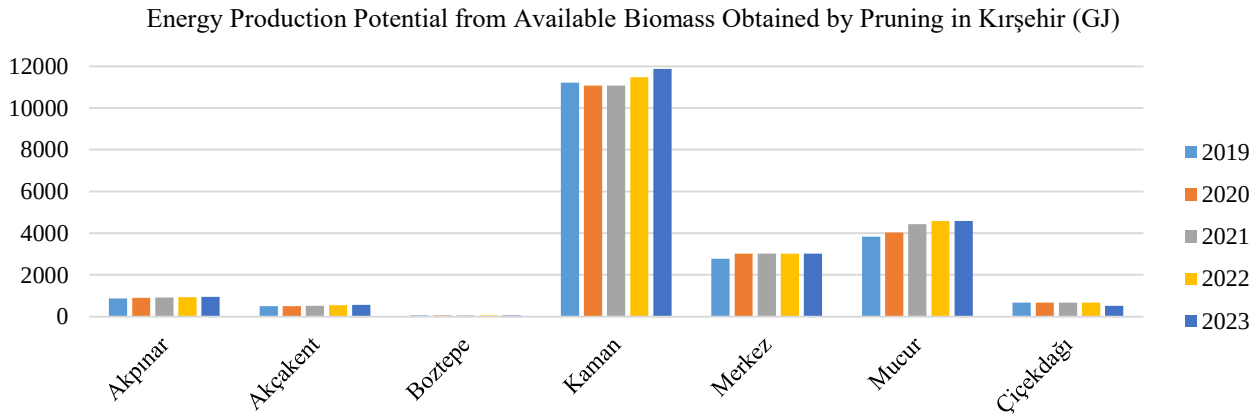


Figure 2. Energy production potential from available biomass obtained by pruning in Kırşehir between 2019-2023

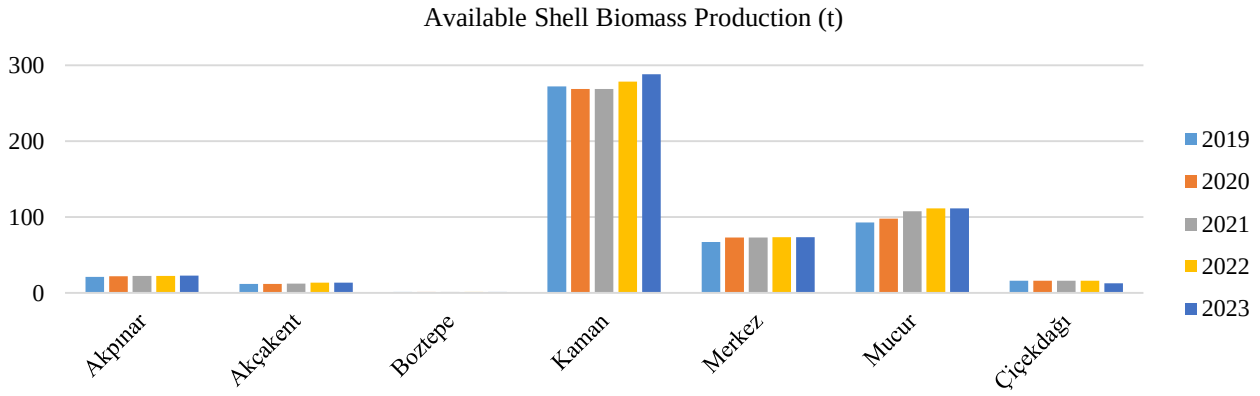


Figure 3. Assumption of available shell biomass production between 2019-2023

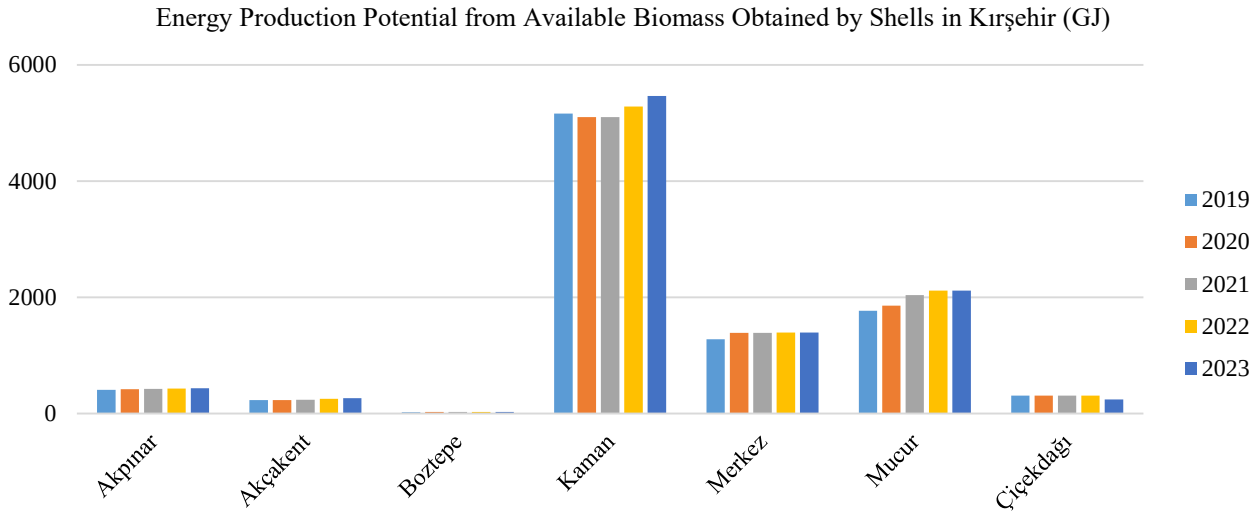


Figure 4. Energy production potential from available biomass obtained by shells in Kırşehir between 2019-2023

Table 2. Total energy production potential from walnut residues in Kırşehir (TJ)

| Year | Akpınar | Akçakent | Boztepe | Kaman | Merkez | Mucur | Çiçekdağı | Total |
|---|---|---|---|---|---|---|---|---|
| 2019 | 1.28 | 0.73 | 0.08 | 16.37 | 4.04 | 5.60 | 0.970 | 29.07 |
| 2020 | 1.33 | 0.73 | 0.08 | 16.17 | 4.41 | 5.89 | 0.970 | 29.58 |
| 2021 | 1.35 | 0.75 | 0.08 | 16.17 | 4.41 | 6.47 | 0.970 | 30.20 |
| 2022 | 1.36 | 0.81 | 0.08 | 16.75 | 4.41 | 6.70 | 0.970 | 31.08 |
| 2023 | 1.39 | 0.83 | 0.08 | 17.33 | 4.42 | 6.70 | 0.761 | 31.51 |

Results show that considering direct combustion method to produce energy, pruning residues contain higher potential than shell residues. Nevertheless, the pyrolysis method for walnut shell can be regarded as a promising biomass source for the production of bio-oil, biochar, and syngas (Goklani et al., 2022).

The total energy production potential from walnut residues rose from 29,068.63 GJ (29.07 TJ) in 2019 to 31,506.22 GJ (31.51 TJ) in 2023, an 8.4% increase. Kaman consistently contributed the largest share, providing over 17,328.89 GJ (17.33 TJ) in 2023 (Table 2). The total contribution of walnut residues is 31,506.22 GJ (31.51 TJ) which is higher than the potential contribution of greenhouse tomato wastes in Kırşehir, 4,046 GJ (Boyacı et al. 2021). Boyacı et al. (2022) determined the potential for bioenergy production from animal manure as 2791.41 GJ in the Kirşehir province which is considerably lower than the possible contribution of walnut residues to energy production. Ünal (2005) found that the total energy potential of walnut residues in Türkiye is 1,790 TJ, which is considerably higher than the 31.51 TJ potential of Kırşehir. It can be concluded that, in the event of losses being disregarded, there exists a possibility of annual electricity production of 8751.74 MWh from walnut residues.

A spatial representation of the total energy production potential from walnut agricultural wastes in 2023 has represented in Figure 5. The map highlights the dominant role of Kaman, which significantly surpasses other districts with 17,328.89 GJ of energy potential. Considerably lower energy potentials as 346.37 GJ for greenhouse tomato wastes (Boyacı et al. 2021) and 258.39 GJ for animal manure (Boyacı et al. 2022) in Kaman have been determined in previous research. Akpınar, Mucur, and Merkez (Central Town) also contribute notably, while Boztepe and Çiçekdağı show lower energy potentials.

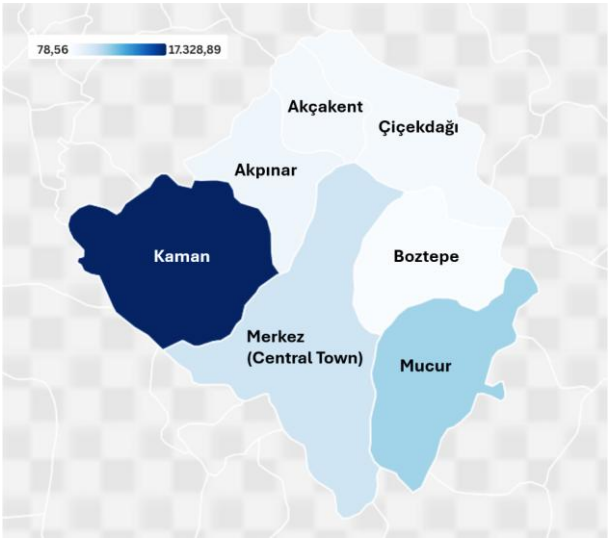


Figure 5. Energy production potential from walnut residues in 2023

The reduction in energy potential observed in Çiçekdağı may reflect regional agricultural shifts or decreased walnut production activities. This geographical distribution emphasizes the regional disparities in walnut biomass availability, guiding future biomass energy development strategies.

## Conclusion

This study highlights the significant energy potential of walnut residues in Kırşehir, particularly the contribution of pruning and shell biomass. The results show a consistent increase in both biomass production and energy potential from 2019 to 2023, with Kaman district emerging as the main contributor. The data indicate that walnut pruning residues have a higher energy potential compared to shell residues, making them a valuable resource for bioenergy production.

The total energy production potential for 2023 was determined to be 31.51 TJ, which corresponds to an estimated electricity generation capacity of 8751.74 MWh. The spatial distribution of energy potential highlights regional disparities, suggesting that targeted strategies could optimize biomass utilization across the province. The decline in energy potential in districts such as Çiçekdağı indicates the need for further research into local agricultural practices and resource management.

Overall, the use of walnut agricultural residues for energy production represents a sustainable approach to reducing dependence on fossil fuels and promoting renewable energy solutions. The findings suggest that targeted incentives for agricultural waste collection and biomass utilization could enhance the feasibility of walnut residue-based bioenergy production. Implementing local subsidies and infrastructure for biomass processing may significantly contribute to sustainable energy strategies.

## Declarations

### *Authors Contributions*

ÖE gathered and analyzed the data, prepared the draft manuscript, reviewed and revised the draft.

### *Data Availability Statement*

The data source of the research is publicly accessible at https://www.tuik.gov.tr/

### *Declaration of Competing Interest*

The researcher stated that no conflicts of interest or interpersonal connections could have impacted the published results.

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# A Study Examining the Potential of the 5S Methodology for Improving Efficiency in Agricultural Production Processes

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ABSTRACT

This study comprehensively examines the applicability of the 5S methodology as a tool for enhancing efficiency, occupational safety, and sustainability in the agricultural sector. The 5S methodology, predicated on the principles of sorting, organising, cleaning, standardising, and sustaining, aims to create safer and more productive workplaces. The research investigates its effects on both indoor agricultural machinery manufacturing processes and outdoor crop production practices. The analysis reveals notable advantages in manufacturing processes, including reduced waste, enhanced occupational safety, and improved product quality, particularly in production, assembly, and quality control processes. However, the methodology faces limitations in outdoor agricultural practices due to seasonal variations and open-field conditions, which challenge the cleaning, sorting, and organising stages. However, its application in maintenance, repair, and equipment storage processes has been shown to prolong the lifespan of machinery and ensure safer working conditions. The 5S methodology aligns with the United Nations Sustainable Development Goals, serving as a practical approach to enhancing efficiency, workplace safety, and resource management in the agricultural sector. This study underscores the 5S methodology's promise as a sustainable solution for enhancing productivity and safety in agriculture.

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## Introduction

This study examines the applicability of the 5S method to enhance productivity, occupational safety and sustainability in the agricultural sector. The 5S method is designed to enhance order, safety and efficiency in working environments through the application of the following principles: sorting, organisation, cleaning, standardisation and sustainability (self-discipline). The application of this method allows for the optimisation of resource utilisation throughout the production process, while simultaneously enhancing occupational safety and reducing waste. The agricultural sector is confronted with a multitude of challenges, both in indoor (production of agricultural tools and machinery) and outdoor (use of machinery in the field) environments. Consequently, this study assesses the benefits and limitations of 5S practices in both environments.

For instance, the implementation of 5S in greenhouse agriculture has significantly improved workflow efficiency by ensuring the systematic organisation of tools and equipment. Similarly, in dairy farms, structured cleaning and sorting protocols within the 5S framework have enhanced hygiene management, thereby positively impacting livestock health and milk quality. As outlined by the US Environmental Protection Agency (EPA), the 5S method represents a Lean Thinking tool designed to reduce waste and enhance efficiency within the workplace. The methodology facilitates the organisation and efficiency of the work environment through the implementation of the following steps: sorting, organisation, cleaning, standardisation and sustainability (EPA, 2024). This methodology enhances workplace organisation and efficiency by implementing key steps such as sorting, organising, cleaning, standardising, and sustaining.

The potential of 5S to contribute to production processes in the agricultural sector was previously discussed by Sidhu et al. (2013). This study posited that the implementation of 5S in conjunction with the Plan-Do-Check-Act (PDCA) cycle has the potential to enhance productivity and quality in the agricultural sector, thereby facilitating continuous improvement. The integration of 5S

with the Plan-Do-Check-Act (PDCA) cycle in agricultural production processes provides information regarding the reduction of waste, the optimisation of business processes and the improvement of decision-making processes. Nevertheless, it is primarily confined to delineating the overarching framework for the advantages of 5S in agricultural production. In contrast, this study provides a comprehensive assessment of the impact of 5S practices on a range of working conditions within the agricultural sector, including indoor agricultural machinery production and outdoor agricultural practices. The distinctive challenges inherent to these disparate work environments serve to diversify the applicability and benefits of the 5S methodology.

It is of particular importance to provide a regular and safe working environment, especially in the agricultural sector, for disadvantaged groups such as the elderly or disabled. Those with disabilities, who are among the most disadvantaged groups, are exposed to a range of risks when a safe and healthy working environment is lacking. The provision of suitable working environments for disabled employees enables them to work safely and in an adequately motivated manner (WHO, 2011). In this context, the 5S methodology can be employed to mitigate hazards and risks for disadvantaged groups by ensuring order and cleanliness in workplaces. The 5S methodology can be regarded as a technique that guarantees the security of disadvantaged employees in the agricultural sector with regard to occupational safety and risk reduction (Kurt et al., 2022).

Tractors represent the most significant source of power in agricultural operations, facilitating the execution of a vast array of agricultural activities. A study of the annual usage time of tractors in Turkey has demonstrated the significant role played by these vehicles in enhancing agricultural productivity (Evcim & Ertuğrul, 2017). In this context, the implementation of methodologies such as 5S represents a significant step towards ensuring the effective use of tractors and enhancing occupational safety. By introducing order, efficiency and safety measures into agricultural work processes, it is possible to optimise the utilisation of tractors and improve the safety of those working with them.

Furthermore, the study considers the psychosocial impacts of 5S, including occupational safety, ergonomics and employee satisfaction in the agricultural sector. It assesses the effects of 5S not only in terms of workplace organisation and productivity, but also on employee health and motivation. In this context, the study offers a comprehensive assessment of 5S in the agricultural sector, providing a more nuanced perspective and further elaborating on the potential contributions of the methodology in achieving sustainable development goals in the sector.

In order to successfully integrate 5S practices and achieve more efficient results, the importance of user-oriented approaches is emphasised in different industries. For instance, Toyota's methodologies, including Kaizen and Kaikaku, facilitate the active involvement of employees in business operations, fostering their sense of ownership over the processes and enhancing the efficacy of improvement initiatives. While Kaizen strives for continuous improvement, Kaikaku contemplates more radical alterations. When these two methods are integrated with workplace organisation and efficiency tools such as 5S, they can facilitate high performance and workplace quality (Moi & Sing, 2021). Such user-centred approaches have the potential to enhance the adaptability of the 5S method and facilitate employee adoption in diverse work environments, including the agricultural sector. Furthermore, the literature indicates that approaches centred on user experience (e.g., design thinking) enhance the efficacy of 5S practices and provide solutions aligned with employees' experiences (Muotka et al., 2023).

The study additionally demonstrates the manner in which 5S practices in agriculture can facilitate occupational safety and productivity in alignment with the United Nations' Sustainable Development Goals (SDGs) (United Nations Türkiye, 2024). In particular, the potential of 5S to contribute to sustainable development in agriculture is assessed in relation to the following goals: Goal 8 (Decent Work and Economic Growth), Goal 9 (Industry, Innovation and Infrastructure), Goal 12 (Responsible Consumption and Production) and Goal 13 (Climate Action). The structure of the 5S method is conducive to the simultaneous enhancement of occupational safety and productivity, as well as environmental sustainability, in alignment with the aforementioned goals (Table 1).

Table 1. Relationship between the 5S Method and United Nations Sustainable Development Goals

| SDGs Number | SDGs Topic | The 5S Method and Its Impact |
|---|---|---|
| Goal 8 | Decent Work and Economic Growth | It provides better conditions for employees by increasing safety and efficiency in the workplace |
| Goal 9 | Industry, Innovation and Infrastructure | It facilitates the implementation of innovative production processes, thereby enhancing efficiency and contributing to the development of sustainable infrastructure. |
| Goal 12 | Responsible Consumption and Production | It encourages the efficient utilisation of resources, thereby reducing waste and supporting environmental sustainability. |
| Goal 13 | Climate Action | It contributes to the global effort to mitigate the effects of climate change by minimising its environmental impact through the reduction of waste and the enhancement of efficiency. |

Table 2. 5S implementation recommendations in different agricultural practices

| Agricultural Application Area | 5S Application Recommendation | Advantages | Implementation Challenges |
|---|---|---|---|
| Greenhouse Agriculture | All steps of 5S are recommended (Sorting, Organisation, Cleaning, Standardisation, Sustainability). | The implementation of a closed working area facilitates the assurance of cleanliness, order and quality management. | The process requires a significant input of labour and the use of sophisticated equipment. |
| Tractor and Agricultural Equipment-Machinery Production | The use of 5S is recommended at all stages to increase efficiency. | Such an environment has been demonstrated to enhance quality, minimise the occurrence of production errors and foster a secure and hygienic working environment. | It is essential to provide training and supervision to ensure the long-term sustainability of the application. |
| Animal Production (On-Farm) | Only the sorting, cleaning and organising steps are recommended; standardisation can be partially implemented. | A clean and well-organised environment is conducive to the health and safety of animals and workers alike. | The open area is subject to variable conditions, with the presence of animals having an impact on the surrounding environment. |
| Open Field Agricultural Production (In Field) | Partial implementation of 5S is recommended only in maintenance and storage areas. | Furthermore, it prolongs the lifespan of machinery, ensures secure storage and maintenance. | The variable field conditions and the difficulty of continuous cleaning represent significant challenges. |
| Storage and Packaging Areas | All 5S steps can be applied, especially order and standardisation are recommended. | Such measures lead to increased productivity, maintained product quality and a reduction in waste. | The level of equipment intensity and the requisite labour input must also be considered. |
| Open field fruit-garden cultivation | Sorting and organising are partially recommended. | The organisation of the work area facilitates the safe utilisation of equipment. | The open area factor and the restrictions imposed by weather conditions. |

## Materials and Methods

This study analyses the effects of the 5S method in two main application areas within the agricultural sector (Table 2). The impact of the 5S methodology on productivity, occupational safety and resource efficiency was evaluated in the context of indoor tractor and agricultural machinery production and outdoor agricultural operations. Furthermore, an analysis was conducted to provide recommendations for the implementation of the 5S method in accordance with the diversity of agricultural activities, with a comparison of different implementation conditions in indoor and outdoor areas.

In this analysis, data provided by the US Environmental Protection Agency (EPA) on the 5S approach was employed to ascertain the potential benefits of 5S in the agricultural sector. The United States Environmental Protection Agency (EPA) defines the 5S method as a tool to ensure that workplaces are organised and efficient. The basic steps of this system, as outlined by the EPA, are sorting, organising, cleaning, standardising and ensuring continuity. These principles provide an effective framework for the creation of order in agricultural workplaces with regard to both organisation and occupational safety (EPA, 2023). Moreover, a study on the implementation of Lean Thinking production principles in the Swedish agricultural sector revealed the benefits of Lean Thinking and 5S methodologies for both the physical and psychosocial work environment. The study indicated that the application of Lean Thinking principles contributes to the establishment of a structured work environment, a reduction in occupational accidents and an enhancement of inter-employee collaboration (Andersson et al., 2020).

In evaluating the effects of 5S in agricultural practices, it was considered that user-oriented methods have the potential to enhance the success of 5S.

Below is a chart entitled 5S Implementation Recommendations. This chart presents the applicability of the 5S method and recommendations for different agricultural activities. Considering the effectiveness and advantages of 5S in different agricultural applications, it is stated in which areas it can be more useful (Table 2).

Additionally, a chart entitled "Indoor and Outdoor 5S Principles Comparison" is provided below. The chart illustrates the manner in which the 5S principles are implemented in the context of indoor tractor and agricultural machinery production, as well as in outdoor agricultural practices (Table 3).

## Results

The results of the study demonstrate that the 5S method has a marked effect on enhancing productivity, quality control and work safety in confined spaces. The optimisation of production processes was achieved through the reduction of waste and the maintenance of order, particularly during the sorting and cleaning stages. Nevertheless, the implementation of 5S in outdoor agricultural contexts was found to be constrained by certain limitations.

Table 3. Comparison of 5S principles in closed and open environments

| 5S Principles | Indoor (Tractor and Agricultural Machinery Production) | Outdoor (The utilisation of agricultural machinery in the field) |
|---|---|---|
| Sorting | The removal of superfluous materials from the environment is achieved with remarkable swiftness, while the rate of workflow is accelerated and the level of safety is increased. | The variability of the terrain presents a challenge to the sequencing of operations, and the organisation of machinery in the field may be constrained by this factor. |
| Systematic Organisation | The positioning of tools and materials in fixed locations on the production line is complemented by the implementation of visual management techniques. | The organisation of the vehicles is challenging due to the lack of a fixed positioning system. Furthermore, the applicability of this system is limited in maintenance and storage contexts. |
| Cleaning | The removal of production waste, such as oil and chips, is facilitated by regular cleaning operations. The implementation of cleaning procedures in closed areas is a relatively straightforward process. | External factors, such as dust and mud, can complicate the cleaning processes, which may require regular attention. |
| Standardisation | The implementation of standardised processes has been demonstrated to enhance quality and reduce the incidence of errors, which is a crucial objective in the context of quality management. | The implementation of a fully standardised approach is challenging due to the influence of climate and terrain variations. Consequently, this approach is only applicable within the context of maintenance processes. |
| Sustainability | The 5S system can be implemented on an ongoing basis through the provision of training and the conducting of audits. It is readily sustainable within the context of a factory environment. | The sustainability of this approach is hindered by seasonal and environmental factors, although it is partially applicable in maintenance and storage processes. |

The cleaning and organisation steps of 5S are rendered more challenging by seasonal changes and environmental factors. Nevertheless, the implementation of 5S practices in maintenance and storage procedures has been demonstrated to extend the lifespan of the equipment and provide a safe working environment.

Nevertheless, certain constraints were identified with regard to the implementation of 5S in outdoor agricultural contexts. The cleaning and organisation steps of 5S are rendered challenging by environmental factors, including seasonal changes, soil conditions and weather. These conditions render it challenging to regulate soil and environmental contamination in open settings, such as fields, thereby hindering the ability to maintain a consistent level of cleanliness. These findings underscore the significance of user-centred methodologies for the efficacious implementation of 5S in agricultural work environments (Muotka et al., 2023).

Nevertheless, the implementation of 5S practices in maintenance and storage processes has resulted in an extension of equipment lifespan and the provision of a safe working environment. The implementation of standardisation and sustainability (self-discipline) measures, particularly in confined spaces, has proven effective in maintaining and storing equipment, leading to long-term cost savings. Similarly, Chourasia and Nema (2019) emphasised the effects of 5S in increasing order, quality and employee motivation in their study on 5S practices in educational institutions. This study demonstrates that 5S not only engenders physical order and efficiency, but also confers benefits in the psychosocial dimensions of employee satisfaction and job satisfaction by fostering collaboration. These findings indicate that 5S is a valuable tool in the agricultural sector, offering benefits beyond mere physical organisation and efficiency. It has also been shown to enhance employee satisfaction and motivation (Andersson et al., 2020).

Figure 1 provides a summary of the contributions of the 5S method in terms of productivity, safety and sustainability in the agricultural sector. The figure illustrates the applicability and benefits of the method in agricultural activities.

Table 2, presented in the Materials and Methods section, provides a detailed account of the advantages and challenges of the 5S method in agricultural activities, as observed across different geographical areas.

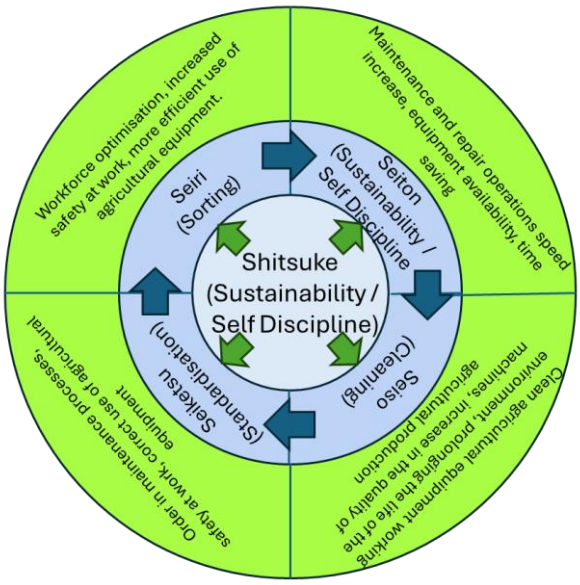


Figure 1. Applicability and contributions of the 5S method in the agricultural sector

Table 4. Effects of 5S implementation recommendations in different agricultural practices (before and after)

| Agricultural Application Area | Situation Before 5S Implementation | Situation After 5S Implementation |
|---|---|---|
| Greenhouse Agriculture | The absence of cleanliness and order, coupled with the irregular placement of equipment and vehicles, resulted in a disruption to the workflow. | The implementation of the 5S methodology ensures the maintenance of cleanliness and orderliness, with equipment positioned in designated locations. This approach has been shown to enhance productivity and promote a safe working environment. |
| Tractor and Agricultural Equipment-Machinery Production | Frequent errors were observed during the production process, and the safety measures in place were deemed to be inadequate. | The implementation of 5S has resulted in an enhancement of quality, a reduction in errors, and the provision of a secure working environment for employees. |
| Animal Production (On-Farm) | The potential risks to animal health and hygiene were significant, and maintaining a hygienic environment was challenging. | The implementation of regular cleaning and organisational procedures has ensured the protection of animal health and occupational safety. |
| Open Field Agricultural Production (In Field) | The equipment was frequently damaged as a result of exposure to the elements, a lack of organisation and maintenance, and other factors. | The organisation of maintenance and storage areas has resulted in an extension of equipment life and the provision of a safe working environment. |
| Storage and Packaging Areas | The productivity of the operation was adversely affected by a lack of clarity regarding the spatial configuration of the facilities, which in turn led to a deterioration in the quality of the products. | The implementation of a storage system resulted in the organisation of storage areas, an increase in productivity and the maintenance of product quality. |
| Open field fruit-garden cultivation | The workflow was disrupted, and it proved challenging to organise according to weather conditions. | The implementation of 5S resulted in the organisation of the workflow and a reduction in the impact of weather-related restrictions. |

The results demonstrate that while the 5S method has a positive impact on productivity and safety in enclosed environments, its effectiveness is constrained by environmental factors in open settings. Table 4 illustrates the enhanced productivity and safety outcomes attained in diverse agricultural settings through the deployment of the 5S methodology. The table demonstrates the efficacy of the methodology by contrasting the circumstances preceding and following the implementation of 5S in each area.

Table 4 illustrates the impact of implementing the 5S methodology in diverse agricultural contexts. It can be observed that the implementation of 5S practices in confined areas has a beneficial impact on aspects such as work safety, productivity, and quality control. Additionally, the organisation and cleaning steps have led to enhanced waste management. In particular, the sorting and standardisation processes contributed to the creation of a safer and more organised work environment for employees.

Conversely, the application of the methodology in open spaces revealed certain limitations due to environmental factors. The impact of seasonal changes and weather conditions on agricultural production makes the continuous implementation of some 5S steps challenging. Furthermore, ensuring consistent cleanliness in such environments is also a significant hurdle. This situation demonstrates the necessity for more flexible and user-oriented approaches to 5S applications in open areas.

As evidenced by the findings presented in Table 4, the 5S methodology has been demonstrated to both extend the lifespan of equipment and reduce costs associated with maintenance and storage processes. It can thus be concluded that the implementation of the 5S methodology in the agricultural sector is more straightforward in enclosed areas, whereas adaptations are required in open areas.

**Discussion and Conclusion**

This study has conducted a comprehensive evaluation of the applicability and benefits of the 5S method in the agricultural sector. While the 5S method has been demonstrated to yield effective results in terms of productivity and occupational safety in closed areas, specifically in the production of agricultural tools and machinery, it has been observed that this method encounters some limitations in open areas, particularly in the context of agricultural applications. In order to overcome these limitations, especially in open field applications, it is becoming increasingly important to adopt a user-oriented approach. In the literature, it is emphasised that methods such as Design Thinking can enhance the applicability of 5S in diverse contexts by prioritising the needs and experiences of employees (Muotka et al., 2023).

Conversely, the beneficial impact of Lean Thinking principles on both physical and psychosocial working conditions in agricultural work environments has been corroborated by studies conducted in the Swedish agricultural sector. The evidence suggests that Lean Thinking and 5S practices not only facilitate order within

the workflow, but also enhance employee cooperation and job satisfaction (Andersson et al., 2020). These findings underscore the significance of 5S benefits in enhancing occupational safety and employee motivation within the agricultural sector, suggesting that the methodology has a broader impact.

The field of ergonomics is becoming increasingly important in agriculture, as a means of improving the safety and comfort of workers engaged in modern farming methods. While traditional farming methods do not prioritise ergonomics, modern agricultural practices have introduced measures to ensure occupational safety and enhance productivity. The implementation of ergonomic practices enables workers to perform their duties in a safer and more productive manner, thereby enhancing the overall efficiency of agricultural production. Ergonomic practices in agriculture are of particular importance for the musculoskeletal health of workers, particularly in roles that require prolonged periods of static postures or involve the manual transportation of heavy loads. The physical demands of agricultural work, coupled with challenging working conditions, render this occupation prone to musculoskeletal discomforts, including back, lumbar and joint pain. The reduction of ergonomic risks has been demonstrated to enhance job satisfaction among workers and to contribute to a reduction in occupational accidents and health problems (Aygün et al., 2022). Consequently, the enhancement of ergonomic standards in agriculture represents a pivotal aspect in the safeguarding of occupational safety and the physical and psychological wellbeing of workers. The implementation of ergonomic principles in agricultural workplaces, in conjunction with workplace organisation methodologies such as 5S, represents an efficacious strategy for the establishment of a sustainable and secure working environment.

The issue of occupational safety in the agricultural sector is of increasing importance, particularly in the context of agricultural activities involving elderly farmers. Öz & Özgünaltay-Ertuğrul (2016) highlight the occupational safety risks faced by elderly farmers. In this study, it was asserted that the primary causes of accidents, such as those involving tractors, pose a significant risk to elderly farmers. In this context, the implementation of the 5S methodology, specifically the cleaning, organising and standardising steps, has the potential to create a safer working environment for older farmers. It is hypothesised that the implementation of the 5S methodology within the workplace will contribute to the prevention of accidents by reducing the potential for distraction.

The field of occupational safety in agricultural production in Turkey is confronted with significant challenges, particularly in the context of tractor accidents. The elevated average age of the tractor fleet in Turkey is a contributing factor to the rising incidence of accidents. The elevated average age of tractors serves to elevate the risks associated with safety, while simultaneously impeding agricultural productivity. Furthermore, data from European Union countries indicate a direct correlation between the age of the tractor and the frequency of accidents. Given that approximately half of the tractors in Turkey are over 25 years old, with an average age of around 40, the necessity for policies aimed at rejuvenating the tractor park in order to prevent accidents becomes evident (Özgünaltay-Ertuğrul et al., 2022). In this context, the implementation of the 5S methodology and regular maintenance of existing equipment represent an additional solution to reduce accident risks.

Furthermore, given the significant impact of tractor accidents on occupational safety in the agricultural sector, it is of paramount importance to ensure the accurate and consistent monitoring of these incidents. There is often a discrepancy between the details of accidents reported in the media and those reflected in official statistics. The aforementioned discrepancies render it challenging to ascertain the true extent of tractor accidents and to implement the requisite preventative measures. It is therefore evident that a more comprehensive and standardised data collection process is required in order to facilitate effective planning of occupational safety measures in the agricultural sector (Öz et al., 2023). A comprehensive approach to the reporting and analysis of tractor accidents will facilitate a more nuanced understanding of the underlying causes, which in turn will inform the development of more effective preventive measures. In this context, the Seiri (Sorting) and Seiton (Organising) steps of the 5S methodology can facilitate the accurate recording and analysis of accidents by regulating the flow of information in the workplace. An orderly and organised work environment can contribute to a reduction in risk by facilitating the implementation of safety measures in a more effective manner. Moreover, the standardisation afforded by the 5S methodology enables a more precise identification of the root causes of accidents and hazards. It is therefore possible to create safe working environments and to minimise risks in order to prevent accidents involving tractors in the agricultural sector.

The agricultural sector is characterised by a number of safety risks, which can be attributed to both biological and chemical hazards. Of particular concern are the risks associated with the use of spraying machines in pesticide applications. As stated by Alkan & Özgünaltay-Ertuğrul (2022), the utilisation of agricultural machinery in pesticide applications may give rise to a multitude of hazards and risks. Such risks include the presence of inadequate warning signage on the machinery itself, as well as the potential for operators to be exposed to chemicals. The 5S methodology has the potential to minimise such risks and can create safe working environments by ensuring the cleanliness and orderliness of machinery and equipment. In particular, the cleaning and organising steps can prevent the accumulation of pesticide residues and ensure that the machinery is properly maintained. Consequently, the probability of worker exposure to pesticides is diminished, thereby enhancing occupational safety.

This study provides a comprehensive examination of the contributions of the 5S method in terms of productivity, occupational safety and sustainability in the agricultural sector. The findings of the study demonstrate that the 5S methodology is particularly efficacious in terms of efficiency and organisation in closed areas. However, in open areas, it encounters challenges due to environmental factors. Nevertheless, it was also noted that the implementation of the 5S methodology has the potential to positively impact various aspects of agricultural operations. These include the extension of equipment

lifespan, a reduction in occupational accidents, and cost savings in maintenance and storage processes. The results demonstrate that the 5S method has considerable potential for enhancing occupational safety and productivity in the agricultural sector.

In particular, the implementation of regular cleaning, sorting and standardisation procedures in enclosed workspaces serves to enhance the safety of personnel and optimise the efficiency of operational processes. Furthermore, the implementation of sophisticated AI-driven systems, such as helmet detection technologies, can significantly enhance safety by facilitating the monitoring of compliance and reducing the incidence of human errors in agricultural machinery production facilities (Özüağ & Ertuğrul, 2024). Conversely, in open environments, it is of the utmost importance to ensure the adaptability of 5S practices in order to accommodate seasonal fluctuations. In this context, the integration of 5S methodology with sustainable and user-oriented approaches in agricultural areas has the potential to positively affect both occupational safety and employee motivation.

In conclusion, this study demonstrates the potential for the application of 5S methodology in the agricultural sector, and emphasises that significant improvements can be achieved in both production processes and occupational health and safety. Further studies should be conducted with the aim of increasing productivity in agricultural activities, with a view to developing a 5S implementation model that is flexible and suitable for environmental conditions, thus enhancing the effectiveness of the methodology.

## Declarations

### *Author Contribution Statement*

In this study, three authors were involved at different stages of the research process. The primary author, G.Ö.E., made significant contributions to the development of the research concept, the synthesis of relevant literature, the general composition of the study, the collection and analysis of data, and the preparation of comparative tables on the applicability of 5S practices in agricultural contexts. Additionally, İ.A. and E.U. contributed to the discussion of the study's findings, their alignment with the Sustainable Development Goals, and the finalisation of the manuscript. All authors were actively engaged in the writing process and approved the final version of the manuscript.

### *Conflict of Interest*

The authors declare no conflict of interest.

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# Development of Land Consolidation Studies in Türkiye (1961-2004)

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ABSTRACT

This study aims to comprehensively evaluate land consolidation studies carried out in Türkiye between 1961 and 2004. Data from 509 land consolidation and on-farm development services projects carried out in 33 provinces of Türkiye were examined using statistical analysis methods. It was determined that land consolidation projects significantly contributed to more efficient use of agricultural lands, reducing the number of parcels and reaching more suitable sizes of agricultural enterprises. The results show that the average consolidation rate of the examined projects was 35.76% and an average increase of 81.45% occurred in parcel sizes. In the regional evaluations, it was determined that the Black Sea Region had the highest consolidation rate. In contrast, relatively lower success rates were obtained in the Eastern Anatolia and Southeastern Anatolia regions. These differences are related to land structure, property relations and agricultural enterprise sizes. It has been observed that technological developments, especially computer-aided mapping techniques and tools such as Geographic Information Systems, have increased the success of consolidation projects. As a result, it has been determined that land consolidation studies play a critical role in the sustainability of agricultural production. Still, more strategic planning should be made by considering regional characteristics. This study is essential for evaluating the historical development and impacts of land consolidation studies in Türkiye. It is also considered a guide for institutions carrying out consolidation studies.

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## Introduction

Land consolidation is a mechanism to eliminate economic, social, and environmental imbalances in rural areas (Acar, 2024). It means arranging fragmented, scattered, or degraded parcels of land where agricultural activities cannot be economically sustained and making them suitable for agriculture. These arrangements, made in accordance with modern agricultural methods, aim to increase agricultural land productivity and the number of arable lands (Arıcı & Akkaya Aslan, 2014; Kirmikil & Akkaya Aslan, 2024).

Land consolidation projects worldwide can provide different positive benefits both within the land consolidation project area and on a larger scale. The numerical ratio of such benefits varies mainly depending on the country where the projects are carried out and the content of the projects. By improving the structure of the parcels and farms within the land consolidation project area, agricultural enterprises can be made more efficient and adapted to rural development, environment and other relevant needs. Legal uncertainties regarding land ownership rights within the project area are eliminated to reallocate the parcels, which also brings legal benefits that increase the functionality of official agricultural land markets. With consolidation projects, in addition to reallocation planning depending on regional needs, other infrastructure works such as agricultural roads, irrigation and drainage, recreation areas and studies for the protection of the ecosystem, social areas and ecotourism can contribute to the improvement of agricultural infrastructure. In addition, improvements in landownership, farm structures, and rural infrastructure in the project area will benefit farmers and society in general. Land consolidation can also provide social benefits such as integrated community development, new social spaces, better opportunities for young farmers, reduced emigration, improved public infrastructure, and strengthened gender equality (Veršinkas et al., 2021).

Land consolidation studies in the world and Türkiye have social and economic importance in agricultural activities. Many factors should be considered for these studies to yield successful results. These studies, which require intensive effort and significant investments, are challenging to implement. It is not always possible for each project area to have the same standards due to its demands and conditions. The success rate in projects varies depending on how much technical requirements are taken into account and how much regional demands are met (Kirmikil & Akkaya Aslan, 2024).

Table 1. Land consolidation projects carried out in Türkiye by year

| Year | Institution | Consolidated Area (ha) |
|---|---|---|
| 1961-2002 | General Directorate of Soil and Water – General Directorate of Rural Services | 450,000 |
| 2003-2007 | Special Provincial Administrations | 132,000 |
| 2008-2018 | General Directorate of Agricultural Reform – Provincial Directorates of Food, Agriculture and Livestock | 3,018,021 |
| 2019-2023 | General Directorate of State Hydraulic Works | 3,673,566 |
| Total | | 7,273,587 |

Land consolidation studies started in Türkiye in 1961 and were carried out entirely by state institutions until 1987. In 1987, land consolidation projects were opened to the private sector for the first time. With the closure of the General Directorate of Rural Services, the duties and authorities of the General Directorate were given to the Provincial Special Administrations. The workload and understanding of the Provincial Special Administrations, their financial situation and the need for expertise in land consolidation caused the studies to end throughout the country. With the closure of the General Directorate of Rural Services, the General Directorate of Agricultural Reform began to focus on land consolidation studies (Arıcı & Akkaya Aslan, 2014). With the Presidential Decree No. 30479 published in the Official Gazette dated July 15, 2018, the responsibility and authority of land consolidation and on-farm development services were transferred to the State Hydraulic Works (DSI, 2024; Tunalı, 2024) (Table 1).

Sustainable rural land management is critical for the correct and efficient use of land, protection of the environment and support of socio-economic development (Ayhan et al., 2024). Land consolidation projects, one of the most important elements contributing to sustainable agriculture, have significant social and economic importance in agriculture in Türkiye and worldwide. These projects require large-scale investments and intensive effort, and have local conditions and demands. Success rates are affected in different ways depending on the extent to which the demands in the regions where the projects are carried out are met, and how much the technical needs are taken into account (Kirmikil & Akkaya Aslan, 2024). In addition, the conditions during the implementation of the projects should also be examined well. It is possible to say that such studies will make significant contributions to the studies of the consolidation projects to be carried out later. This study aims to evaluate the land consolidation studies by the General Directorate of Rural Services in Türkiye in the first 40 years. In this way, the development status of the land consolidation projects carried out in Türkiye in the first period was examined, and the impact of the projects carried out on the present day was evaluated.

## Materials and Methods

This study examined 509 land consolidation and on-farm development services projects completed in 33 provinces in Türkiye between 1961 and 2004. For this purpose, land consolidation project data belonging to the repealed Ministry of Agriculture and Rural Affairs, General Directorate of Rural Services, were evaluated in the study. These data were entered into the Microsoft Excel program, and a single-variable statistical report was created with the help of the descriptive statistics analysis tool. Thus, the central tendency and diversity of the data belonging to land consolidation projects were examined in general. In addition, the total number of parcels, average parcel area, and the change in the average number of parcels per enterprise for the projects carried out in different regions were also evaluated. The changes in the Consolidation Rate (CR, %), Parcel Area Increase Rate (PAIR, %), Parcel Reduction Index (PRI) and Consolidation Coefficient (CC) values, which are important success criteria in land consolidation projects, were also examined regionally and depending on the years. Equations 1, 2, 3 and 4 were used regarding the success indicators of land consolidation projects (Arıcı, 1994; Crecente et al., 2002):

$$CR = \frac{PreCPN - PostPCN}{PreCPN} \times 100 \quad (1)$$

PreCPN : Pre consolidation parcel numbers
PostCPN: Post consolidation parcel numbers

$$PAIR = \left(\frac{PostCPA}{PreCPA} \times 100\right) - 100 \quad (2)$$

PostCPA: Post consolidation parcel area
PreCPA : Pre consolidation parcel area

$$PRI = \frac{PreCPN}{PostCPN} \quad (3)$$

$$CC = \frac{PreCPN}{PostCPN} \times \frac{100}{\frac{PreCPN}{Number\ of\ landowners}} \quad (4)$$

## Results

Descriptive statistics of land consolidation project data are given in Table 2.

The study evaluated 509 consolidation projects completed in 33 provinces in different regions of Türkiye between 1961 and 2004. The total project area is 405,869 ha, which is given to farmers after the deduction rate.

When the table data is examined, it is seen that the average project area is 797.39 ha. The lowest project area is recorded as 14 ha in Sinop – Boyabat – Osman village, the consolidation of which was completed in 2000. The largest project area is the 14,394-ha in Aksaray – Eskil – Eşmekaya village, which was completed in 2002. In addition, the mode value (132 ha) given among the descriptive statistics shows that most projects are small-scale. When the descriptive statistics of the number of enterprises in the project areas are examined, it is seen that the average enterprise number is 326 and the maximum enterprise number is 2,869. The standard deviation value is high since some projects have high enterprise numbers.

Table 2. Descriptive Statistics

| | PA | NE | NP | TPN | | APA | |
|---|---|---|---|---|---|---|---|
| | | | | Before | After | Before | After |
| Mean | 797.39 | 325.52 | 415.77 | 848.23 | 472.24 | 1.49 | 2.26 |
| Standard Error | 46.79 | 15.87 | 19.21 | 59.65 | 40.44 | 0.06 | 0.08 |
| Median | 534.00 | 217.00 | 294.00 | 461.00 | 275.00 | 1.09 | 1.78 |
| Mode | 132.00 | 115.00 | 192.00 | 236.00 | 181.00 | 0.60 | 2.02 |
| Standard Deviation | 1,055.68 | 358.06 | 433.39 | 1,345.65 | 912.42 | 1.41 | 1.76 |
| Sample Variance | 1,114,459.91 | 128,208.66 | 187,829.83 | 1,810,786.88 | 832,509.22 | 1.98 | 3.11 |
| Kurtosis | 59.70 | 13.88 | 15.13 | 43.59 | 90.58 | 11.86 | 18.40 |
| Skewness | 6.00 | 3.19 | 3.27 | 5.60 | 8.56 | 2.95 | 3.22 |
| Range | 14,380.00 | 2,865.00 | 3,741.00 | 15,404.00 | 11,824.00 | 10.81 | 18.39 |
| Minimum | 14.00 | 4.00 | 4.00 | 11.00 | 10.00 | 0.01 | 0.01 |
| Maximum | 14,394.00 | 2,869.00 | 3,745.00 | 15,415.00 | 11,834.00 | 10.82 | 18.40 |
| Sum | 405,869.00 | 165,690.00 | 211,629.00 | 431,748.00 | 240,369.00 | 758.71 | 1,148.22 |
| Count | 509.00 | 509.00 | 509.00 | 509.00 | 509.00 | 509.00 | 509.00 |
| Largest (1) | 14,394.00 | 2,869.00 | 3,745.00 | 15,415.00 | 11,834.00 | 10.82 | 18.40 |
| Smallest (1) | 14.00 | 4.00 | 4.00 | 11.00 | 10.00 | 0.01 | 0.01 |
| Confidence Level (95%) | 91.93 | 31.18 | 37.74 | 117.18 | 79.45 | 0.12 | 0.15 |

| | AAP | ANE | | CR | PRI | PAIR | CC |
|---|---|---|---|---|---|---|---|
| | | Before | After | | | | |
| Mean | 2.31 | 3.05 | 1.78 | 35.76 | 1.81 | 81.45 | 90.14 |
| Standard Error | 0.09 | 0.23 | 0.18 | 0.96 | 0.04 | 4.32 | 2.09 |
| Median | 1.73 | 1.97 | 1.18 | 36.60 | 1.58 | 57.73 | 84.47 |
| Mode | 1.80 | 1.00 | 1.00 | 33.33 | 1.50 | 24.52 | 100.00 |
| Standard Deviation | 2.11 | 5.11 | 3.95 | 21.56 | 0.97 | 97.37 | 47.17 |
| Sample Variance | 4.45 | 26.16 | 15.60 | 464.94 | 0.95 | 9,481.30 | 2,225.43 |
| Kurtosis | 12.24 | 88.38 | 179.02 | 0.47 | 45.23 | 45.23 | 8.41 |
| Skewness | 2.96 | 8.45 | 12.38 | -0.19 | 4.83 | 4.83 | 1.80 |
| Range | 18.06 | 67.71 | 67.26 | 159.32 | 13.02 | 1,301.85 | 458.08 |
| Minimum | 0.19 | 0.34 | 0.22 | -66.67 | 0.60 | -40.00 | 1.48 |
| Maximum | 18.25 | 68.05 | 67.47 | 92.66 | 13.62 | 1,261.85 | 459.56 |
| Sum | 1,177.76 | 1,553.27 | 904.02 | 18,203.63 | 923.56 | 41,455.94 | 45,881.71 |
| Count | 509.00 | 509.00 | 509.00 | 509.00 | 509.00 | 509.00 | 509.00 |
| Largest (1) | 18.25 | 68.05 | 67.47 | 92.66 | 13.62 | 1,261.85 | 459.56 |
| Smallest (1) | 0.19 | 0.34 | 0.22 | -66.67 | 0.60 | -40.00 | 1.48 |
| Confidence Level (95%) | 0.18 | 0.45 | 0.34 | 1.88 | 0.08 | 8.48 | 4.11 |

PA: Project Area (ha); NE: Number of Enterprises (pcs); NP: Number of Person (pcs); TPN: Total Parcel Number (pcs); APA: Average Parcel Area (ha); AAE: Average Parcel Area per Enterprise (ha); AAP: Average Parcel Area per Person (ha); ANE: Average Parcel Number per Enterprise (pcs); CR: Consolidation Rate (%); PRI: Parcel Reduction Index; PAIR: Parcel Area Increase Rate (%); CC: Consolidation Coefficient

The average of the consolidation rate values in the evaluated project areas was found to be 35.76%. The negative consolidation ratio values in some projects decreased this rate. However, the maximum value was seen in Isparta – Atabey – Harmanören Village (92.66%) in 1976. Out of 509 projects, only 12 had a negative consolidation rate.

The average parcel size increase rate was 81.45%, and the highest increase rate was observed in the largest project area, Isparta – Atabey – Harmanören village, with 1,261%. In addition, the increase rate in the parcel area was negative, as in the 12 villages with a negative project area consolidation rate. The average parcel size per person in the projects is 2.31 ha. The highest average parcel area was seen in the consolidation project in Aydın – Karacasu – Tepecik Village in 1990 with 18.25 ha.

The average parcel reduction index value was found to be 1.81. The smallest parcel reduction rate value (0.60) occurred in Konya – Ereğli – Taşağıl Village, while the largest parcel reduction rate occurred in Isparta – Atabey – Harmanören Village, just like the consolidation rate and average parcel size increase rate. The fact that the difference between the minimum and maximum values is so high shows that the differences between the projects are also very high. When the consolidation coefficient values, which should be examined together with the parcel reduction index, are examined, it is seen that the average consolidation coefficient value is 90.14. As the parcel reduction rate increases, the consolidation coefficient increases somewhat.

In addition to the statistical evaluation made nationwide in the study, the characteristics of the parcels at the geographical region level were also evaluated. For this purpose, the total number of parcels, the average parcel area and the average number of parcels per enterprise were calculated separately for each region (Table 3). When Table 3 is examined, it is seen that the region with the most projects is the Aegean Region, while the region with the fewest projects is the Southeastern Anatolia Region. The number of projects has changed proportionally with the regional land size values. While the region with the highest average parcel area before and after consolidation is the Southeastern Anatolia Region, the regions with the lowest are the Marmara Region with 1.00 ha before consolidation and the Black Sea Region with 1.28 ha after consolidation.

Table 3. Changes in some parcel characteristics in different geographical regions

| Row Labels | NP | Project Area | Total Parcel Number | | Average Parcel Area (ha) | | Average Parcel Number per Enterprise | |
|---|---|---|---|---|---|---|---|---|
| | | | Before | After | Before | After | Before | After |
| Mediterranean | 16 | 16,247 | 1,826 | 839 | 1.07 | 1.61 | 3.07 | 1.34 |
| Eastern Anatolia | 13 | 15,794 | 703 | 525 | 2.04 | 2.94 | 3.47 | 2.02 |
| Aegean | 225 | 148,662 | 683 | 434 | 1.50 | 2.12 | 2.16 | 1.43 |
| Southeast Anatolia | 3 | 2,680 | 270 | 172 | 3.33 | 5.46 | 2.54 | 1.63 |
| Central Anatolia | 116 | 135,123 | 881 | 399 | 2.10 | 3.18 | 3.51 | 1.60 |
| Black Sea | 40 | 17,136 | 851 | 375 | 0.71 | 1.28 | 2.92 | 1.42 |
| Marmara | 96 | 70,227 | 1,070 | 632 | 1.00 | 1.78 | 4.59 | 3.00 |
| Total | 509 | 405,869 | 848 | 472 | 1.49 | 2.26 | 3.05 | 1.78 |

NP: Number of Project

Table 4. Changes in different geographical regions according to some consolidation evaluation criteria

| Row Labels | Consolidation Rate (%) | Parcel Area Increase Rate (%) | Parcel Reduction Index | Consolidation Coefficient |
|---|---|---|---|---|
| Mediterranean | 41.20 | 159.89 | 2.60 | 94.45 |
| Eastern Anatolia | 29.04 | 57.43 | 1.57 | 80.62 |
| Aegean | 29.72 | 52.12 | 1.52 | 90.04 |
| Southeast Anatolia | 34.91 | 66.44 | 1.66 | 69.60 |
| Central Anatolia | 38.30 | 96.41 | 1.96 | 86.40 |
| Black Sea | 47.33 | 126.11 | 2.26 | 113.80 |
| Marmara | 42.08 | 104.14 | 2.04 | 86.25 |
| Total | 35.76 | 81.45 | 1.81 | 90.14 |

The highest values for the average number of parcels per enterprise are in the Marmara Region, and the lowest values are in the Aegean Region before consolidation and in the Black Sea Region after consolidation. However, after consolidation, the average number of parcels per enterprise in the Aegean region is very close to the Black Sea region.

The study evaluates some important success indicators of consolidation projects at the regional level. Table 4 provides the consolidation rate, parcel area reduction rate, parcel reduction index, and consolidation coefficient values.

The region with the highest consolidation rate and consolidation coefficient values, which are accepted as success indicators, is the Black Sea Region. The Mediterranean Region has the highest parcel area reduction rate and parcel reduction index. When the lowest values are examined, the Eastern and Southeastern Anatolia Regions stand out regarding consolidation rate and consolidation coefficient, respectively. In contrast, the region with the lowest parcel area reduction rate and parcel reduction index values is the Aegean Region. When the average values of Türkiye are considered, it is seen that the consolidation rate values of the Black Sea, Marmara and Central Anatolia Regions are above the average ones of Türkiye. Again, while the parcel area reduction rate is above the average of Türkiye in the Black Sea and Marmara Regions, the parcel reduction rate values of the Aegean, Eastern Anatolia and Southeastern Anatolia Regions are above average. The regions with a consolidation coefficient higher than the average are the Black Sea and Aegean Regions. In general, it is seen that the Black Sea Region, especially the parcel area reduction rate and consolidation coefficient values, are much higher than other regions. It can be said that the merging of parcels, which are much smaller in size compared to other regions, increases the success rates of the projects even more compared to other regions. It is also expected that the Southeastern Anatolia region, which has much larger parcels compared to other regions of Türkiye, has lower values compared to other regions. It would be correct to say that these data are due to parcel sizes based on regional characteristics of agricultural areas rather than project performance (Table 4).

The change values according to the years in which land consolidation projects were carried out in Türkiye are given in Figures 1-6. In 1961-2004, 509 projects were completed on a total area of 405,869 hectares, and an increase was observed in the projects according to the years. Just as in the number of projects and project area values, an increase was observed in the consolidation rate, the parcel reduction index, the parcel area increase rate, and the consolidation coefficient values according to the years. The reason for this is the increasing specialization of the technical team carrying out the project in the field and the use of computer technologies over time. The fluctuations in the graphs are due to the periods when the number of completed projects was lower compared to other years. However, these values do not mean a decrease in consolidation projects in those years. Since consolidation projects are projects completed in the long term, it is normal for there to be decreases in the graphs in these years. Therefore, it would be more accurate to consider the trend lines instead of the results in the graphs.

When Figure 1 is examined, it is seen that the highest number of completed projects was in 1997 (50 projects). Although there was a specific decrease in the number of projects after this year, an increase was observed again after 2000. However, no land consolidation projects were completed in 1963, 1965, 1980, and 1981. This situation is not due to the complete cessation of work on land consolidation projects. Since these projects are long-term, the emergence of such a situation should be considered normal.

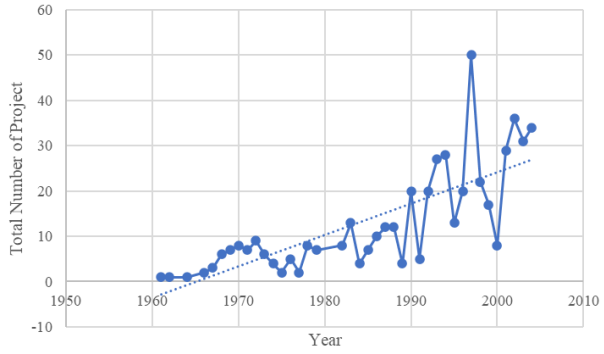


Figure 1. Change in the total number of land consolidation projects by year

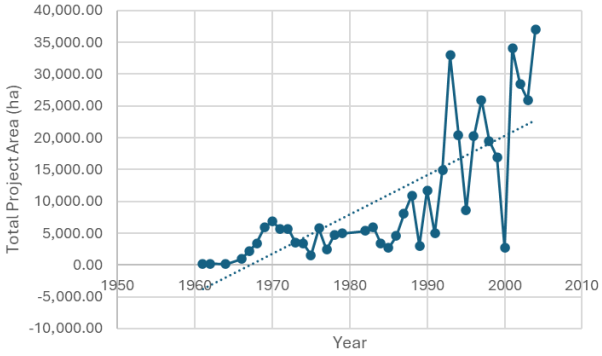


Figure 2. Change in total project area by year

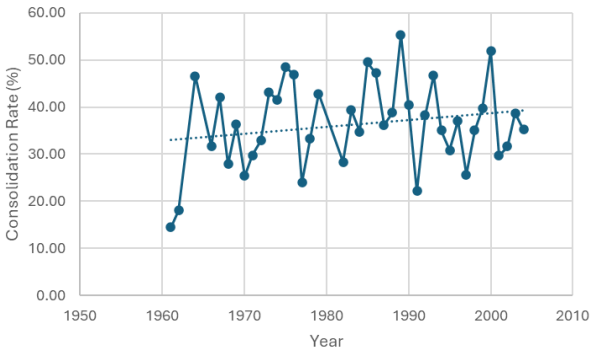


Figure 3. Change in consolidation rates by year

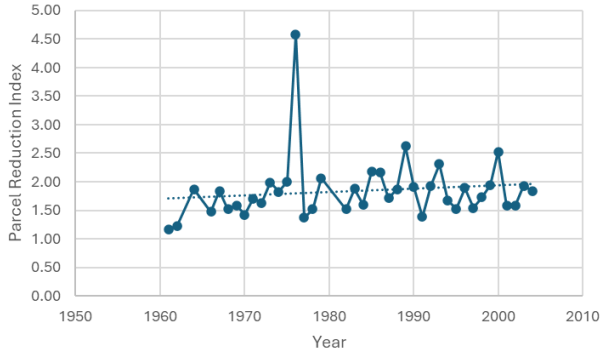


Figure 4. Changes in parcel reduction index by year

Just like the total number of projects whose consolidation has been completed, there has also been an increase in area values over the years. When we look at the trend lines of the increase in project areas, it is seen that it is more than the total number of projects. This situation also shows that project engineers have started to work on projects in larger areas, which can indicate that they have started to specialize in their fields (Figure 2).

Although it is lower compared to the total number of projects and project areas, it has been observed that there has been a slight increase in the consolidation rates over the years. The lowest consolidation rate average obtained from land consolidation projects was observed in 1961, while the highest average occurred in 1989. Although the consolidation rate values were low in specific years after 1989, this situation did not change the upward trend. In this context, it is thought that evaluating each project on its own would be more accurate. For example, the consolidation rate value of 78.39%, the land consolidation project completed in Gökdere Village of Eskişehir Central District in 1989, provided a significant increase in the 1989 average compared to other years. Since the consolidation rate values of the other three projects completed in 1989 were above the national average, they ensured the annual average was high (Figure 3).

When the parcel reduction index values, which reflect the technical quality of the consolidation project, belonging to the land consolidation projects completed by year, are examined in Figure 4, it is seen that there has been a low increase, just like the consolidation rate averages. The most significant increase in parcel reduction index averages occurred in 1976. The parcel reduction index value that affected this increase the most was the reduction index value 13.62, which was carried out on a 569-ha area in the Harmanören village of the Atabey district of Isparta province. The highest consolidation rate, parcel reduction index, and parcel area increase rate values in the projects evaluated were obtained from this project. As expected, the lowest parcel reduction index value was obtained from the project completed in 1961. Based on this, it is possible to say that the technical quality and efficiency of the projects have increased over the years.

When Figure 5, which shows parcel area increase rate values, is examined, a similar change occurs in parcel reduction index values. Based on this, it is possible to say that an evaluation using only one of the parcel reduction index and parcel area increase rate values will be sufficient.

When Figure 6, which shows the change in the consolidation coefficient values depending on the years, is examined, a higher increase is observed in the consolidation coefficient values compared to the consolidation rate, parcel reduction index and parcel area increase rate values. The highest consolidation coefficient averages were obtained from the eight projects completed in 2000. Although these values do not include maximum values, they are above the general average. The lowest consolidation coefficient averages were obtained from the two projects completed in the Central Anatolia Region in 1975.

## Discussion

Many studies have been conducted in Türkiye to evaluate land consolidation projects. However, these studies generally include project-based studies covering only one or a few villages. In addition, limited studies evaluated land consolidation projects from before 2005.

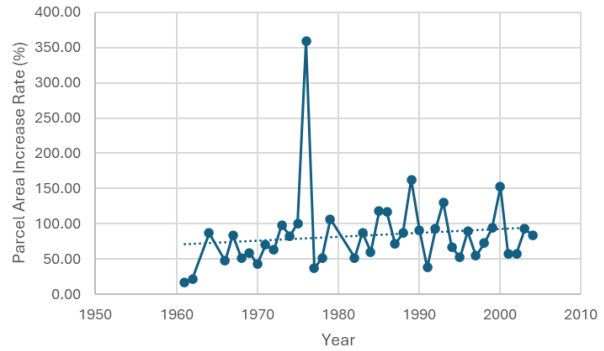


Figure 5. Change in parcel area increase rates by the year

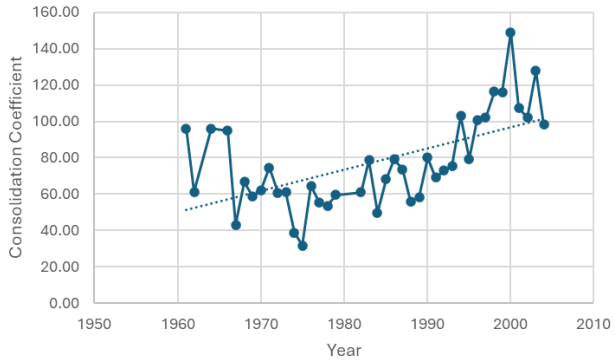


Figure 6. Changes in consolidation coefficient by year

In a study examining land consolidation projects in Karkın village of Konya province by Çevik (1974), it was reported that there was a 44% decrease in the total number of parcels and a 72% increase in the average parcel size (Gündoğdu, 1993). Takka (1993) reported that in the land consolidation studies carried out in Türkiye between 1961 and 1992, the average parcel size increased by 84%, the average number of parcels per farm decreased by 62%, and an average agricultural land size of 3.14 ha per farm emerged.

In the study conducted by Tulukcu & Çağla (2005), in which land consolidation projects were examined in Çumra district, it was determined that many social, economic and natural factors affected the implementation of the applications for the first time in Çumra. In their study in Galicia - Spain, Crecente et al. (2002) determined the reduction index value as 6.1 and the consolidation coefficient value as 90. Akkaya Aslan et al. (2007) determined the parcel reduction index value as 4.28 for Serem (Thrace District) and 1.51 for Beyköy (Marmara Region) in the consolidation projects they evaluated. The consolidation coefficient values for the same projects were 100.00 (Serem) and 76.45 (Beyköy), respectively. Arıcı & Akkaya Aslan (2014) reported that although the land consolidation studies carried out within the General Directorate of Soil, Water and Rural Services were distributed to various irrigation areas of the country, many of them consisted of projects implemented in Manisa, Aydın, Denizli, İzmir, Aksaray, Tokat, Konya, Eskişehir and Bursa. The results obtained from the studies conducted in general are parallel to those obtained from this study. However, it was observed that changes in parcel size distribution and consolidation rate values were generally considered in Türkiye's studies. When the consolidation rate values, one of the most important outputs of land consolidation studies, are examined, it is seen that even today, examples from the world have not yet been reached. For example, in Germany, which has made significant progress in rural area planning, the consolidation rate is 80%, and in Spain, it is 82.14% (Arıcı & Akkaya Aslan, 2014). However, this situation should not be accepted as an indication that the consolidation studies conducted in Türkiye have failed. In the studies conducted today, instead of aiming for a high consolidation rate, the aim is to prevent the hidden fragmentation that occurs due to shareholding, one of rural areas' most important problems. Therefore, this study is considered an important source regarding the historical development of consolidation projects in Türkiye in terms of periodic evaluation.

It has been observed that technological developments play an important role in land consolidation processes. Since the 1980s, computer-aided mapping techniques, geographical information systems, and remote sensing methods have enabled projects to be completed more successfully and quickly. When the increasing trend over the years is examined, it is observed that technological developments increase project success. However, it has been observed that some difficulties such as the participation of farmers in the consolidation processes, legal regulations, financing and infrastructure deficiencies limit the success of the projects. Informing the local people and ensuring their active participation in the projects is important for the more efficient implementation of future projects. In addition, integrating other rural development elements such as irrigation infrastructure and transportation facilities with consolidation projects will increase long-term success.

As a result, to implement land consolidation projects more effectively in Türkiye, projects should be planned and implemented taking regional differences into account. In future studies, detailed research on the socio-economic effects of consolidation projects and farmers' perspectives on the project is recommended.

## Conclusion

In this study, 509 land consolidation and on-farm development services projects carried out in 33 provinces of Türkiye between 1961-2004 were statistically evaluated. It was determined that land consolidation projects provided significant gains in more effective and efficient use of agricultural lands. The data obtained show that due to the projects, there was an increase in average parcel sizes, a decrease in the number of parcels, and a significant improvement in consolidation coefficient values.

When the study results are evaluated in general, the average consolidation rate is calculated as 35.76% and it is observed that this rate reaches relatively high levels in some projects. The average parcel area increase rate is 81.45%, and higher rates are reached especially in regions where small-scale agricultural lands are located. However, the average value of the parcel decrease index is determined as 1.81.

When regional differences are examined, it is determined that the Black Sea Region is the most prosperous in consolidation rate and parcel area increase rate. In contrast, relatively lower success is achieved in the Eastern Anatolia and Southeastern Anatolia regions. This situation is due to differences in land structure and agricultural enterprise sizes between the regions.

This study's results are important in providing a broader assessment compared to previous project-based studies. Considering that many previous studies were related to a limited number of projects or regions, this research more comprehensively reveals the success of consolidation projects throughout Türkiye.

To increase the success of land consolidation projects, it is recommended that more detailed analyses be conducted before the project, farmers should be more involved in the processes, and modern technologies should be used effectively. In addition, consolidation processes should focus on parcel size and have an integrated structure with irrigation infrastructure, transportation facilities, and rural development goals.

To increase the effectiveness and sustainability of land consolidation projects, it is recommended to use Geographic Information Systems and remote sensing technologies, implement artificial intelligence-based decision support systems, and create digital cadastral and blockchain-based property records. In addition, long-term monitoring systems that evaluate the socio-economic impacts of consolidation projects should be established, irrigation infrastructure and consolidation processes should be integrated, and farmers' participation in the process should be increased. A model that includes local governments and stakeholders should be adopted with a participatory planning approach, and digital platforms and mobile applications should be developed to ensure that consolidation processes are carried out transparently. In addition, some legal and structural regulations need to be implemented. Legislative changes should make consolidation mandatory throughout the country in irrigation areas outside agricultural reform regions. In addition, necessary legal measures should be taken to prevent land fragmentation again and relevant regulations should be implemented. For consolidation projects to be carried out more efficiently, the number of technical personnel to work in the field should be increased. These personnel should be trained in Turkey and abroad and their expertise should be ensured. Communication channels such as written, visual, and social media should be used more effectively to explain the consolidation process's benefits to wider audiences and raise awareness among farmers.

In conclusion, land consolidation projects are critical for the sustainability of agricultural production in Türkiye. However, more regional strategies should be developed, considering each region's specific conditions, and consolidation projects should be supported by longer-term planning. The results of this study are believed to shed light on future projects and guide policy makers.

## Declarations

### *Ethical Approval Certificate*

This study does not require an Ethical Approval Certificate.

### *Author Contribution Statement*

Safiye Pınar Tunalı: Data collection, investigation, formal analysis, and writing the original draft, project administration, supervision, conceptualization, methodology, review and editing.

### *Fund Statement*

This study has not been funded.

### *Conflict of Interest*

The authors declare no conflict of interest.

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# The Economic Determinants of Agricultural Value Added: A Panel Data Analysis on E7 Countries

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ABSTRACT

The agricultural sector plays a crucial role in economic growth, employment, and food security. Although E7 countries (Brazil, China, India, Indonesia, Mexico, Russia, and Turkey) hold a significant share in global agricultural production, there is limited literature on the determinants of agricultural value added. This study aims to analyze the key economic factors affecting agricultural value added in E7 countries and assess the effectiveness of agricultural policies. The study covers the period 2001-2022 and employs the Panel ARDL method to examine long-term relationships. The findings indicate that the proportion of agricultural land and agricultural employment positively impact agricultural value added, whereas government expenditures may have a negative effect. Governments intervene in the agricultural sector through both direct and indirect measures. It can be suggested that direct income support policies for farmers may have the potential to increase dependency rather than enhance productivity. Although a detailed distinction regarding the implementation of direct payments during the analyzed period could not be made, the impact of government support is likely to vary depending on the type and implementation of the assistance provided. The results emphasize the need for more effective planning of agricultural support mechanisms. Redirecting public expenditures towards infrastructure investments, agricultural technology adoption, and rural development projects could enhance the sector's long-term sustainability. Additionally, improving the transparency and measurability of support policies may increase their effectiveness and strengthen agricultural productivity. Policymakers should conduct a more detailed analysis of the effects of different support mechanisms to develop appropriate intervention strategies.

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## Introduction

The agricultural sector plays a crucial role in economic growth, employment, and food security. Beyond food production, it provides raw materials for industries, contributes to export revenues, and supports rural development (Soyyiğit & Yavuzaslan, 2019). The sector's capacity to generate employment is particularly significant in developing economies, where it enhances social welfare (Kaya, 2020). Additionally, the agricultural sector establishes strong economic linkages with industries such as logistics, trade, and finance, reinforcing overall economic stability (Aydoğan & Vardar, 2020). Due to these factors, agriculture holds great significance in terms of both direct and indirect economic contributions.

E7 countries (Brazil, China, India, Indonesia, Mexico, Russia, and Turkey) occupy a crucial position in global agricultural production and trade. These nations, which comprise approximately 45% of the world's population, account for over 30% of global Gross Domestic Product (GDP) (Doğdu, 2022). However, studies analyzing the determinants of agricultural value added in these economies remain limited. The impact of government expenditures on agriculture, the contribution of agricultural employment to sectoral growth, and the economic implications of agricultural land use have not been sufficiently examined. This study aims to identify the key economic factors affecting agricultural value added and evaluate the effectiveness of agricultural policies.

Accordingly, this study investigates the economic determinants of agricultural value added in E7 countries. Specifically, it examines the effects of the agricultural employment rate, the share of agricultural land in total land, and government expenditures on agriculture. The study covers the period from 2001 to 2022 and employs the Panel ARDL method to analyze long-term relationships. Given the diverse economic structures of E7 countries, the comparative analysis offers significant insights into the effectiveness of agricultural development policies.

Agricultural value added represents the economic contribution derived from agricultural production. The key elements influencing agricultural value added include

agricultural employment, land use, and government support (Soyyiğit & Yavuzaslan, 2019). While the extent of agricultural land determines production capacity, employment levels influence labor productivity within the sector. Government expenditures, on the other hand, affect agricultural productivity through infrastructure investments, subsidies, and direct support payments (Aydoğan & Vardar, 2020). However, the impact of public support may vary depending on its implementation, and in some cases, it may foster dependency rather than increase efficiency (Adedoyin et al., 2021).

This study makes a significant contribution to the literature by being one of the first comprehensive panel data analyses on the economic determinants of agricultural value added in E7 countries. While previous research has often focused on individual countries or specific variables, this study adopts a broader approach by jointly analyzing government expenditures, employment, and land use. Furthermore, using the Panel ARDL model, it assesses the long-term relationships between these variables and provides policy recommendations to enhance sustainable agricultural development.

The structure of the study is as follows: The second section presents the theoretical framework on the determinants of agricultural value added. The third section provides a literature review, summarizing relevant studies. The fourth section details the data set, methodology, empirical findings, and discussion. The fifth section concludes with an overview of the study's findings and presents policy recommendations.

The agricultural sector holds critical importance not only for food production and rural development but also for environmental sustainability and energy security. Agricultural activities contribute to the environment by acting as carbon sinks and preserving biodiversity, while simultaneously playing a fundamental role in the development of renewable energy sources (Adedoyin et al., 2021). These characteristics position the agricultural sector as a central actor not only in economic but also in social and environmental policies. Thus, the agricultural sector occupies a strategic position that integrates environmental sustainability, energy policies, and economic development goals.

In E7 countries, the agricultural sector stands out as one of the cornerstones of their economies. In countries such as Brazil and India, the agricultural sector constitutes a significant portion of export revenues, whereas in nations like China and Indonesia, agricultural modernization projects aim to enhance productivity (Aydoğan & Vardar, 2020). For example, Brazil, as a leading global agricultural exporter, plays a pivotal role in food production, while India focuses on balancing workforce dependency in agriculture with local consumption policies. In these countries, the agricultural sector not only meets domestic consumption demand but also provides strategic advantages in international trade.

Labor utilization, as one of the fundamental components of the agricultural sector, is crucial for ensuring social stability in rural areas. Moreover, agricultural productivity is directly related to factors such as technological investments and government support (Kaya, 2020). For instance, investments in irrigation systems, modernization of agricultural machinery, and infrastructure improvements enhance production capacity while also positively contributing to rural economies (Doğdu, 2022). These factors establish a solid foundation not only for economic growth but also for social development in rural regions.

Consequently, the components of the agricultural sector extend beyond economic contributions to directly serve social and environmental development goals. In this context, core elements such as the employment rate in agriculture, the efficiency of agricultural land, and public expenditures provide a critical basis for analyzing the dynamics of the agricultural sector in E7 countries. These analyses will facilitate a deeper understanding of the economic, social, and environmental impacts of the agricultural sector

Figure 1 illustrates the changes in the share of agricultural value added in GDP across E7 countries between 2001 and 2022. India stands out as the country with the highest agricultural value added during this period, starting at approximately 21% in 2001.

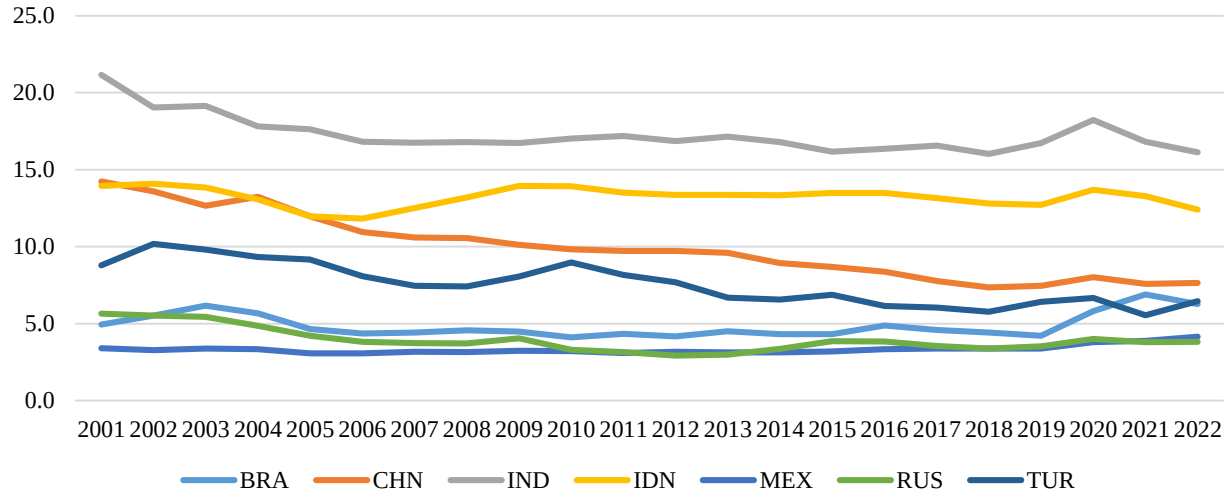


Figure 1. Agricultural Value Added (as % of GDP)
Source: https://databank.worldbank.org/source/world-development-indicators Adapted by the author from

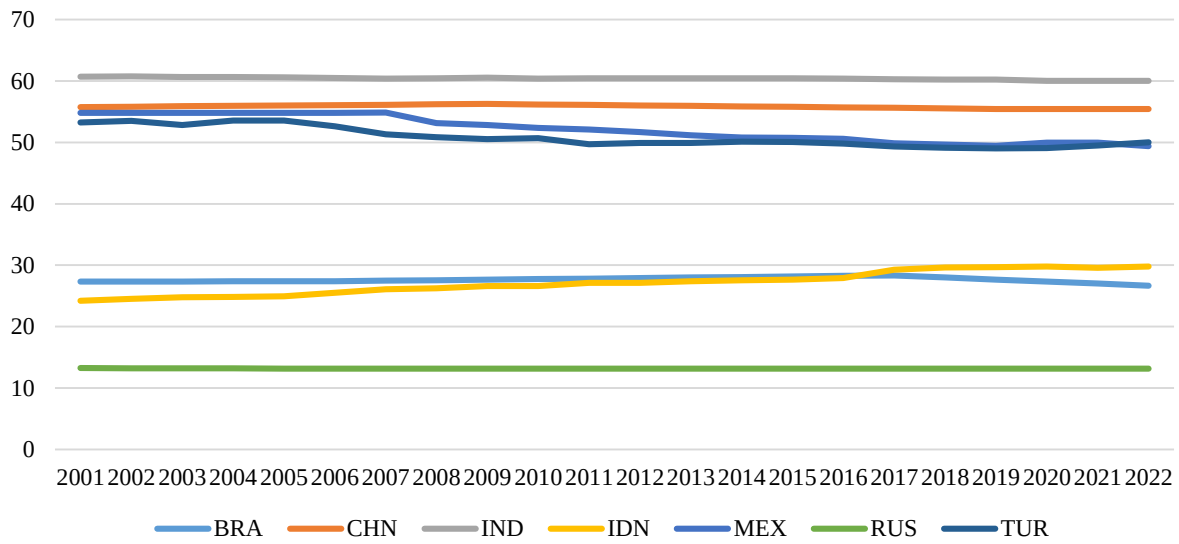


Figure 2. Agricultural Land Proportion (as % of Total Land)
Source: https://databank.worldbank.org/source/world-development-indicators Adapted by the author from

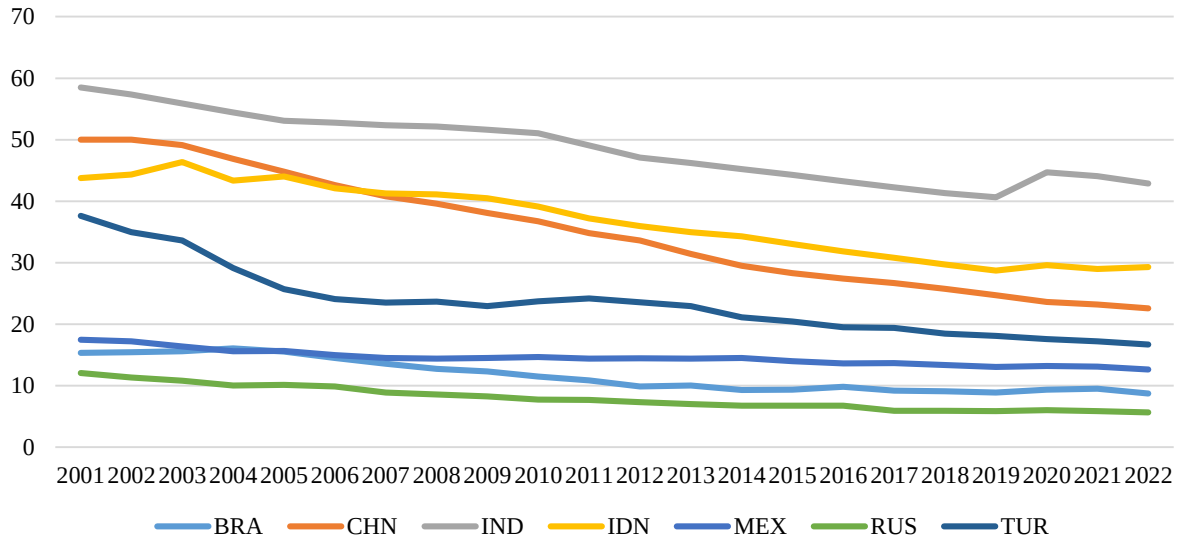


Figure 3. Agricultural Employment (as % of Total Employment)
Source: https://databank.worldbank.org/source/world-development-indicators Adapted by the author from

Although it has shown a gradual decline over the years, it still remains at a significantly higher level compared to other countries in 2022. Indonesia has maintained a stable trajectory, with agriculture's contribution to GDP fluctuating within the 12-14% range. In contrast, China has exhibited a consistent downward trend in agricultural value added since 2001, though occasional fluctuations have slowed this decline. In Turkey and Brazil, the share of agriculture in GDP declined between 2001 and 2010, followed by fluctuations and a relative increase after 2019. Mexico and Russia have consistently recorded the lowest shares of agricultural value added throughout the analyzed period, with figures remaining below 5%. Overall, Figure 1 highlights the evolving economic significance of the agricultural sector in E7 countries while also revealing differing trends across nations.

Figure 2 illustrates the changes in the proportion of agricultural land as a percentage of total land across E7 countries between 2001 and 2022. India has the highest share of agricultural land, consistently remaining above 60%. Similarly, China also maintains a high proportion, with minimal fluctuations over the years. In Turkey and Brazil, the share of agricultural land has gradually declined since 2001 but has stabilized towards the end of the period. In contrast, Indonesia has experienced a gradual increase in the proportion of agricultural land from 2001 to 2022, approaching Brazil's level after 2017. Russia has the lowest agricultural land proportion among the E7 countries, maintaining a stable level slightly above 10% throughout the period. Mexico falls into the mid-range, showing relatively little variation over time. Figure 2 highlights the trends in agricultural land usage across E7 countries, indicating a decline in some nations while others continue to preserve their agricultural land.

Figure 3 illustrates the changes in the share of agricultural employment as a percentage of total employment in E7 countries between 2001 and 2022. India stands out as the country with the highest agricultural employment rate, which was approximately 60% in 2001 but has steadily declined over the years.

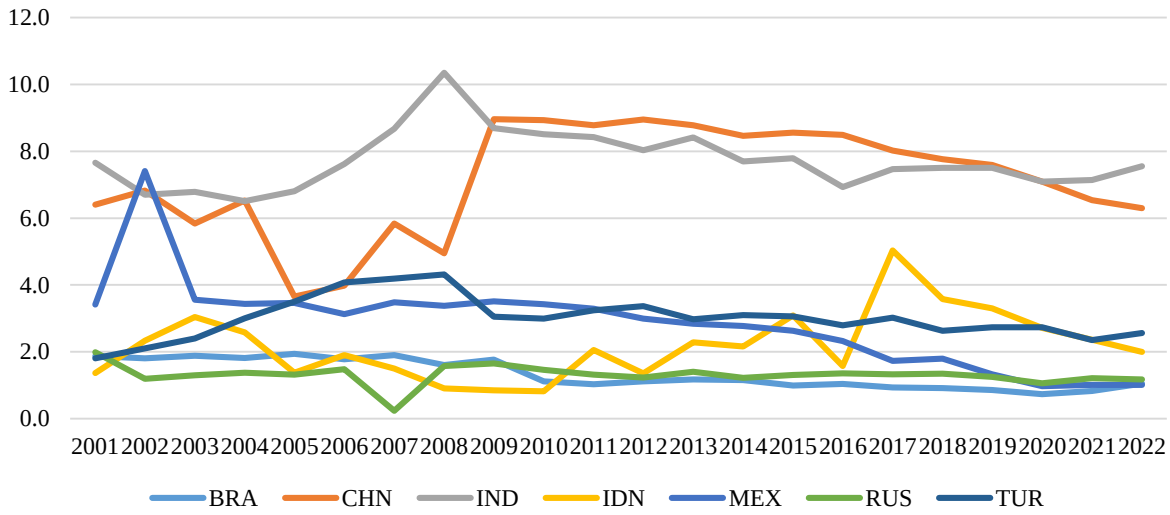


Figure 4. Share of Government Expenditures on Agriculture
Source: https://www.fao.org/faostat/en/#dataAdapted by the author from

A significant decrease in agricultural employment is also observed in China and Indonesia, with China experiencing a more rapid decline. In Brazil and Turkey, the share of agricultural employment has also decreased considerably, albeit at a slower pace compared to other countries. In Mexico and Russia, agricultural employment started at relatively lower levels and exhibited a gradual downward trend throughout the period. Figure 3 highlights the overall decline in the share of agricultural employment across E7 countries, indicating a shrinking role of agriculture in the labor market. However, variations among countries suggest that the rate and extent of this decline differ across economies.

Figure 4 illustrates the changes in the share of government expenditures on agriculture in E7 countries between 2001 and 2022. India and China stand out as the countries with the highest public spending on agriculture throughout the period. In India, a sharp increase was observed between 2006 and 2012, followed by a gradual decline after 2013, although expenditures remained at relatively high levels. In China, the share of government expenditures on agriculture increased after 2005, peaked between 2010 and 2014, and then entered a declining trend. In Turkey and Brazil, public spending on agriculture remained relatively stable, with Brazil experiencing fluctuations in the early years before stabilizing at lower levels. Indonesia saw a notable increase in government expenditures on agriculture between 2015 and 2017, followed by a fluctuating pattern in subsequent years. Mexico and Russia recorded the lowest shares of agricultural public spending throughout the analyzed period. Figure 4 highlights the variability of government support for agriculture in E7 countries, with some nations increasing agricultural subsidies during specific periods.

## Literature Review

The agricultural sector plays an important role in economic growth, environmental sustainability, and social development. However, analyses of the agricultural sector in E7 countries (Brazil, Russia, India, China, Indonesia, Mexico, and Turkey) are quite limited. While these countries draw attention with their rising roles in the global economy and the contributions of the agricultural sector to economic performance, the lack of sufficient academic literature on the subject creates a gap. Understanding the economic and social impacts of the agricultural sector in E7 countries can contribute to achieving sustainable development goals in these countries. In this context, the present study aims to shed light on policies for economic growth and development by focusing on the agricultural sector in E7 countries. In addition to the agricultural sector, this study also addresses non-agricultural issues in these countries and agricultural studies in other countries. These analyses are crucial in filling a significant gap in the literature and emphasizing the necessity of new studies in the context of E7 countries.

In studies focusing on the agricultural sector in E7 countries, Tıraşoğlu and Karasaç (2018) analyzed the middle-income trap between 1960 and 2016 and found that macroeconomic stability was effective in overcoming this trap. The study particularly stated that Indonesia, Mexico, and Russia were caught in the middle-income trap. Soyyiğit and Yavuzaslan (2019) examined the relationships between agricultural value added, economic complexity, political stability, and government effectiveness, emphasizing the importance of government effectiveness and political stability in increasing agricultural value added. This study is based on data from the 1996-2017 period. Aydoğan and Vardar (2020) analyzed the relationships between renewable energy consumption, economic growth, agricultural value added, and CO2 emissions, highlighting the importance of the agricultural sector in environmental sustainability. The findings underscore the critical role of agriculture in this context. Ağır et al. (2020) explored the relationships between financial development and income inequality and analyzed the positive effects of agricultural value added on social balance using data from 1988-2016. Özşahin and Güven (2023) assessed the impacts of agricultural subsidies and government stability on agricultural value added. Their findings indicate positive effects of agricultural employment and raw material imports.

In studies focusing on non-agricultural issues in E7 countries, Bozgeyik (2020) examined unemployment hysteresis and stated that unemployment rates in E7 countries generally tend to revert to the mean. This study analyzed unemployment rate data for the 1991-2018 period. Topçuoğlu and Ayyıldız (2020) identified key sectors supporting economic growth and development, highlighting the importance of agriculture and industry for development. This study is based on 2014 data. Han (2022) examined the relationships between renewable energy consumption and economic growth, finding that energy consumption has a significant impact on economic growth. This study used data from 1990-2018. Tekin and Merdivenci (2022) analyzed trade volumes between Turkey and E7 countries, revealing that economic growth and trade are strongly connected. This study focuses on the 2000-2018 period. Gyamfi et al., (2023) analyzed the environmental impacts of economic globalization and emphasized the importance of sustainable environmental policies. This study used data from 1990-2019. Additionally, Doğdu (2022) investigated the causality between renewable energy production and economic growth in G7 and E7 countries, finding that renewable energy investments promote economic growth.

In studies on other countries, Akyol (2018) analyzed the effects of agricultural incentives on agricultural value added in Turkey, South Africa, Mexico, China, and Brazil. The study demonstrated that these incentives positively contribute to economic growth, using data from 2000-2016. Kaya (2020) examined agricultural value added convergence between Turkey, China, the United States, India, Brazil, and Indonesia, emphasizing the importance of technological advancement. The study concluded that Turkey showed convergence with China and the United States but not with Brazil, India, and Indonesia. This study covers the 1960-2018 period. Erdinç and Aydınbaş (2021) examined the determinants of agricultural value added in 20 different countries, evaluating the impacts of economic, social, and legal regulations on the agricultural sector. This study uses data from the 2000-2018 period. Additionally, Adedoyin et al. (2021) analyzed the effects of agricultural development, energy consumption, and economic growth on CO2 emissions in E7 countries. The findings revealed that renewable energy consumption reduces emissions.

Benin et al. (2007) found that agricultural diversity in Ethiopia is shaped by land size, labor, and market access. Oyetade et al. (2014) highlighted the positive impact of fisheries and food production on economic growth in Nigeria. Kakar et al. (2016) found that agricultural land use, fertilizer, and credit utilization improve productivity in Pakistan, while agricultural employment and pesticide use have no significant long-term effects. Onoja et al. (2017) indicated that agricultural growth in Nigeria and Kenya is affected by macroeconomic factors such as capital investments, infrastructure spending, and exchange rates. Muraya et al. (2017) observed that while exchange rate fluctuations and inflation constrain agricultural production in Kenya, infrastructure investments enhance productivity.

Teshome et al. (2018) suggested that Ethiopia's agricultural GDP can be increased through agricultural land expansion and financial support, whereas Mutunga et al. (2018) pointed out the need to reassess agricultural subsidies in Kenya. Mocanu et al. (2018) demonstrated that infrastructure development and incentives for young labor could support agricultural growth in Romania. Overall, market access, financial support, and macroeconomic stability emerge as key determinants of agricultural productivity and large-scale agricultural economies. Czyżewski et al. (2018) stated that agricultural incomes in the EU are related to production scale, subsidies, and economic indicators, while the impact of labor remains limited in new EU member states. Coca et al. (2023) emphasized the critical role of agricultural capital investments in enhancing productivity. Abdi and Mohamed (2025) revealed that exchange rates, foreign investments, and institutional quality influence agricultural exports in Somalia.

The literature on the agricultural sector in E7 countries highlights its significance in economic growth, environmental sustainability, and social development. However, despite the increasing global economic influence of these countries, research on the economic determinants of agricultural value added remains limited. Existing studies have examined various factors, including government effectiveness, political stability, economic complexity, and environmental sustainability, in relation to agricultural sector performance. Additionally, studies focusing on agricultural subsidies, employment, and trade suggest that macroeconomic stability and sector-specific policies play crucial roles in enhancing agricultural productivity. While some research extends beyond E7 countries to assess global agricultural trends, there is a noticeable gap in comparative analyses specific to E7 economies. This study aims to address this gap by providing a comprehensive evaluation of the determinants of agricultural value added in these nations, contributing to a more nuanced understanding of sectoral dynamics and policy implications.

## Materials and Methods

This study aims to understand the determinants of agricultural value added in E7 countries for the period between 2001 and 2022. The selected period is significant as it encompasses transformations in agricultural policies and economic turbulence. In this study, the key determinants of the agricultural value-added rate are considered as the agricultural employment rate, the proportion of agricultural land, and the share of government expenditures allocated to the agricultural sector. The E7 countries (Brazil, China, India, Indonesia, Mexico, Russia, and Turkey) hold a significant position in the global economy due to their large populations and substantial economic potential. The agricultural sector plays a critical role in these countries' economic structures, providing a fundamental contribution to economic development and rural prosperity. Therefore, understanding the determinants of agricultural value added is crucial not only for the agricultural development of these countries but also for achieving global goals such as food security and economic sustainability. However, the number of studies in the literature on the comparative analysis of the fundamental economic factors influencing the agricultural value-added rates in these countries is quite limited.

The econometric analysis of the study initially examines the stationarity of the series in the panel data set using the Pesaran unit root test. Subsequently, the panel ARDL method is employed to test for long-term relationships. The model selection is determined using the Hausman test, and error correction models are applied to analyze the speed of adjustment to equilibrium. The findings provide a striking perspective on the agricultural policies of E7 countries and the differences in their economic structures.

The model to be used in this study is as follows:

$$AVA_{it} = \beta_1 AL_{it} + \beta_2 AGE_{it} + \beta_3 EA_{it} + \varepsilon_{it}$$

Where, $\beta_1, \beta_2$ and $\beta_3$ are the coefficients, $\varepsilon$ is the error term, i represents the countries and t represents time.

***Stationarity Testing***

In panel data analysis, as in time series analysis, the stationarity of variables plays a critical role. If the stationarity of variables is not checked, spurious regression problems may emerge due to incorrect model selection (Karadaş, 2021). Panel unit root tests are divided into two main groups: first-generation tests assume independence between cross-sectional units, while second-generation tests allow for dependence between these units. If an inappropriate test is chosen, incorrect conclusions regarding the stationarity of the series may be reached, leading to erroneous analyses. Initially, cross-sectional dependence tests should be conducted, and the appropriate model should be determined accordingly. Common tests for examining cross-sectional dependence include the Breusch-Pagan LM test, Pesaran scaled LM test, bias-corrected scaled LM test, and Pesaran CD test. These tests should be chosen based on the characteristics of the data set (Arslan & Karadaş, 2021).

The choice of test depends on the dimensions of the panel data set, specifically the size of the cross-section (N) and the time dimension (T). For instance, the Breusch-Pagan LM test provides meaningful results when T>N, while the Pesaran scaled LM test is preferred when both T and N are large. The bias-corrected scaled LM test and Pesaran CD test are generally used in cases where N>T. In all these tests, the null hypothesis posits that there is no cross-sectional dependence between units. Significant results indicate the presence of cross-sectional dependence in the panel data set (Pesaran, 2004).

In the panel data set used in this study, the time dimension (T=22) is greater than the cross-section dimension (N=7). Therefore, the Breusch-Pagan LM test was applied to examine cross-sectional dependence, and the results are presented in the relevant table.

The Breusch-Pagan LM test results clearly demonstrate the presence of cross-sectional dependence for the variables of agricultural value added, agricultural land ratio, government spending on the agricultural sector, and agricultural employment rate. For all variables, the significance level (p-value) of the test statistics is below 0.05, indicating that the null hypothesis of "no cross-sectional dependence among units" should be rejected. Given that all variables exhibit cross-sectional dependence, a second-generation unit root test will be applied. The results of the Pesaran panel unit root test (CIPS) are presented in Table 3.

The results of the panel unit root tests indicate varying stationarity levels for the variables: agricultural value added, agricultural employment rate, agricultural land ratio, and government spending on the agricultural sector. The AVA, EA and AL variables were found to be non-stationary but became their first differences are stationary. This suggests that these there variables are integrated of order one, I(1). The AGE variable, on the other hand, is the only variable found to be stationary at level ($p<0.05$).

Table 1. Variables

| Variable | Definition | Source |
|---|---|---|
| AVA | Agricultural value added as a share of GDP | World Bank Databank |
| AL | Agricultural land (% of land area) | World Bank Databank |
| AGE | Share of government expenditures on agriculture | FAO Statistics |
| EA | Employment in agriculture (% of total employment) (ILO modeled estimate) | World Bank Databank |

Table 2. Breusch-Pagan LM Cross-sectional Dependence Test

| Variable | Statistic | Probability |
|---|---|---|
| AVA | 111.4977* | 0.0000 |
| AL | 182.9394* | 0.0000 |
| AGE | 45.31639* | 0.0016 |
| EA | 405.1928* | 0.0000 |

Note: * indicates significance at the 1% level.

Table 3. CIPS unit root test results

| Variable | Specification without trend | | Specification with trend | |
|---|---|---|---|---|
| | Zt-bar | Probability | Zt-bar (First Differences) | Probability |
| AVA | -0.030 | 0.488 | -0.302 | 0.381 |
| d.AVA | -4.075* | 0.000 | -2.739* | 0.003 |
| EA | -0.166 | 0.434 | -0.445 | 0.328 |
| d.EA | -3.619* | 0.000 | -2.428* | 0.008 |
| AL | -0.541 | 0.294 | 0.865 | 0.807 |
| d.AL | -1.942** | 0.026 | -2.843* | 0.002 |
| AGE | -2.303** | 0.011 | -3.777* | 0.000 |

Note: * and ** denote significance levels at 1% and 5%, respectively.

Table 4. Cross-Sectional Dependence in the Equation

| Test | Statistic | Prob. |
|---|---|---|
| Breusch-Pagan LM | 20.16060 | 0.5111 |

Table 5. Hausman Test (MG vs. PMG)

| | (b) | (B) | (b-B) | sqrt(diag(V_b-V_B)) |
|---|---|---|---|---|
| | MG | PMG | Difference | S.E. |
| AL | 6.338517 | .5462269 | 5.79229 | 9.1934 |
| AGE | -0.5173357 | -0.09045 | -0.4268857 | 0.3424212 |
| EA | 0.2455498 | 0.1317203 | 0.1138295 | 0.2368086 |

chi-square = 1.74; Prob> chi-square = 0.6279

Table 6. Hausman Test (PMG and DFE)

| | (b) | (B) | (b-B) | sqrt(diag(V_b-V_B)) |
|---|---|---|---|---|
| | PMG | DFE | Difference | S.E. |
| AL | 0.5462269 | 0.3034526 | 0.2427743 | 1.316956 |
| AGE | -0.09045 | -0.369192 | 0.278742 | 1.963458 |
| EA | 0.1317203 | 0.1120318 | 0.0196886 | 0.2372065 |

chi-square = 0.08; Prob> chi-square = 0.9941

These findings provide critical guidance for determining the methods to be used in the panel data model. The presence of variables with different stationarity levels necessitates the use of models such as panel ARDL, which can accommodate both I(0) and I(1) variables. Furthermore, the significant results for all first-difference stationary variables suggest the potential existence of long-term relationships among the series.

***Long-Term Relationships***

In this study, the long-term relationships between variables are analyzed. The results of the panel unit root tests reveal that the variables exhibit different levels of stationarity. Therefore, the panel ARDL method has been selected to examine these long-term relationships. However, before proceeding with the panel ARDL analysis, it is critical to verify the model's suitability in terms of cross-sectional dependence. The presence of cross-sectional dependence can lead to misleading estimation results and must be considered. Accordingly, similar methods used for testing cross-sectional dependence among variables have also been applied to the equation. Given that the time dimension (T=22) exceeds the cross-sectional dimension (N=7) in the dataset, the Breusch-Pagan LM test was used to evaluate cross-sectional dependence. The test results are presented in table 4.

According to the test results, the Breusch-Pagan LM test statistic is over 0.05 which suggests that the null hypothesis of "no cross-sectional dependence" cannot be rejected, indicating no cross-sectional dependence among the variables in the equation. This outcome demonstrates independence among the cross-sectional units in the analysis. Consequently, standard panel ARDL models can be applied without the need to account for cross-sectional dependence explicitly.

In panel ARDL analysis, three estimators—Mean Group (MG), Pooled Mean Group (PMG), and Dynamic Fixed Effects (DFE)—are commonly utilized. The primary differences among these estimators lie in the assumptions regarding homogeneity or heterogeneity in short- and long-term relationships.

The MG estimator assumes heterogeneity in both short- and long-term relationships across cross-sections, making it suitable when relationships between variables differ across countries. On the other hand, the PMG estimator assumes heterogeneity in short-term coefficients but homogeneity in long-term coefficients (Pesaran et al., 1999; Asteriou et al., 2020; Lee and Wang, 2015). This estimator is particularly advantageous when long-term relationships are expected to be similar across countries (Sohag et al., 2015).

The DFE estimator, however, assumes that intercepts may vary across cross-sections while the short- and long-term coefficients remain constant for all cross-sections. In this method, slopes are assumed to be the same for all units (Sohag et al., 2015; Lee and Wang, 2015). In summary, with the MG method, the short- and long-term coefficients are different across panel cross-sections, while with the PMG method, only short-term coefficients vary, and long-term coefficients are constant. The DFE method assumes both short- and long-term coefficients are constant (Pesaran et al., 1999).

The choice among these estimators depends on examining the homogeneity of the series or using the Hausman test. The Hausman test evaluates the performance of different estimators by testing the validity of the null hypothesis (H0) and the alternative hypothesis (Ha). In this context, the first estimator (b) is considered consistent under both H0 and Ha, while the second estimator (B) is efficient under H0 but inconsistent under Ha (Hausman, 1978). The results of the Hausman tests applied in this study are presented in table 5 and 6.

The results of the Hausman test presented in Table 6 compare the performance of the MG and PMG estimators. According to the test results, the chi-square value is 1.74, with a probability of 0.6279. The results of the Hausman test comparing the MG and PMG estimators show that the null hypothesis is not rejected. Consequently, the second estimator, PMG, is found to be more consistent than the first, MG.

Table 7. Panel ARDL Results According to the DFE Estimator

| | Coefficient | Standard Error | z | P>\|z\| | [95% Confidence Interval] | |
|---|---|---|---|---|---|---|
| ECT | -0.3276842* | 0.057372 | -5.71 | 0.000 | -0.4401313 | -0.2152371 |
| Long-term Equation | | | | | | |
| AL | 0.3034526* | 0.1154253 | 2.63 | 0.009 | 0.0772232 | 0.5296819 |
| AGE | -0.369192** | 0.1541683 | -2.39 | 0.017 | -0.6713564 | -0.0670276 |
| EA | 0.1120318* | 0.0269273 | 4.16 | 0.000 | 0.0592552 | 0.1648083 |

Note: * and ** denote statistical significance at the 1% and 5% levels, respectively.

The results of the Hausman test presented in Table 6 compare the performance of the PMG and DFE estimators. According to the test results, the chi-square value is 0.08, with a probability of 0.9941. These findings indicate that the null hypothesis cannot be rejected. Therefore, the results of the Hausman test comparing the PMG and DFE estimators show that the null hypothesis is not rejected. Consequently, the second estimator, DFE, is found to be more consistent than the first, PMG.

Based on the comparison of the three estimators, the DFE estimator is concluded to be more consistent than the other two estimators. The preference for the DFE estimator implies that policy recommendations for the agricultural sector may be consistent and generally applicable across countries. This result provides a clearer framework regarding the overall dynamics of the agricultural sector in E7 countries. The panel ARDL results obtained using the DFE estimator are presented in the table 7.

The panel ARDL analysis results presented in Table 7 detail the long-term relationships among variables. First, the error correction term (ECT) is calculated as -0.3276842 and found to be statistically significant at the 1% level. This result indicates that the model can correct short-term shocks in the long run and restore approximately 32.76% of the system to equilibrium within a given period. The significance and negative value of the error correction mechanism validate the model's ability to accurately represent long-term relationships and confirm that short-term imbalances dissipate over time.

Looking at the long-term coefficients, the coefficient for the agricultural land ratio (AL) is 0.3034526, which is statistically significant at the 1% level. This finding suggests that a 1% increase in the agricultural land ratio positively impacts agricultural value-added by 0.30%. This result underscores the importance of policies aimed at improving the efficient use of agricultural land. Expanding agricultural land or enhancing its productivity emerges as a key strategy for economic growth.

The coefficient for government expenditures on agriculture (AGE) is calculated as -0.369192 and is statistically significant at the 5% level. The negative coefficient indicates that government spending on the agricultural sector has not yielded the expected benefits. This may be attributed to inefficient use of public funds, allocation of expenditures to short-term support rather than infrastructure, or prioritization of other sectors. Additionally, it suggests that government policies in the sector may temporarily address structural issues rather than provide sustainable solutions.

The agricultural employment ratio (EA) variable shows a positive relationship with a coefficient of 0.1120318, significant at the 1% level. This result highlights the positive impact of agricultural employment on agricultural value-added and underscores the importance of rural development policies. Increasing employment in the agricultural sector contributes to economic activity in rural areas and supports the sustainable continuation of agricultural production. The role of human labor in the agricultural sector is critical for both social welfare and economic growth.

Overall, the findings from Table 7 reveal that the agricultural land ratio and agricultural employment positively contribute to agricultural value-added, while government expenditures negatively impact it. This highlights the need to reevaluate policies targeting the agricultural sector and emphasizes the importance of adopting efficiency-focused approaches. The findings also underline the necessity for implementing sustainable agricultural policies and allocating resources more effectively.

Based on the analysis results, this study has identified the distinct effects of various factors on agricultural value-added in E7 countries. The findings demonstrate that agricultural employment and the share of agricultural land have a significant and positive influence on agricultural value-added. Conversely, the impact of government expenditures is more complex and varies across countries. While some studies in the literature emphasize the positive effects of government support, this study finds that its influence is contingent upon the type of support mechanisms in place and their implementation effectiveness.

E7 countries exhibit diverse agricultural policies, reflecting their economic structures and policy priorities. Countries with large-scale agricultural economies, such as China and Brazil, allocate substantial investments in infrastructure and direct subsidies to enhance productivity. In contrast, Turkey, Indonesia, and Mexico focus on supporting small-scale farmers through financial aid, cooperative development, and market access initiatives. Meanwhile, Russia and India periodically adjust their agricultural policies, reflecting shifting government intervention strategies. These variations indicate that a uniform agricultural policy approach is not applicable across all E7 countries.

The findings highlight the significant contribution of agricultural employment to agricultural value-added. However, sustaining this impact requires improving the quality of agricultural employment. The adoption of modern production techniques and advancements in agricultural technology can enhance labor efficiency and ensure the sector's long-term viability. Additionally, while the availability of agricultural land remains a crucial determinant of production capacity, its efficient and sustainable utilization is equally important for maximizing productivity and ensuring food security.

The findings of this study largely align with existing literature on E7 countries, while diverging in certain aspects. For instance, the positive effect of the agricultural land ratio on agricultural value-added is consistent with the findings of Tıraşoğlu and Karasaç (2018) and Soyyiğit and Yavuzaslan (2019). These studies emphasize the importance of efficient land use in boosting agricultural production and contributing to economic growth. Similarly, this study confirms that agricultural land positively contributes to agricultural value-added.

The positive impact of agricultural employment aligns with the studies of Ağır et al. (2020) and Kaya (2020). Ağır et al. (2020) highlight the positive social equilibrium effects of agriculture, while Kaya (2020) underscores the role of agricultural employment in supporting economic growth. This study also finds that agricultural employment increases agricultural value-added and further suggests that modernization and technological support can amplify this effect.

However, the negative impact of government expenditures contrasts with much of the existing literature. Notably, studies by Soyyiğit and Yavuzaslan (2019) and Özşahin and Güven (2023) emphasize the positive contributions of government support to agricultural value-added. The negative findings in this study could be attributed to shortcomings in the planning and implementation of support mechanisms, insufficient monitoring systems, or the misallocation of resources for purposes other than intended. These issues suggest that operational problems may hinder agricultural support from achieving its expected impact.

In conclusion, this study is largely consistent with the existing literature but highlights significant practical challenges concerning the effectiveness of government spending. These findings suggest that agricultural support policies in E7 countries need to be revisited and improved to ensure that these mechanisms effectively contribute to agricultural productivity and sustainability.

## Conclusion and Policy Recommendations

This study analyzed the determinants of agricultural value-added in E7 countries, evaluating the impacts of agricultural employment, agricultural land, and government expenditures on the sector. The findings indicate that agricultural employment and agricultural land have a significant and positive effect on agricultural value-added. However, the impact of government expenditures varies across countries and, in some cases, fails to provide the expected contribution. This highlights the importance of implementation differences in the effectiveness of agricultural support policies.

The research reveals that agricultural policies in E7 countries are not homogeneous and that there are significant differences in agricultural support mechanisms across countries. While agricultural employment is a critical component for sustaining agricultural production, the quality of labor and access to modern agricultural techniques are also determining factors for its sustainability. The extent of agricultural land is a key determinant of production capacity; however, its efficient use and sustainability policies should also be taken into account. The impact of government expenditures varies depending on the form of support and monitoring mechanisms, indicating the need for a review of agricultural policies.

Based on the findings, country-specific policy recommendations for E7 countries are proposed:

- China and Brazil: Increasing investments in agricultural infrastructure and strengthening logistical support can enhance production efficiency. Additionally, expanding incentives for sustainable agricultural practices will contribute to long-term growth.
- Turkey, Indonesia and Mexico: Supporting small-scale farmers and promoting cooperatives will enhance the sustainability of agricultural production. Facilitating farmers' access to financial instruments and expanding agricultural insurance can strengthen risk management.
- Russia, India: Strengthening monitoring and evaluation mechanisms is essential to assess the effectiveness of agricultural support. Additionally, promoting digitization, smart farming applications, and improved water management systems can enhance productivity and resilience against climate variability.

In addition, several fundamental strategies are suggested to enhance the efficiency of general agricultural policies in E7 countries. Encouraging the conservation and sustainable use of agricultural land will ensure the efficient utilization of natural resources. Instead of relying solely on financial incentives, support programs should incorporate long-term, high-impact policies such as technical assistance, education, and innovative solutions. Moreover, increasing agricultural knowledge-sharing and regional cooperation among E7 countries can help disseminate innovative solutions within the agricultural sector.

In conclusion, this study provides a detailed analysis of the factors affecting agricultural value-added in E7 countries and presents policy recommendations based on the findings. To sustain agricultural growth, policymakers must develop long-term and targeted strategies that address the specific needs of each country.

## Declarations

### *Ethical Approval Certificate*

This study does not require approval from an ethics committee.

### *Author Contribution Statement*

Please indicate how each author contributed to this work and at what stage. For example:

Author 1: Data collection (100%), investigation (100%), formal analysis(100%), and writing the original draft (100%), Project administration(100%), supervision(100%), conceptualization (100%), methodology(100%), review and editing (100%),

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### *Conflict of Interest*

"The authors declare no conflict of interest."

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# Morphological, Thermal and Functional Properties of Horse Chestnut Starches Produced by Alkaline and Ultrasound Assisted Methods

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ABSTRACT

Global climate change and a growing population are driving research into alternative starch sources. The aim of this study was to produce starch from horse chestnut seeds by alkali and ultrasound assisted methods and to determine the morphological, thermal and functional properties of the produced starches. The ultrasound-assisted method increased the swelling power and water/oil holding values of the starches and decreased the syneresis values. The samples showed gel formation at a minimum starch content of 6% and 12%. The amylose contents of the starches produced by the alkaline and ultrasonic methods were 25.41% and 29.86%, respectively. The highest temperatures of thermal degradation of the starches were determined in the range 221-343°C. The ultrasonic method increased the $\lambda_{max}$ value of starch from 523,0 nm to 583 nm and decreased the specific rotation angle from 59.90 to 21.75. Thermogravimetric data showed that 50% of the mass was lost in the range 302-312°C for the alkaline method and 303-304°C for the ultrasonic assisted method. Ultrasonication caused a partial change in the structure of starches with orthorhombic crystal structure. This study comprehensively investigates alternative starch sources. The starches produced have the potential to ortorombik be used in the production of gluten-free products and in products where a gel structure is desired.

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 13(3): 611-619, 2025

# Alkali ve Ultrasonik Destekli Yöntemlerle Üretilen At Kestanesi Nişastalarının Morfolojik, Termal ve Fonksiyonel Özellikleri

MAKALE BİLGİSİ

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*Anahtar Kelimeler:*
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At kestanesi
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Nişasta
Ekstraksiyon

ÖZ

Küresel iklim değişiklikleri ve artan nüfus alternatif nişasta kaynaklarına yönelik araştırmaları artırmaktadır. Bu çalışmanın amacı, at kestanesi tohumlarından alkali ve ultrason destekli yöntemlerle nişasta üretimi gerçekleştirmek ve üretilen nişastaların morfolojik, termal ve fonksiyonel özelliklerini belirlemektir. Ultrason destekli yöntem nişastaların şişme gücü ve su/yağ tutma kapasite değerlerini yükseltmiş, sineresis değerlerini ise düşürmüştür. Örnekler minimum %6 ve %12 nişasta oranlarına sahipken jel oluşumu göstermiştir. Alkali ve ultrasonik yöntemlerle üretilen nişastaların amiloz içerikleri sırasıyla %25,41 ve %29,86'dır. Nişastaların termal bozunmasının en yüksek olduğu sıcaklıklar 221-343°C aralığında tespit edilmiştir. Ultrasonik yöntem nişastanın $\lambda_{max}$ değerini 523,0 nm'den 583 nm'ye yükseltmiş ve spesifik dönme açısını 59,90'dan 21,75'e düşürmüştür. Termogravimetrik veriler kütlenin %50'sinin alkali yöntem için 302-312°C ve ultrason destekli yöntem için 303-304°C aralığında kaybolduğunu göstermiştir. Ultrasonikasyon, ortorombik kristal yapıya sahip nişastaların yapısında kısmi bir değişikliğe neden olmuştur. Çalışmamız alternatif nişasta kaynakları ile ilgili kapsamlı bir çalışmadır. Üretilen nişastaların başta glutensiz ürün üretiminde ve jel yapısı istenen ürünlerde kullanılma potansiyeli bulunmaktadır.

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## Giriş

*Aesculus hippocastanum* L., yaprak ve çiçeklerinin dekoratif özellikleri ile 16. yüzyılın sonlarından günümüze kadar park, bahçe ve yol kenarlarında süs ağacı olarak yaygın bir şekilde kullanılmaktadır. Aynı zamanda tohum kabukları ve çiçekleri geleneksel ve tıbbi tedavilerde kullanılan değerli bir bitkidir (Dudek-Makuch & Matławska, 2011). Önemli bir nişasta (%30-40) ve yağ (%5,5-7,7) kaynağı olan at kestanesinin ana biyoaktif bileşenleri, triterpen saponinler (escin), flavonoidler, kumarinler ve proantosiyanidinler dahil olmak üzere 190 farklı bileşen olarak tanımlanmıştır (Amiri ve ark., 2019; Owczarek-Januszkiewicz ve ark., 2023).

Nişasta, α-1,4 bağları ile bağlanmış α-D-glukoz birimlerinden oluşan düz amiloz zincirleri ve α-1,6 bağları ile bağlanmış dallanmış amilopektin zincirlerinden oluşan, bitki dokularında yarı kristal granüller halinde bulunan kompleks bir karbonhidrattır (Lemos ve ark., 2015). Nişasta, tahılların, baklagillerin, yumruların ve köklerin ana bileşenidir (Lu ve ark., 2012). Gıda maddelerinde jelatinleştirici, dolgu maddesi, stabilizatör, sorbent polimer ve yapıştırıcı olarak kullanılmaktadır (Vishal Banyal ve ark., 2023). Yaygın olarak buğday, patates, manyok, pirinç ve mısır gibi endüstriyel ürünlerden üretilen nişastalar farklı yapısal ve teknolojik kalite özelliklerine sahiptir. Bitki türü, hasat zamanı ve çevresel/iklimsel faktörlere bağlı olarak nişastaların özelliklerinde değişimler meydana geldiği bildirilmektedir (Vishal Banyal ve ark., 2023). Nişasta üretiminde genellikle geleneksel yöntemler kullanılmaktadır. Zaman ve kimyasal tüketimi açısından dezavantajları olan geleneksel ekstraksiyon uygulamalarının popüleritesi her geçen gün azalmaktadır. Geleneksel optimizasyon yöntemleri her seferinde bir değişkeni değiştirmektedir. Fakat bu durum analiz süresini ve reaktif tüketimini arttırmaktadır. Ayrıca ekstraksiyon parametrelerinin bir arada gösterebileceği potansiyel sinerjik etkisinin tespiti zorlaşmaktadır. Ultrason destekli ekstraksiyon, bileşiklerin yapısal ve moleküler özelliklerini korurken, bitkilerden hedeflenen bir maddenin ekstrakte edilmesi için kullanılan modern yöntemlerden bir tanesidir. Bu yöntem, solvent ve enerji tüketimini azaltmakta, ekstraksiyon süresini kısaltmakta ve prosesi basitleştirmektedir (Amiri ve ark., 2019).

Yapılan literatür taramalarında at kestanesinden antioksidan, glikoz ve lignin gibi katma değeri yüksek ürünlerin üretilmesine yönelik çalışmalar tespit edilmiştir (Çiçek Özkan & Güner, 2022; Gullón ve ark., 2020). Ayrıca literatürde alkali yöntem kullanılarak kestane ve at kestanesinden nişasta üretimi ve ultrasonik destekli yöntemin nişasta partiküllerinin özellikleri üzerindeki etkisini araştıran çalışmalar bulunmaktadır (Castaño ve ark., 2014; Correia ve Beirão-da-Costa, 2012; Lemos & ark., 2015; Rafiq ve ark., 2016; Shah ve ark., 2016; Ahmad ve ark., 2020). Literatürde at kestanesi nişastasının fiziko-kimyasal özelliklerinin belirlendiği çalışmalar mevcuttur. Fakat fonksiyonel (teknolojik) özelliklerinin, aflatoksin içeriğinin ve morfolojik özelliklerinin kapsamlı olarak belirlendiği sınırlı sayıda çalışma tespit edilmiştir. Bu çalışmada at kestanesinden iki farklı yöntem kullanılarak nişasta ekstraksiyonu gerçekleştirilmiş ve elde edilen nişastaların fonksiyonel (teknolojik), morfolojik ve termal özellikleri belirlenmiştir. Ayrıca at kestanesinde ve nişastasında aflatoksin varlığı araştırılmıştır.

## Materyal ve Metot

### *Materyal*

Nişasta eldesi için at kestanesi (*Aesculum hippocastanum*) kullanılmıştır. At kestanesi İstanbul'daki (Türkiye) park ve bahçelerden toplanmıştır. Toplanan tohumlar -18°C'de depolanmıştır.

### *Nişasta Ekstraksiyonu*

At kestanesi tohumları kabuğundan ayrılmış ve 3-4 mm kalınlığında parçalar halinde doğranmıştır. Dilimlenen parçalar %0,5 potasyum metabisülfit ve %1 sitrik asit çözeltisinde 30 dakika bekletildikten sonra 70°C'de 12 saat kurutulmuştur (Singh ve ark., 2008). Alkali ve ultrason destekli ekstraksiyon yöntemlerinde seçilen parametreler literatür taramaları ve ön denemeler sonucunda belirlenmiştir. Alkali yöntemle nişasta ekstraksiyonunda dilimlenmiş ve kurutulmuş 100 g at kestanesi 300 mL %1'lik NaOH çözeltisi içine ilave edilmiş ve burada +4°C'de 12 saat bekletilmiştir. Süre sonunda karışım 5 dakika boyunca yüksek hızlı bir öğütücüde parçalanmış, 60 mikronluk bir süzgeçten geçirilmiş ve süzgecin üstü 2-3 kez distile su ile yıkanmıştır. Elde edilen süzüntü 3000 rpm'de 15 dakika santrifüj (Boeco U-32R, Almanya) edilmiştir. Santrifüj işlemi dört kez tekrarlanmış ve her tekrardan sonra distile su ile yıkanmıştır. Son santrifüj işleminden sonra elde edilen süpernatant büyük bir cam kaba alınmış, 40-50°C'de 24 saat boyunca etüvde (Memmert 100-800, Almanya) kurutulmuş ve ardından öğütülerek kapalı kaplarda saklanmıştır (Rafiq ve ark., 2015). Ultrason destekli yöntem ile nişasta ekstraksiyonunda ise 100 g at kestanesi 300 mL %0,68'lik NaOH çözeltisi içine ilave edilmiş ve burada 37W ultrason gücünde 2 saat ultrasonik su banyosunda (Elma, E30H, Schmidbauer, Almanya) muamele edilmiştir. Süre sonunda alkali yöntemle aynı işlemler tekrarlanarak nişasta üretimi gerçekleştirilmiştir.

### *Fonksiyonel Özellikler*

Örneklerin su ve yağ tutma kapasitesi, şişme gücü ve çözünürlük analizleri Wani ve ark., (2014) tarafından açıklanan yönteme göre, yığın yoğunluğu analizleri Wani ve ark., (2013) tarafından açıklanan yönteme göre, sediment hacmi ve bulanıklık analizleri Rafiq ve ark., (2015) tarafından açıklanan yönteme göre yapılmıştır. Işık geçirgenliği ise Wani ve ark., (2013) tarafından tanımlanan yöntem kullanılarak yapılmıştır. Örneklerin jelleşme özellikleri Coffmann & Garcia (1977) tarafından açıklanan yönteme göre belirlenmiştir.

### *Donma-Çözünme Stabilitesi*

%6'lık nişasta çözeltisi 90°C'deki kaynar su banyosunda 30 dakika boyunca çalkalanarak bekletilmiştir. Elde edilen jel 16 saat boyunca 4°C'de depolanmış ve ardından -18°C'de dondurulmuştur. Bu sıcaklıkta 24 saat kaldıktan sonra, 25°C'de 6 saat çözdürülmüş ve ardından -18°C'de 24 saat boyunca tekrar dondurulmuştur. Bu döngü 5 kez tekrarlanmıştır. Her çözdürme işleminden sonra tüpler 10°C'de 20 dakika boyunca 1000×g'de santrifüj edilmiş ve ayrılan su miktarı tespit edilmiştir (Hoover & Ratnayake, 2002).

***Sineresis***

%6'lık nişasta çözeltisi su banyosunda 90°C'de 30 dakika boyunca çalkalanarak bekletilmiştir. Ardından soğutulan örnekler 4°C'de 0, 24, 48, 72, 96 ve 120 saat bekletilmiştir. Süre sonunda 3000*g'de 10 dakika santrifüj edildikten sonra ayrılan su miktarı % olarak hesaplanmıştır (Wani ve ark., 2010).

***Saponin Analizi***

0,5 g nişasta örneği üzerine 0,5 mL distile su eklenerek 15 dakika çalkalanmıştır. Süre sonunda yaklaşık 0,25 cm köpük oluşumu saponin varlığını göstermektedir (Janarthanan ve ark., 2012).

***Glikozit Analizi***

1 g nişasta örneği üzerine 1,5 mL kloroform ve %10'luk amonyak damla damla eklenmektedir. Pembe renk oluşumu glikozit varlığını göstermektedir (Janarthanan ve ark., 2012).

***Spesifik Rotasyon (α)***

Spesifik çevirme açısı bir polarimetre (Atago, AP-100, Japonya) kullanılarak ölçülmüş ve Eşitlik 1 kullanılarak hesaplanmıştır (Polavarapu, 2002).

$$\alpha = a \times 100 / (L \times C) \quad (1)$$

α= belirli dönme derecesi
a= gözlenen dönüş açısı
L= desimetre cinsinden tüp uzunluğu
C= 100 mL başına gram cinsinden konsantrasyon

***$\lambda_{max}$ Değeri, Mavi Değer ve Amiloz İçeriği***

Nişastanın max absorpsiyon spektrumları Nwokocha & Williams (2011) tarafından bildirilen yönteme göre ölçülmüştür. Mavi değeri, Takeda ve ark. (1983) tarafından bildirilen yönteme göre 680 nm'de ölçülmüş ve Eşitlik 2 kullanılarak hesaplanmıştır.

$$Mavi\ değer = \frac{Abs_{680} \times 4}{\text{Nişasta Konsantrasyonu } (\frac{mg}{dL})} \quad (2)$$

Amiloz içeriğinin tespitinde nişasta örneği 12 saat boyunca %90 dimetil sülfoksit (DMSO) çözeltisinde çözdürülmüş ve sıcak izopropanol ile çöktürülerek saflaştırılmıştır (Stevenson ve ark., 2006).

***Aflatoksin Analizi***

Pietri ve ark. (2012) tarafından bildirilen aflatoksin yöntemi modifiye edilerek kullanılmıştır. At kestanesi tohumundan ve nişastadan 25 g alınarak 250 mL aseton/su (70 + 30 v/v) karışımı ile 45 dakika boyunca çalkalamalı su banyosunda ekstrakte edilmiştir. Karışım filtre kağıdından süzüldükten sonra, süzüntüden 5 mL alınmış ve distile su ile 50 mL'ye seyreltilmiştir. Çözelti daha sonra bir immünoafinite kolonu (Rhone Diagnostic Technologies) aracılığıyla saflaştırılmıştır. Elüat azot akışı altında konsantre edilmiş ve asetonitril su (25 + 75 v/v) karışımı ile 2 mL'ye tamamlanarak vortex ile karıştırılmış ve ardından filtrelenmiştir (Millex HV 0.45 µm, Millipore). Analiz HPLC cihazında (Perkin Elmer, ABD) gerçekleştirilmiştir. Aflatoksinler Macharey Nagel CC 250/4.6 Nucleosil 100-5 C18 HD kolonunda 40°C'de su-metanol-asetonitril (64 + 23 + 13, v/v/v) mobil fazı (pompa akış hızı: 1 mL/dak, enjeksiyon hacmi: 100 µL) ile ayrıştırılmıştır. AFB1, AFB2, AFG1 ve AFG2'nin farklı konsantrasyonlardaki (0.025 ve 6.0 ng/mL) altı kalibrasyon çözeltisi enjekte edilmiş ve bir kalibrasyon eğrisi oluşturulmuştur.

***Nişastanın Granül Yapısı***

%1'lik nişasta çözeltileri ışık mikroskobu (Olympus, Japonya) altında görüntülenmiş ve granül yapılarının fotoğrafları çekilmiştir (Canon 80D, Japonya).

***FT-IR Analizi***

FT-IR spektrumları Jasco FT-IR 4700 spektrometresinde 400-4000 $cm^{-1}$ aralığında kaydedilmiştir. Tüm spektrumlar Jasco Spectra Manager Version 2 yazılımı (v 2.14.05) kullanılarak analiz edilmiştir (Akbaş, 2020).

***Termogravimetrik Analiz (TGA)***

At kestanesi nişastası örnekleri TA Instruments SDT Q600 TG/DSC (ABD) tipi bir termogravimetrik analizör ile analiz edilmiştir. Nişasta örnekleri açık alümina krozeler kullanılarak 30 ila 900°C arasında ısıtılmıştır. 10,0 mg örnek miktarı, 10°C/dak ısıtma hızında 100 mL/dak sentetik hava akışında kullanılmıştır (Wendlandt, 1986).

***Taramalı Elektron Mikroskobu (SEM) Analizi***

At kestanesi nişastalarının yapısal ve morfolojik özelliklerini SEM analizi JEOL JSM-7001F Schottky SEM (Japonya) ile incelemiştir. Görüntüler ×1000 boyutunda kaydedilmiştir.

***İstatistiksel Analiz***

SPSS istatistik programı (SPSS, Inc., Chicago, IL, ABD) kullanılmış, sonuçların varyans analizi (ANOVA) yapılmış ve gruplar arasındaki farklar Duncan çoklu karşılaştırma testi ile %95 güven aralığında istatistiksel olarak değerlendirilmiştir.

**Bulgular ve Tartışma**

İki farklı yöntemle üretilen at kestanesi nişastalarının fonksiyonel (teknolojik) özellikleri Çizelge 1'de sunulmuştur. At kestanesinin yapısında bulunan glikozit ve saponinler büyük oranda kabuk kısmında bulunmakta olup tohumda bulunan glikozit ve saponinlerin nişastaya geçmediği tespit edilmiştir. Nişasta ekstraksiyonunda ultrason destekli yöntemlerin kullanılması yağ ve su tutma kapasitesini ve şişme gücünü artırmıştır. Su tutma kapasitesi, viskoziteyi etkileyen önemli bir işleme parametresidir (Ahmad ve ark., 2020). Alkali yöntemle üretilen at kestanesi nişastasının (A-HCS) su tutma kapasitesi 234,60 g/g iken, ultrason destekli yöntemle üretilen at kestanesi nişastasının (U-HCS) su tutma kapasitesi 329 g/g'a yükselmiştir. Nişasta molekül boyutundaki azalma su tutma kapasitesini artırmaktadır (Ahmad ve ark., 2020). Ultrason destekli yöntemde su tutma kapasitesindeki artış, nişasta moleküllerinin artan yüzey alanından kaynaklanmaktadır. Kullanılan NaOH ve ultrason destekli yöntemin sinerjik etkisi ile amiopektinin kristal yapısı azalmakta veya kırılmaktadır. Bu durumda su tutma kapasitesinde artışa neden olmaktadır.

Çizelge 1. At kestanesi nişastalarının fonksiyonel özellikleri
*Table 1. Functional properties of horse chestnut starches*

| Parametreler Parameter | A-HCS | U-HCS |
|---|---|---|
| Yağ tutma kapasitesi (g/g) | 175,26±4,47[b] | 189,05±4,23[a] |
| Su tutma kapasitesi (g/g) | 234,60±1,23[b] | 329,00±13,42[a] |
| Şişme Gücü (%) | 273,87±3,23[b] | 481,35±18,54[a] |
| Çözünürlük (%) | 8,82±0,04[a] | 2,67±0,10[b] |
| Yığın yoğunluğu (g/mL) | 0,48±0,03[a] | 0,51±0,01[a] |
| Sediment hacmi (mL) | 10,00±0,01[b] | 17,33±2,31[a] |
| Bulanıklık (%T) | 6,24±2,92[a] | 0,72±0,19[b] |
| Spesifik çevirme açısı (α) | 59,90 | 21,75 |
| Mavi değer | 0,182±0,001[b] | 0,253±0,001[a] |
| Amiloz içeriği | 25,41±0,18[b] | 29,86±0,23[a] |
| $\lambda_{max}$ (nm) | 523,0±1,0[b] | 583,0±4,0[a] |
| Aflatoksin (B1, B2, G1 ve G2) | Tespit edilememiştir | Tespit edilememiştir |

a, b, c, ... harfleri aynı satırdaki örneklerin P<0.05 düzeyinde istatistiksel farklılıklarını göstermektedir.

Nişasta moleküllerinin boyutundaki ve polimerik moleküllerin ağ yapısındaki değişiklikler nişasta partiküllerinin hidrofobikliğini etkilemektedir (Ahmad ve ark., 2020). A-HCS ve U-HCS'nin yağ tutma kapasiteleri sırasıyla 175,26 ve 189,05 g/g olarak belirlenmiştir. Yağ tutma kapasitesi, suyu iyi tutamayan sarmal amiloz zincirlerinin hidrofobik iç yüzeyi ile ilişkilidir (Kong ve ark., 2014). Yapılan çalışmalarda at kestanesi nişastasının su tutma kapasitesinin %85-94, yağ tutma kapasitesinin ise %62-65 arasında olduğu bildirilmiştir (Rafiq ve ark., 2016; Wani ve ark., 2014). Ultrason destekli yöntemle elde edilen nişastaların alkali yönteme kıyasla daha yüksek viskoziteye, daha iyi termal stabiliteye, artan su tutma kapasitesine sahip olduğu bildirilmiştir (Ahmad ve ark., 2020). A-HCS'nin şişme gücü %273,87 ve U-HCS'nin şişme gücü %481,35'tir. Nişastanın sudaki şişme gücü ve çözünürlüğü amilopektinin zincir dal uzunluğundaki dağılım ve fosfor içeriğindeki değişikliklerle ilgilidir. Amilopektinin yapısındaki kısa zincirler, kısa ve zayıf çift sarmallar oluşturarak nişasta granüllerinin şişme gücünü arttırmaktadır (Castaño ve ark., 2014). Ultrason uygulaması nişastalarda amiloz ve özellikle amilopektin yapısında kırılma yada kopmalara neden olmaktadır. Bu durumda ortamdaki kısa zincirli yapıları arttırmaktadır. Castaño ve ark. (2014) at kestanesi nişastasının çözünürlüğünü %3,1 ve şişme gücünü %65 olarak bildirmişlerdir. Nişastaların yığın yoğunluğu 0,48 g/mL ve 0,51 g/mL olup, literatür verileri olan 0,85 g/mL (Ahmad ve ark., 2020) ve 0,86 g/mL (Wani ve ark., 2014) değerlerinden daha düşük tespit edilmiştir.

Mililitrede 1 g madde içeren bir çözeltinin spesifik rotasyonu, uzun bir tüpte ölçülen spesifik çevirme açısı olarak tanımlanmaktadır. Her aktif maddenin karakteristik bir spesifik çevirme açısı bulunmaktadır (Hossain ve ark., 2015). A-HCS'nin spesifik rotasyon (α) değeri 59,90 ve U-HCS'nin 21,75 olarak tespit edilmiştir. Mavi değer (BV), iyot komplekslerinin oluşumundaki farklılıklardan yararlanarak amiloz içeriğini hesaplamak için kullanılan yöntemlerden birisidir (Stawski, 2008). Nişasta-iyot absorpsiyon spektrumları, maksimum absorpsiyon dalga boyu ($\lambda_{max}$)'nu göstermektedir. A-HCS'nin mavi değeri, $\lambda_{max}$ değeri ve amiloz içeriği sırasıyla 0,182, 25,41 ve 523,0 nm iken, U-HCS'ninki 0,253, 29,86 ve 583,0 nm'dir. Amiloz içeriği arttıkça, mavi değer ve $\lambda_{max}$ da artmaktadır (Yu ve ark., 2012). Nişastanın $\lambda_{max}$ değerinin polimerizasyon derecesi ve amiloz ve amilopektinin ortalama zincir uzunluğu ile ilişkili olduğu bildirilmiştir (Hung ve ark., 2008). Ayrıca $\lambda_{max}$'taki artış, sulu çözeltide iyot-amiloz kompleksi oluşturmak üzere amilozun bulunabilirliğinde bir artış olduğunu göstermektedir (Nwokocha & Williams, 2011).

At kestanesi nişastalarının ışık geçirgenliği, donma-çözünme kararlılığı ve sineresis değerleri Çizelge 2'de verilmiştir. Nişasta çözeltilerinin depolanması sırasında, yapıdan salınan amiloz ve amilopektin miktarı, amiloz ve amilopektinin moleküler ağırlığı ve zincir uzunluğu ve nişasta granüllerinin şişme özellikleri gibi faktörler bulanıklığı arttırmakta ve ışık geçirgenliğinin azalmasına neden olmaktadır (Rafiq ve ark., 2015; Wani ve ark., 2014). Nişasta örneklerinin ışık geçirgenliği 120 saatlik depolama süresince incelenmiş ve 48 saat sonunda daha opak bir karışım elde edilmiştir. Nişasta örneklerinin sediment hacmi A-HCS ve U-HCS için sırasıyla 10,00 ve 17,33 mL olarak belirlenmiştir. Ultrason uygulaması ile nişasta moleküllerinin amorf yapısının bozulması su tutma kapasitesini yükseltmekte ve bu durumda da sediment hacminde artışa neden olmaktadır. Başlangıç ışık geçirgenliği (%T) değerleri alkali yöntemle elde edilen nişastalarda daha yüksek bulunmuştur. Ultrason uygulaması ile nişastada meydana gelen mikro yapısal değişiklikler ortamdaki karboksil gruplarının miktarında artışa neden olmaktadır. Oluşan karboksil grupları su molekülleri ile hidrojen bağı oluşturmaktadır. Bu da nişasta jelinin ışık geçirgenliğini ve berraklığını artırmaktadır (Singh ve ark., 2006). Opak jeller daha düşük %T vermektedirler (Rafiq ve ark., 2015). U-HCS'nin A-HCS'ye kıyasla daha düşük ışık geçirgenliği göstermesi karboksil gruplarının oluşumundan kaynaklanmaktadır. Çizelge 2'de gösterildiği gibi, nişastaların sineresisi depolama süresiyle birlikte artmış ve 96 saatte en yüksek seviyeye ulaşmıştır. Ultrason uygulaması nişasta moleküllerinin amiloz ve amilopektin birleşme bölgelerini etkilemekte buda yapıda ki suyun serbest aklmasına neden olmaktadır (Perera & Hoover, 1999). Ultrason destekli ekstraksiyon yönteminin sineresis değerlerinde düşüşe neden olduğu belirlenmiştir. Depolama nedeniyle sineresis yüzdesindeki artışın, sızan amiloz ve amilopektin zincirleri arasındaki etkileşimden kaynaklandığı, bunun da birleşme bölgelerinin gelişmesine ve suyun serbest kalmasına yol açtığı bildirilmektedir (Perera & Hoover, 1999). Jelleşmiş

nişasta karışımlarının donma-çözünme stabilitesi A-HCS için %0-33,65 ve U-HCS için %0,22-37,41 arasında belirlenmiştir. Donma çözünme işlemi esnasında yapıdan ayrılan su miktarındaki artış, nişasta moleküler yapısındaki değişim ve partikül boyutundaki azalmadan kaynaklanmaktadır. Jellerden ayrılan su miktarı depolama süresi ile artmıştır. Alkali yöntemle üretilen at kestanesi nişastalarının minimum %6 konsantrasyonda jel oluşturduğu, ultrason destekli yöntemle üretilen nişastaların ise minimum %12 konsantrasyonda jel oluşturduğu tespit edilmiştir (Çizelge 3). Literatürde at kestanesi nişastasının minimum jel oluşturma konsantrasyonuna ilişkin veri bulunmamaktadır.

***Aflatoksin içeriği***

Çeşitli tarımsal ürünlerde bulunan mikotoksinler, hasattan tüketime kadar bazı küf türleri tarafından çevresel koşullara bağlı olarak üretilen ikincil metabolitlerdir. Mikotoksinler arasında aflatoksinler en toksik, tehlikeli ve yaygın olanıdır (Atasoy ve ark., 2017). Park ve bahçe atığı olarak sulama suyu ve yağmura maruz kalan at kestanesi tohumlarında küflenme ve bozulma gibi sorunlar ortaya çıkabilmektedir. At kestanesinden üretilen nişastalar aflatoksin kontaminasyonu açısından kontrol edilmiştir. Her iki yöntemle üretilen nişastalarda ve at kestanelerinde B1, B2, G1 ve G2 tipi aflatoksinler tespit edilmemiştir (Çizelge 1). Nişasta örnekleri aflatoksin açısından güvenli bulunmuştur.

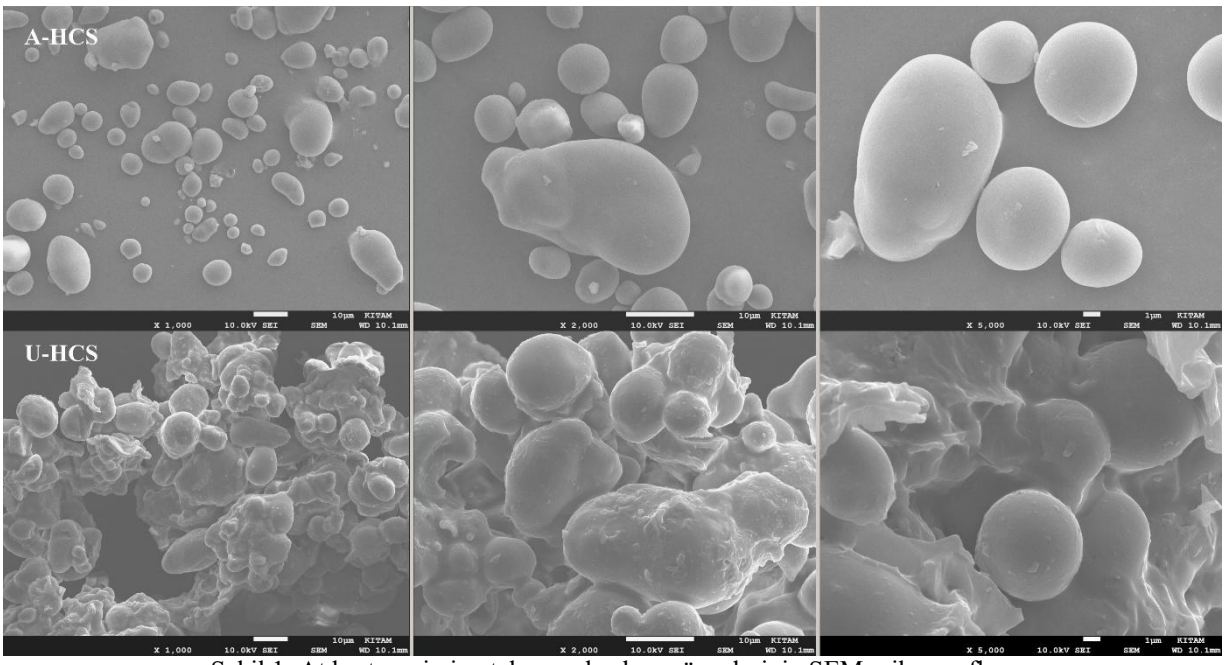
Çizelge 2. At kestanesi nişastalarının ışık geçirgenliği, donma-çözünme stabilitesi ve sineresis değerleri
*Table 2. Light transmittance, freeze-thaw stability and syneresis values of horse chestnut starches*

| Parametreler Parameter | A-HCS | U-HCS |
|---|---|---|
| Işık geçirgenliği (%) | | |
| 0h | 37,31±6,80$^{a}$ | 25,33±1,34$^{b}$ |
| 24h | 66,02±0,89$^{a}$ | 33,97±2,93$^{b}$ |
| 48h | 68,90±1,26$^{a}$ | 38,48±1,33$^{b}$ |
| 72h | 62,40±1,63$^{a}$ | 39,37±3,45$^{b}$ |
| 96h | 55,95±22,45$^{a}$ | 38,47±1,26$^{b}$ |
| 120h | 22,74 ± 4,11$^{a}$ | 13,63±8,54$^{b}$ |
| Donma-çözünme stabilitesi (%) | | |
| 0. çözdürme *0 thaw* | 0,00±0,00$^{b}$ | 0,22±0,00$^{a}$ |
| 1. çözdürme *1 thaw* | 0,18±0,01$^{b}$ | 1,48±0,02$^{a}$ |
| 2. çözdürme *2 thaw* | 2,40±0,04$^{b}$ | 5,85±0,07$^{a}$ |
| 3. çözdürme *3 thaw* | 10,31±0,07$^{b}$ | 10,55±0,12$^{a}$ |
| 4. çözdürme *4 thaw* | 33,65±0,18$^{b}$ | 37,41±0,12$^{a}$ |
| Sineresis (%) | | |
| 0h | 76,6±0,8$^{a}$ | 72,7±1,3$^{b}$ |
| 24h | 80,0±1,9$^{a}$ | 74,2±2,3$^{b}$ |
| 48h | 82,7±1,2$^{a}$ | 73,5±1,8$^{b}$ |
| 72h | 84,7±0,7$^{a}$ | 73,7±1,5$^{b}$ |
| 96h | 85,0±1,4$^{a}$ | 72,4±1,8$^{b}$ |
| 120h | 83,2±0,3$^{a}$ | 66,7±0,4$^{b}$ |

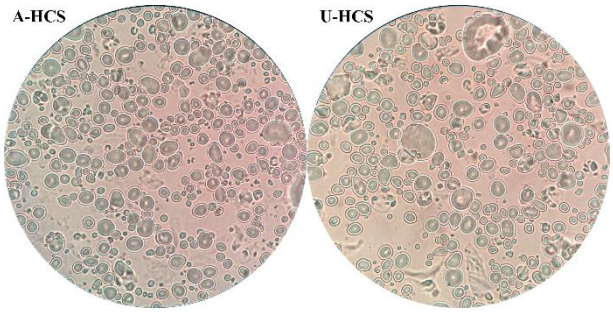
a, b, c, ... harfleri aynı satırdaki örneklerin P<0.05 düzeyinde istatistiksel farklılıklarını göstermektedir.

Çizelge 3. At kestanesi nişastalarının minimum jel oluşturma konsantrasyonları
*Table 3. Minimum gel forming concentration of horse chestnut starches*

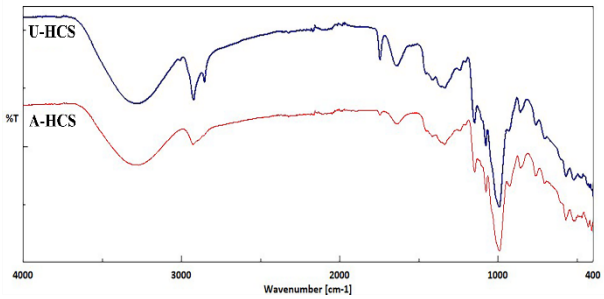
| | A-HCS | | | U-HCS | | |
|---|---|---|---|---|---|---|
| Konsantrasyon (%) | 1 | 2 | 3 | 1 | 2 | 3 |
| 1 | - | - | - | - | - | - |
| 2 | - | - | - | - | - | - |
| 3 | - | - | - | - | - | - |
| 4 | - | - | - | - | - | - |
| 5 | - | - | + | - | - | - |
| 6 | + | + | + | - | - | - |
| 7 | + | + | + | - | - | - |
| 8 | + | + | + | - | - | - |
| 9 | + | + | + | - | - | - |
| 10 | + | + | + | - | - | - |
| 11 | + | + | + | - | - | - |
| 12 | + | + | + | + | + | + |
| 13 | + | + | + | + | + | + |
| 14 | + | + | + | + | + | + |




Şekil 1. At kestanesi nişastalarının kırılma yüzeylerinin SEM mikrografları
*Figure 1. SEM micrographs of fracture surfaces of horse chestnut starches*




Şekil 2. At kestanesi nişastalarının ışık mikroskopu görüntüleri
*Figure 2. Polarised light microscope images of horse chestnut starches*




Şekil 3. At kestanesi nişastalarının IR spektrumları
*Figure 3. IR spectrum of horse chestnut starches*

**Taramalı elektron mikroskobu (SEM)**

Alkali ve ultrason destekli yöntemlerle üretilen nişastalardaki değişiklikleri ve nişasta moleküllerinin şekil ve gözeneklilik gibi morfolojik özelliklerini gözlemlemek için taramalı elektron mikroskobu (Şekil 1) ve ışık mikroskobu (Şekil 2) kullanılmıştır. Işık mikroskobu ile tespit edilen görüntülere göre ultrason uygulamasının nişasta bouyutunu küçülttüğü tespit edilmiştir. SEM görüntüleri dikkate alındığında oluşan nişasta moleküllerinin poligonal yapıda olduğu görülmüştür. Alkali ve ultrason destekli yöntemlerin kombinasyonu nişasta moleküllerinin hücre duvarlarını kırarak daha küçük ve küresel parçaların oluşmasına neden olmaktadır (Zhu ve ark., 2016). Ultrason işlemi nişasta moleküllerinin yüzeylerinde aşınmalara neden olmakta ve nişasta şekillerini değiştirmektedir. Mikrograflar, nişasta granüllerinin şekil ve boyut olarak düzensiz olduğunu ve tek tip bir desene sahip olmadığını göstermektedir. Ayrıca, ultrason işlemi küçük kavitasyonlar nedeniyle nişasta granüllerinin amorf katmanlarını bozmaktadır. Bozulan amorf katmanlar nişastanın jel yapısının içinde görüntülenmesine neden olmaktadır. SEM sonuçları Ahmad ve ark. (2020) tarafından yapılan çalışma ile benzerlik göstermektedir.

**FT-IR analizi**

FT-IR spektroskopisi, nişasta granülleri içindeki yarı kristal ve amorf alanlarda meydana gelen değişiklikleri karakterize ederek nişastanın kristalliğini belirlemek için de kullanılmaktadır (Ahmad ve ark., 2020). Alkali ve ultrasonik destekli yöntemlerle üretilen nişastaların IR spektrumlarında 4000 ve 400 $cm^{-1}$ arasında meydana gelen değişimler Şekil 3'te gösterilmektedir. A-HCS ve U-HCS IR spektrumları 3271 $cm^{-1}$'de -OH bozunmasına atfedilen güçlü absorpsiyon bantları göstermektedir (Ahmad ve ark., 2017). IR spektrumlarının genişliği, moleküller arası ve molekül içi hidrojen oluşumunun derecesini göstermektedir. Alkalizasyon ve ultrason işlemleri -OH gruplarının serbest kalmasına yol açtığı bildirilmektedir (Chun ve ark., 2015). Rodriguez-Garcia ve ark. (2021) nişastanın ortorombik ve hekzagonal kristal yapılara sahip, nanokristallerden oluştuğunu göstermiştir. Ortorombik kristal yapı kristal kafes yapılarından biridir. Bu sistemde a, b ve c eksenleri farklı boylarda, bunlar arasındaki açılar da 90°'e eşittir (Cabrera-Ramírez ve ark., 2021). Nişasta moleküllerindeki O-H germe bantları A-HCS'de daha yüksek bir dalga boyuna kaymıştır. Diğer karakteristik bantlar susuz glikoz halkasındaki C-O bağı, C-O-H ve C-O-C grupları ile ilişkili 1148, 1077 ve 992 $cm^{-1}$ dalga

boylarında ve -$CH_2$ grubu ile ilişkili 2923 ve 2926 $cm^{-1}$ dalga sayısındaki bantlarda gözlenmiştir. 1739 $cm^{-1}$'deki bant esterleşmiş nişastadaki ester bağı ile ilişkilidir ve bu bant U-HCS'de azalmaktadır. 1643 $cm^{-1}$'deki karakteristik bant, nişastadaki bağlı sudan kaynaklanmaktadır ve nişasta boyutunun küçültülmesinden sonra bant yoğunluğunda önemli bir değişiklik göstermemektedir (Ahmad ve ark., 2020). Alkali yöntemle üretilen nişastalarda 1148 ile 992 $cm^{-1}$ arasındaki ve ultrason destekli yöntemle üretilen nişastalarda 1337 ile 993 $cm^{-1}$ arasındaki bantların yoğunluğu, nişastanın parçalanması ve kristalinitenin azalması nedeniyle azalmıştır. U-HCS'de parçalanma seviyesi daha yüksek rapor edilmiştir (Van Soest ve ark., 1995). Özellikle, nişastalardaki C-O bağı ile ilişkili 1019 $cm^{-1}$'deki C-O-C bandı spektrumlarda gözlenmemiştir (Jiugao ve ark., 2005). 859 $cm^{-1}$'deki karakteristik bantlar örneklerdeki β-glikozidik bağların varlığına işaret etmektedir. Nişasta molekülünün yapısındaki şeker fraksiyonlarının bağlarındaki titreşimler, 480-950 $cm^{-1}$ aralığındaki bantların yoğunluğundaki değişikliklerle tespit edilmektedir (Wójcik ve ark., 2023).

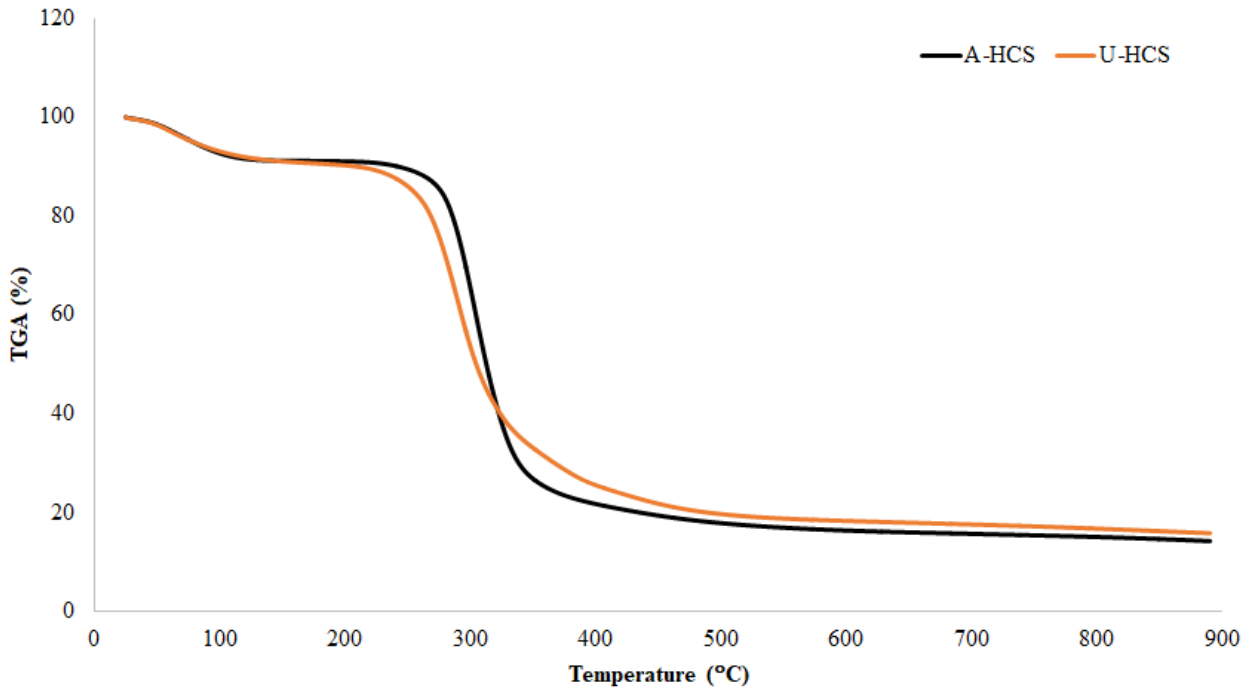
**Termogravimetrik analiz (TGA)**

At kestanesi nişastası örneklerinin termogravimetrik analiz (TGA) eğrileri Şekil 4'te termogravimetresi ise Çizelge 4'de gösterilmektedir. Dehidrasyon aşaması olarak bilinen ilk aşama (25-132°C) da her iki nişasta da stabilite göstermiştir. İkinci aşamada, A-HCS nişastaları 243-343°C'de hızlı ağırlık kaybı gösterirken, U-HCS nişastaları 221-326°C'de hızlı ağırlık kaybı göstermiştir. Bu aşamadaki kayıplar organik maddenin (amiloz ve amilopektin) bozunmasına karşılık gelmektedir (Boukhelkhal & Moulai-Mostefa, 2017). Ağırlık kaybının üçüncü aşaması A-HCS için 343°C'yi U-HCS içinse 326°C'yi aşan sıcaklıklarda nihai kalıntıların (kül) oluşumu gerçekleşmektedir. Bu sonuçlar homopolisakkaritin özelliklerini ortaya koymaktadır. 900°C işleminin sonunda, A-HCS'nin toplam kütlesinin %85,82'si ve U-HCS'nin %84,23'ü kaybolduğu belirlenmiştir. Kütlenin %50'sinin kaybolduğu sıcaklık (%T50) değeri A-HCS ve U-HCS için sırasıyla 302-312°C ve 303-304°C aralığında tespit edilmiştir. Ultrason uygulaması örneklerin T50 değerinde azalmaya neden olmaktadır.

Çizelge 4. Termogravimetrik (TGA) analiz sonuçları
*Table 4. Thermogravimetric (TGA) analysis results*

| Örnek | Aşama | Δm (%) | ΔT (°C) |
|---|---|---|---|
| A-HCS | 1st | 8,49±0,12 | 25-132 |
| | Stabilite | - | 132-243 |
| | 2nd | 67,42±0,16 | 243-343 |
| | 3rd | 24,01±0,08 | 350-900 |
| | T50% | | 302-312 |
| U-HCS | 1st | 8,47±0,03 | 25-132 |
| | Stabilite | - | 132-221 |
| | 2nd | 65,50±0,21 | 221-326 |
| | 3rd | 25,80±0,16 | 350-900 |
| | T50% | | 303-304 |

Δm: kütle kaybı (%), ΔT: sıcaklık aralığı (°C), T50%: Kütlenin %50'sinin kaybolduğu sıcaklık




Şekil 4. At kestanesi nişastalarının TGA kurveleri
*Figure 4. TGA curves of horse chestnut starches*

## Sonuç

Bu çalışma, at kestanesi tohumlarından katma değeri yüksek bir bileşen olan nişastanın iki farklı yöntemle üretimini ve üretilen nişastaların morfolojik, termal ve fonksiyonel özelliklerinin belirlenmesini kapsamaktadır. Nişasta üretimi için alkali ve ultrason destekli olmak üzere iki farklı yöntem kullanılmıştır. Ultrason destekli yöntem, nişastaların su ve yağ tutma kapasitesini artırmakta, şişme gücünü yükseltmekte ve sineresisi azaltmaktadır. Alkali ekstraksiyona kıyasla ultrason destekli ekstraksiyonda nişastaların donma-çözülme stabilitesinde daha fazla su kaybettiği ve nişastaların minimum jel oluşturma konsantrasyonunun arttığı tespit edilmiştir. Ultrasonik yöntem nişastanın $\lambda_{max}$ değerini 523,0 nm'den 583 nm'ye yükseltmiş ve spesifik dönme açısını 59,90'dan 21,75'e düşürmüştür. Termogravimetrik veriler kütlenin %50'sinin 303-304°C aralığında kaybolduğunu göstermiştir. Ultrasonikasyon, ortorombik kristal yapıya sahip nişastaların yapısında kısmi bir değişikliğe neden olmuştur. Bu çalışma, alternatif nişasta kaynaklarına ilişkin kapsamlı bir karşılaştırmalı çalışmadır. Bu çalışmadan elde edilen bilgilerin araştırmacılara yol gösterebileceğine inanılmaktadır. Üretilen nişastaların endüstriyel uygulama potansiyeli için glutensiz kek üretiminde, jelleşme istenen pudding, muhallebi gibi ürünlerde uygulanabilirliği araştırılabilir. Ayrıca atık bir hammaddeden katma değeri yüksek ürün üretilerek ülke ekonomisine katkıda bulunulacaktır.

## Beyanlar

### *Etik Onay Belgesi*

Bu çalışmada etik onay belgesine ihtiyaç bulunmamaktadır.

### *Yazar Katkı Beyanı*

Ali Cingöz; projenin tasarlanması, metodoloji, bulguların değerlendirilmesi ve makale yazımını gerçekleştirmiştir. Zeynep İnatçı; Metodoloji, bulguların değerlendirilmesi ve makalenin düzenlenmesine katkıda bulunmuştur.

### *Fon Beyanı*

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# Natural Preservatives as Medicinal Aromatic Plants: Implications for Sustainable and Functional Bread

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ABSTRACT

In this study, the plants *Melissa officinalis* (*Melissa*), *Elaeagnus angustifolia* (*Elaeagnus*), *Styrax officinalis* (*Styrax*) and *Echinops ritro* (*Echinops*) were firstly used to prepare enriched bread and to study their effects on the shelf life of bread. Water and alcohol extracts of the plants were also prepared to determine their antibacterial and antifungal activities *in-vitro*. The focus is on their potential applications as natural preservatives in sustainable functional bread production. The antimicrobial activity was evaluated using the agar well diffusion assay. Results showed that alcohol extracts of medicinal and aromatic plants exhibited significantly higher antimicrobial activity than water extracts, with inhibition zones diameters ranging from 15-22 mm for alcohol extracts compared to 8-13 mm for water extracts. Gram-negative bacteria, such as *Salmonella* Paratyphi A, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, showed resistance, with inhibition zone diameters below 10 mm. However, alcohol extracts from *Styrax* and *Elaeagnus* achieved inhibition zone diameters of 12-15 mm against these pathogens. Gram-positive bacteria, including *Staphylococcus aureus*, showed promising results, with alcohol extracts zones averaging 20 mm and water extracts inhibition of zone diameters averaging 14 mm. Fungal inhibition zone diameters was effective, with extracts reducing *Aspergillus niger* growth by 85%. A shelf life experiment revealed that bread enriched with *Elaeagnus* and *Melissa* extracts remained mold-free for 7 days, while control samples developed mold within 3-4 days. Sensory analysis indicated that 80% of participants preferred the taste and aroma of *Elaeagnus* seed bread, with an average score of 4.5-5. The incorporation of medicinal and aromatics plants not only enhances bread flavour but also provides health benefits besides sell life of bread. These plants serve as valuable natural preservatives, improving nutritional value, extending shelf life, and inhibiting harmful microorganisms in sustainable bread production.

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## Introduction

The history of medicinal and aromatic plants (MAP) is as old as human history. Sumerians and Assyrians used these plants for therapeutic purposes in 5000-3000 BC. Following this, it is stated that Greeks, Egyptians, Hittites used a total of 250 different medicinal plants (Giesecke, 2023). According to The World Health Organization (WHO), there are around 20.000 medicinal plants in the world (Yücel et al., 2019). In Turkiye 3.035 of the 11.707 taxa growing naturally are in the endemic plant group (Demiray, 2021; MNPS, 2021). Around 80% of people in developing countries rely on traditional herbal remedies, according to the WHO. Out of the 374,000 species of plants (MNPS, 2021), over than 28,000 are used for medicinal purposes, on the other hand about to 30,000 of these species showing antimicrobial effect are identified. (Tunca-Pinarli, et al., 2023). Medicinal plants are an important source of new drugs, and over 100 countries regulate about to their use. Ethnomedicinal knowledge has led to the discovery of 74% of bioactive plant compounds (Picking, 2024). Recently, most researchers have focused on the effect of antimicrobial activity of medicinal plants (Castronovo et al., 2021; Al-Qura'n, 2009).

### *Antimicrobial Activity of Medicinal Plant Extracts*

Medicinal plants are those that contain various parts or bioactive components, which can be directly obtained and used either internally or externally to treat diseases in humans and animals. (Stéphane et al., 2021; Özdemir et al., 2024) Today, it is widely acknowledged that MAPs contain compounds with antimicrobial properties (Vaou et al., 2021). The MAPs contain essential oils or alkaloids, flavonoids, sesquiterpene, lactones, diterpenes, triterpenes

or naphthoquinones (Azeem et al., 2020; Karthikeyan et al., 2020; Ohiagu et al., 2021), and are popular both in the medical field such as potential antimicrobial, anticancer and actively function in many fields. This compounds belonging antimicrobial properties, inhibit bacteria, fungi, viruses and protozoa with different mechanisms than conventional drugs. On the other hand, the compounds extracted from plants offer potential in the treatment of antimicrobial resistant strains and may either have intrinsic antimicrobial effects or enhance th e efficacy of antimicrobials by modifying resistance mechanisms (Álvarez Martinez, et al., 2020; Biharee et al., 2020; Özdemir et al., 2023; Tehranizadeh et al., 2016). Although the some of them can not effect on their own, they can work in synergy with antibiotics to overcome bacterial resistance (Onyancha et al., 2021).

Herbal treatments are generally safer than synthetic drugs, with fewer side effects and a lower risk of resistance development. However, similar to antibiotics, if only one compound targets a specific pathway, resistance can still occur (Tehranizadeh et al., 2016; Umaru, et al., 2020). The efficacy of medicinal plant extracts depends on the synergistic interactions of multiple active compounds, which increase the bioavailability, solubility and absorption of the drug while suppressing bacterial resistance and minimising toxicity (Choudhury, 2022). There is a need to better elucidate mechanisms of resistance and optimal use of medicinal plants to control microbial infectious diseases (Tiwari et al., 2023).

*Styrax officinalis* L. (*Sytrax*) and *Elaeagnus* composed a Important compounds such as egonol, egonol oleate, americanin, various phenolic acids and benzofuran derivatives have been isolated from its leaves, fruits, seeds, flowers and stems. The extracts containing these compounds exhibit significant biological activities, including antibacterial, antifungal, antioxidant, antitumour, anti-leukaemic, haemolytic and tyrosinase inhibitory effects. In addition to these, for therapeutic applications, both natural compounds and their synthetic derivatives are used (Demiray, 2021; Jaradat, 2020). In Turkiye, *Elaeagnus* is employed in traditional medicine for its tonic and antipyretic properties, as well as for the treatment of diarrhea and kidney disorders (Altundag & Ozturk, 2011). Okmen and Turkcan (2014) reported that methanol extracts of *Elaeagnus*, while having no effect on *Escherichia coli* ATCC 1122 and *C. albicans* RSKK 02029, showed the highest antimicrobial activity with a 16 mm inhibition zone diameters against *Yersinia enterocolitica*. The minimum inhibitory concentration (MIC) for the extract was 3.5 mg/mL for all bacterial strains tested except *Y. enterocolitica* NCTC 11174. The *Rheum ribes* chloroform extract exhibited strong antimicrobial activity, with MIC values of 0.89 mg/L for *S.* Paratyphi A (50.81% inhibition) and up to 14.17 mg/L for *B. subtilis* (96.04% inhibition), demonstrating higher efficacy than tetracycline against several pathogens and varying effects on Gram-positive, Gram-negative, and probiotic bacteria due to potential surface tension interactions (Özdemir et al., 2022-2023).

*Melissa* contains bioactive compounds such as volatile compounds (monoterpenes, sesquiterpenes, and monoterpene alcohols, giving it a lemon-like scent), triterpenes, phenolic acids (benzoic acid derivatives (e.g., gallic acid) and cinnamic acid derivatives) and flavonoid being a major component (Mabrouki & Duarte, 2018; Mencherini et al., 2007; Moradkhani et al., 2010).

*Echinops* species have been traditionally used to treat a variety of ailments. These include bacterial and fungal infections, fever, respiratory and cardiac problems (Bitew & Hymete, 2019). Phytochemical analysis suggests *Echinops* has antioxidant, antimicrobial and immunomodulatory properties (Falah et al., 2021). This genus, which belongs to the Asteraceae family, comprises over 120 species characterised by uniflorous capitula arranged in spherical or oval heads (Bitew & Hymete, 2019). Chemical profiling of *Echinops* roots reveals a predominance of thiophenes, such as α-terthiophene, while flavonoids isolated from *E. grijsii* roots and *E. echinatus* plants may contribute to hepatoprotective effects (Wang et al., 2015). Studies on *E. heterophyllus* extracts have shown significant hepatoprotective activity against methotrexate-induced hepatotoxicity. Ethanolic extracts were superior to flavonoid fractions (Abdulmohsin et al., 2019). In vitro evaluations of extracts from *E. giganteus, E. ritro* and *E. tournefortii* showed remarkable free radical scavenging effects, but current assessments of antioxidant activity lack comprehensive in vivo models (Anvari & Jamei, 2018; Sytar et al., 2022).

This study aims to evaluate the *in vitro* antimicrobial properties of selected MAPs such as *Melissa*, *Elaeagnus*, *Styrax* and *Echinops* and investigate their potential as natural bread preservatives. This research seeks to enhance the shelf life, safety, and sensory quality of functional bread, contributing to sustainable and innovative food preservation methods.

## Materials and Methods

### *Indicator Microorganism*

All of the indicator microorganisms were obtained from Cukurova University, Biotechnology Research and Application Center. Gram negative bacteria were *Klebsiella pneumonia* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella* Parathypi A NCTC13, *E. coli* ATCC 25922, Gram positive bacteria were *Listeria monocytogenes* ATCC 7677, *Bacillus subtilus* B-354, *Staphylococcus aureus* ATCC 29213, Molds were *Aspergilus niger* 10 ph k, *Aspergilus niger* ATCC 1015, *Candida utilis, Candida sakazakii, Candida albicans, and Yeasts were Saccharomyces cerevisiae*_sauch_VL1, *Saccharomyces cerevisiae_zymaflore and* Pneumonia-Associated Respiratory Pathogens (*Klebsiella pneumonia*-1, *Acinetobacter baumannii*-5, *Pseudomonas aeruginosa*-3).

### *Medicinal and Aromatic Plants*

*Melissa*, *Elaeagnus*, *Styrax* and *Echinops* plants were collected from the Çukurova region of Turkey. Different organs (whole plant, seed, fruit, leaf) of these plants were used in the experiment. These samples were *Eleganus* seeds and fruit, *Echinops* fruit, *Styrax* seeds and fruit, *Melissa* whole and *Melissa* and *Styrax* juices. The collected MAPs (Figure 1) were immediately stored in a -80°C cold chain until analysis was performed.


Figure 1. Images of medicinal and aromatic plants (MAP)s used in the study

Figure 2. The dough was prepared with the incorporation of seeds of *Melissa* with *Echinops* and fruit organs of *Echinops*, *Styrax and Melissa*

***Solvent Extracts of MAPs***

The extracts were prepared using ethyl alcohol and water as solvents for the different plant organs. The seeds of *Echinops* and *Styrax*, flowers of *Echinops*, leaf of *Melissa* were ground in a blender, the seeds of *Styrax* and *E. angustifolia* were separated from the fruit, dried in an oven, ground in a mill and pulverised. For this purpose, extraction solutions containing 5 g of each plant and 45 mL of solvent were prepared. The same procedure was followed for the second extract, but ethyl alcohol was used as solvent (Tufekci et al., 2018). The solutions were filtered and used in the study as alcohol and water extracts of the plants. Only the extracts of *Styrax* and *Melissa* were obtained.

As a result, (Table 2) the 10% and 5% water extract (WE) and alcohol extracts (AE) of *Melissa* leaves, *Echinops flowers*, *Styrax* (seed and fruit), *Elaeagnus* (seed and fruit) were prepared. Determination of antimicrobial effect of water and alcohol extracts of aromatic plants on gram-positive bacteria (inhibition zone diameter (mm) measurement results. Water extract groups (1-6): 1- M*elissa*. 2- *Elaeagnus* seed, 3- *Elaeagnus* fruit, 4- *Styrax* seed, 5- *Sytrax* fruit, 6- *Echinops*, Alcohol extract groups (7-12) :7- M*elissa*, 8- *Elaeagnus* seed, 9- *Elaeagnus* fruit,10- *Sytrax* seed, 11- *Styrax* fruits, 12- *Echinops*, 13- *Sytrax* juice, 14- M*elissa* juice.

***Determination of Antibacterial Activity***

The well diffusion agar assay test that was performed according to the methods designed and modified by Moghimi et al. (2016). Indicator bacteria subcultured overnight in triptic soy broth at 37 °C. It was poured into 90 mm diameter petri dishes with 20 mL Mueller-Hinton agar (Merck 1.05437) and added 0.5 Mc-Farland 1 mL fresh indicator bacteria and kept at room temperature for 30 minutes. Later, aseptically wells with a diameter of 6 mm were opened to frozen agar petries. 100 µL each plant extract were added to each well. Then inhibition zone diameters formed around the wells as a result of the incubation process of the indicator bacteria were measured in millimeters with the digital caliper (Mitutoyo 500-181-30, 0-150 mm) and interpreted CLSL 2018.

***Preparation of Bread with MAPs Additive***

Visuals of the plants used in the experiments were taken and shown in Figure 2. To ensure optimal incorporation of MAPs into the dough, the preparation process for herb-enriched bread included several steps. First, the aromatic plants were carefully washed to remove any impurities. The plants were then ground in a food processor. This was done until a homogeneous mixture was obtained. The seeds of *Styrax* and *Elaeagnus* were separated from the fruit before processing. The seeds were then dried in an oven to remove moisture. They were then ground to a fine powder in a mill. To produce experimental bread samples, the prepared plant powders were incorporated into the dough mix in specific proportions. For each bread, 5 g of MAP fruits and seeds were added to 200 g of flour by weighing 5 g on a precision balance. The dough was left to rest and then baked in an oven at 180 °C (Figure 2). This ensures even heat distribution for consistent texture and flavour development.

After baking, the bread samples contained with / without herbal additives were sliced. Following, they were exposed to air and placed in polyethylene bags and stored at room temperature in a dark environment to assess their shelf life. A portion of the bread was also cut into small pieces to facilitate the sensory evaluation, in which the participants rated the taste and texture.

## Results

MAPs were rich in potential antioxidants and antimicrobial compounds, including carotenoids, phenolics, phenols, and flavonoids (Özdemir et al., 2022; Sarkar, 2020). Each plant's biological effect is related to its chemical profile, hence chemical composition. The inhibition zone diameters formed in plate assays of water and alcohol extracts of MAPs against Gram-negative bacteria, Gram-positive bacteria, molds, and yeasts were measured and calculated.

***In-vitro Antibacterial Activiy of MAPs***

*Gram-negative pathogens*

Inhibition zone diameters measurements of water extracts of aromatic plants numbered from 1 to 6, alcohol extracts from 7 to 12 and plant juices such as *Melissa* and *Styrax* from 13 to 14 are as shown in Figure 3- 5b.

*Melissa officinalis* extracts (except water extract of whole plant) suppressed *S.* Paratyphi A and were highly effective against *Klebsiella* spp., *E. coli*, and *K. pneumonia*-1 with the alcohol extract showing the highest effect. Both alcohol and water extracts of *Styrax* seed inhibited *K. pneumonia*-1, *P. aeruginosa*, and *S.* Paratyphi A, while only alcohol extract of *Echinops* and *Elaeagnus* were effective against *E. coli*. The alcohol extracts of all plants showed inhibition against *A. baumannii*-5, while both water and alcohol extracts were effective against *S.* Paratyphi A. The presence of triterpenoids, tannins and saponins was identified in an initial phytochemical analysis of the fruit pericarp extract of *Styrax*. Tayoub et al. (2006) reported that bioactivity study evaluated the plant's potential as a biopesticide, with essential oil ratios ranging from 0.01-0.02%, lower than other studies. Key components identified in three development stages included (E)-2-hexenal, geraniol, octanol, nonanal, α-terpineol, tridecanal, trans-cubebol, and geranyl acetone. Another studies, infusions from the leaves and flowers of the plant have been used to treat coughs, diphtheria, and leucorrhoea, while the plant also possesses antibacterial, antifungal, and wound-healing properties and is used in India for conditions such as scabies and skin ulcers. Additionally, a tincture of the plant can serve as a mouthwash for asthma and is applied in the treatment of coughs, gonorrhoea, oedema, and tuberculosis (Al-Qura'n, 2015; Paşa, 2023).

*Gram-positive pathogens*

All groups showed inhibitory effect on *Listeria monocytogenes* except juice of *Melissa* and *Styrax* fruit. Inhibitory effect was observed more on *Bacillus. Styrax* juice did not show any inhibitory effect on any gram positive organism (Figure 4).

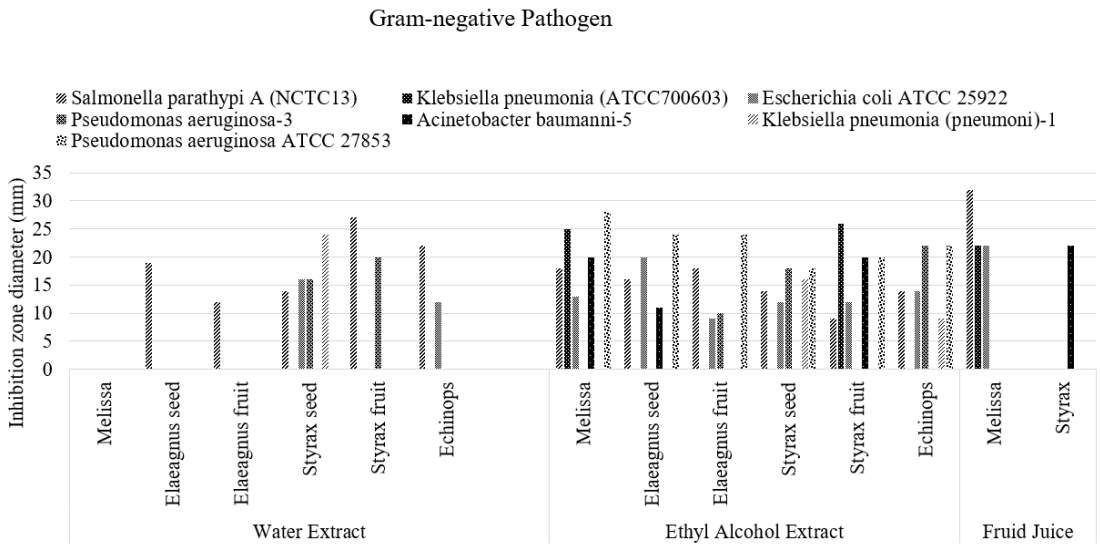


Figure 3. Inhibition zone measurement of MAPs on Gam-negative bacteria

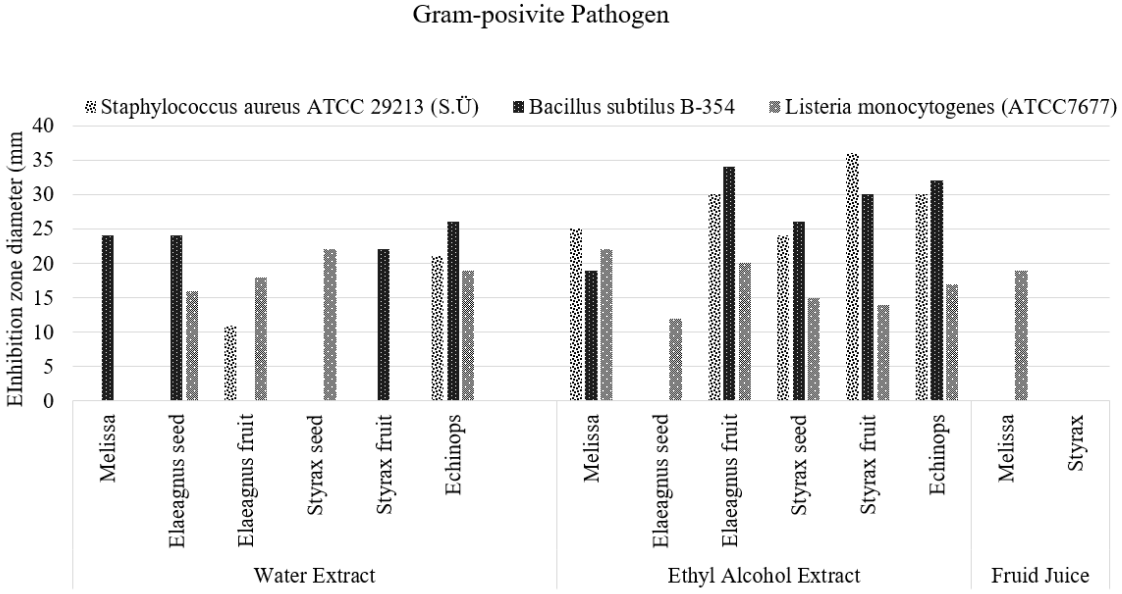


Figure 4. Inhibition zone measurement of MAPs on Gam-positive bacteria

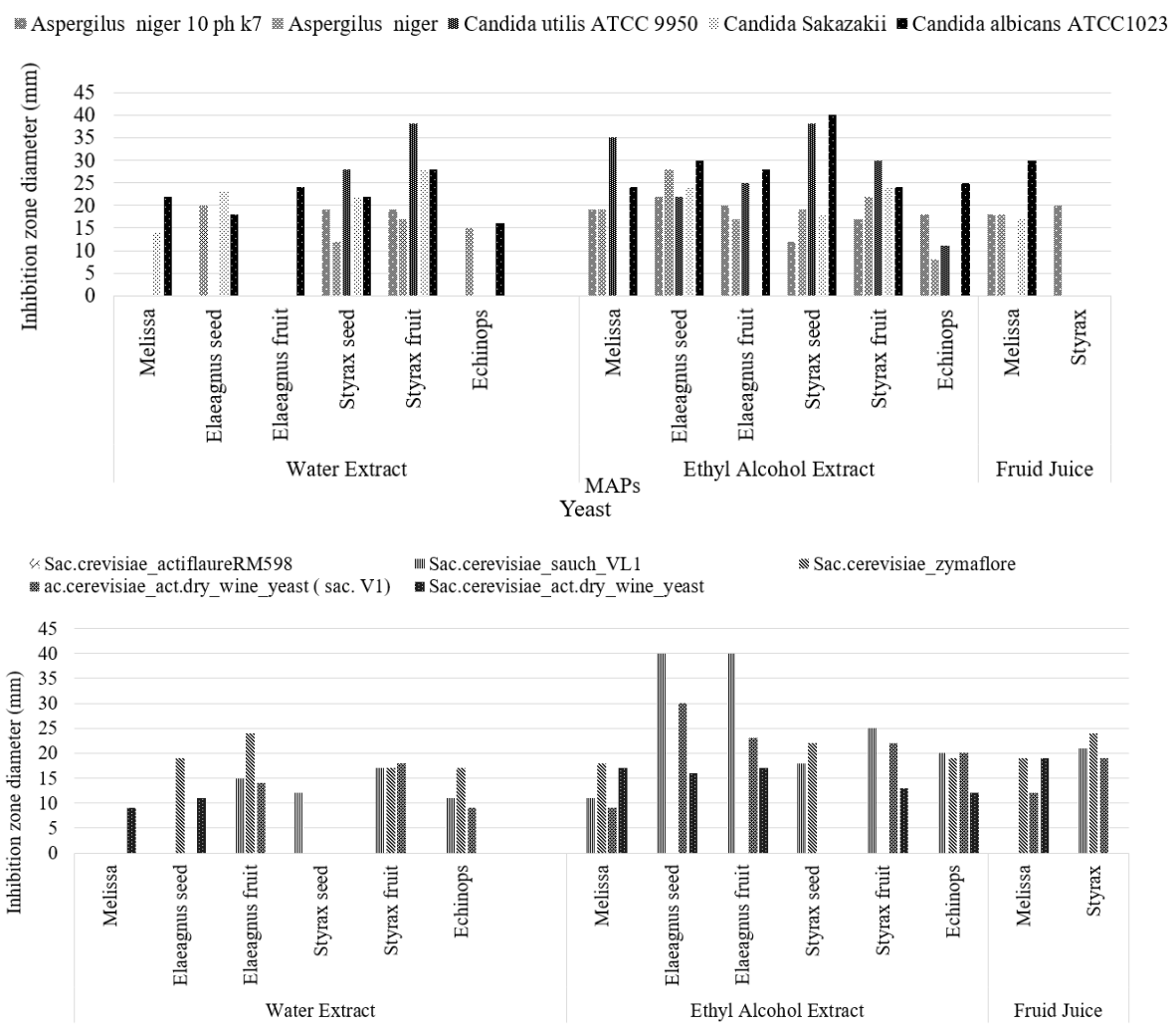


Figure 5b. Inhibition zone measurement of aromatic plants on yeast.

Bertanha et al. (2013) and Öztürk et al. (2008) reported the antibacterial activity of *Melissa* compounds (Egonol, Homoegonol, Americanin) against *Bacillus subtilis*, *E. coli*, *S. aureus*, *Haemophilus influenzae*, *K. pneumoniae*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*. Celebi et al. (2021) assessed the antimicrobial efficacy of *Elaeagnus* extracts against *Proteus mirabilis* (≥ 12.500 µg/mL), *C. albicans* (1.562 µg/mL), *Enterococcus faecalis* (1.562 µg/mL), *S. aureus* (3.125 µg/mL), *S. epidermidis* (1.562 µg/mL), *E. coli* (≥ 12.500 µg/mL), and *P. aeruginosa* (≥ 12.500 µg/mL), showing strong activity, especially against gram-positive bacteria and yeast. Additionally, ZnO nanoparticles with plant extract *Eleagnus angustifolia* showed antimicrobial activity with MIC values of ≥ 1.6 mg/mL.

*Echinops* species are traditionally used for bacterial and fungal infections, fever, and respiratory and heart problems (Bitew & Hymete, 2019). They have antioxidant, antimicrobial, and therapeutic properties, with thiophenes acting against fungi, bacteria, and insects, and terpenes and flavonoids offering anti-inflammatory and liver protection effects (Bitew & Hymete, 2019; Erenler et al., 2014). Extracts from *E. grijsii* root (300 mg/kg) and *E. echinatus* aerial parts (500 and 750 mg/kg) reduced liver function markers ASAT and ALAT. Aqueous methanol extracts of *E. ritro* L. were also studied.

The study by Hosseini & Gholipour (2020) tested plant extracts from *Quercus brantii*, *Elaeagnus angustifolia*, *Satureja montana*, *Tragopogon dubius*, and *Sonchus asper* for antimicrobial and anti-biofilm properties. *Quercus brantii* showed the strongest antibacterial and anti-biofilm effects, including against *Pseudomonas* and *Staphylococcus*. *Tragopogon dubius* and *Sonchus asper* had limited anti-biofilm activity. Özdemir et al. (2024) reported that *Rheum ribes* extract had strong antimicrobial effects. It inhibited *K. pneumoniae* (Pneumonia-Associated Respiratory Pathogens) and *P. aeruginosa* at 0.5 ppm, with inhibition rates of 94.88–100% against *K. pneumoniae* isolates and 82.82% against *P. aeruginosa*. Jalal et al. (2015) tested the antibacterial activity of essential oils against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Citrobacter koseri*. The oil showed strong antibacterial activity, with citronellal, β-caryophyllene oxide, and geraniol acetate as the main components.

***In-Vitro Antifungal Activity of MAPs***

Figure 5a and 5b showed the zone measurement (mm) graph on mould and yeast in water and alcohol extracts of MAPs. The MAPs tested displayed varying degrees of antifungal activity, with alcohol extracts generally outperforming water extracts in inhibiting mould (Figure 5a) and yeast growth (Figure 5b).

Alcohol extracts (AEs) of MAPs strongly inhibited *Candida albicans* and *Aspergillus niger*, while water extracts (WEs) showed milder effects. *Styrax*, *Melissa*, *Echinops*, and *Elaeagnus* displayed strong antifungal properties, with *Elaeagnus* being especially effective against *Aspergillus* spp. *Styrax* contains triterpenoids, tannins, saponins, and essential oils like geraniol, and is traditionally used for coughs, diphtheria, and skin conditions (Dehghan et al., 2014; Al-Qura'n, 2015; Paşa, 2023). MAPs, rich in essential oils and flavonoids, show antimicrobial effects, including against resistant strains, with *Styrax* and *Melissa* having antifungal activity (Jaradat, 2020).

*Echinops* essential oil showed strong antibacterial and antifungal activity, with low MIC values against *E. coli*, *S. aureus*, and *S. enteritidis* (Jiang et al., 2017). *Styrax suberifolius* bark inhibited *Phomopsis cytospore*, *Fusarium oxysporum*, and *Alternaria solani* (Zheleva-Dimitrova et al., 2023). *Styrax* saponins, including saponin A, showed antifungal activity against *Trichoderma viride*, *Fusarium oxysporum*, *A. niger*, and *Rhizopus mucor* (Mansour et al., 2016; Sak et al., 2024). *Elaeagnus* demonstrated antibacterial and antifungal activity, particularly against *Alternaria solani*, *Botrytis cinerea*, and *Aspergillus* species (Khan et al., 2016). *Styrax* species are known for over 150 bioactive compounds with insecticidal, antibacterial, and anti-inflammatory properties (Liu et al., 2018).

*Elaeagnus angustifolia* is rich in nutrients, antioxidants, and minerals, with antimicrobial activity against *E. coli*, *S. aureus*, *P. aeruginosa*, *Y. enterocolitica*, coagulase-negative *Staphylococci*, and fungal pathogens like *Alternaria solani* and *Aspergillus* species (Farzaei et al., 2015a; Khan et al., 2016; Okmen & Turkcan, 2014; Bahraminejad et al., 2015). High levels of caffeoylquinic acids and flavonoids were found in Elaeagnus (Zengin et al., 2022), and 95 secondary metabolites were identified, including chlorogenic acid, apigenin, and hyperoside (Bitew & Hymete, 2019), with antimicrobial activities Uzelac et al., 2023). *Elaeagnus* grows in dry, stony habitats in southern and eastern Europe and western Asia (Loizeau & Jackson, 2017), *Melissa* which extensively distributed in the Mediterranean region (Pouyanfar et al., 2018), *Styrax* species habitats in the Asian region (Paparella et al., 2024) Echinops habitas in primarily in Africa and Asia (Elserag et al., 2024).

***Shelf Life and Sensory Testing of Bread Enriched With MAPS***

The shelf life of unadulterated bread and bread made from TAP were observed for 7 days in two different conditions as in polythene packaging (Table 1) and outdoor (Table 2). The shelf life of bread with MAPs was tested over 7 days. The results of the functional bread trial; these plants were used in bread making and the results are given in Table 1.

Table 1. Mould formation on the functional bread by MAPs kept in the polyethylene packaging and out-door for 1 week

| Fonctional Bread | Additive MAP form | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day7 |
|---|---|---|---|---|---|---|---|---|
| In-door (polythene packaging) | | | | | | | | |
| Control | - | -* | - | X | | | | |
| *Styrax* | Fruit | - | - | - | - | X | - | - |
| | Seed | - | - | X | | - | | |
| *Elaeagnus* | Fruit | - | - | - | X | - | | |
| | Seed | - | - | - | - | - | - | X |
| *Melissa* | Leaf | - | - | - | - | - | - | X |
| *Echinops* | Flower | - | - | - | - | X | | |
| Out-door | | | | | | | | |
| Control | - | -* | - | - | - | - | - | - |
| *Styrax* | Fruit | - | - | - | - | X | - | - |
| | Seed | - | - | - | - | X | - | - |
| *Elaeagnus* | Fruit | - | - | - | - | - | - | - |
| | Seed | - | - | - | - | - | - | - |
| *Melissa* | Leaf | - | - | - | - | - | - | - |
| *Echinops* | Flower | - | - | - | - | - | X | - |

*: Not mouldy (-), Mouldy (X).

The shelf life of unadulterated bread and bread made with TAP was monitored over 7 days under two conditions: polythene packaging and outdoor exposure (Table 1). Outdoors, additive-free bread developed mould on day 3, *Styrax* fruit extract delayed mould to day 5, and seed extract showed mould on day 3. *Elaeagnus* fruit extract prevented mould until day 4, while the seed extract and *Melissa* remained mould-free for 7 days. *Echinops* developed mould on day 5.

In open air, additive-free bread developed mould on the 1st day. Bread with *Styrax* extracts developed mould on day 5, while *Elaeagnus* and *Melissa* prevented mould throughout the 7-day period. *Echinops* showed mould on day 6. Overall, *Elaeagnus* and *Melissa* were the most effective, particularly outdoors. Additive-free bread was the most susceptible to mould. Plant extracts, especially seeds, enhanced mould resistance, with better results in indoor storage.

Table 2. Sensory test of functional bread enriched with MAPs in comparison with a standard bread. 12 Participants and percent likeability

| | MAPs | How did the bread taste? | | | | |
|---|---|---|---|---|---|---|
| MAPs | | 5* | 4 | 3 | 2 | 1 |
| *Styrax* | Fruit | 25 | 58 | 17 | | |
| | Seed | 42 | 58 | - | - | - |
| *Elaeagnus* | Fruit | 17 | 83 | - | - | - |
| | Seed | 25 | 67 | 8 | - | - |
| *Melissa* | Leaf | 67 | 33 | - | - | - |
| *Echinops* | Flower | 25 | 42 | - | 33 | - |

*I like it very much: 5, I like it: 4, Undecided: 3, I don't like it: 2, I don't like it at all: 1

*Elaeagnus* and *Melissa* emerged as the most effective and preferred components for both shelf life enhancement and sensory qualities. They demonstrated strong mould resistance, especially outdoors, and received high ratings from participants. Additive-free bread was the least durable and received moderate sensory acceptance, highlighting the potential of plant extracts as functional additives in bread formulations. Mahboubi (2018) found *E. angustifolia* extracts (200–600 mg daily) beneficial for osteoarthritis. *Melissa* (Lemon balm) has antibacterial, sedative, and antispasmodic properties, aiding with stress and gastrointestinal issues (Zam et al., 2022; Ohiagu et al., 2021).

The observations of the breads evaluated in terms of shelf life, which continued for 7 days by looking at the breads 2 times every day in the morning and evening to see whether mould formed on them or not, are given in Tables 2

The breads with herbs were firstly subjected to sensory test. Twelve person participated in the sensory test of bread enriched with MAPs. *Styrax* seeds were similarly well received, with 42% giving them a 5 and 58% a 4. Bread with *Elaeagnus* seeds had the highest acceptance, with 17% rating it 5 and 83% rating it 4. The seeds of *Elaeagnus* received a slightly lower score, with 25% giving it a 5, 67% giving it a 4 and 8% being undecided. Bread made with *Melissa* received very positive feedback, with 67% of participants giving it a 5 and 33% a 4. However, bread made with *Echinops* showed mixed reactions, with only 25% giving it a 5, 42% giving it a 4 and 33% giving it a 1 ("don't like it at all"). The results show the most favourable components to be *Elaeagnus* fruit and *Melissa*.

Melissa officinalis, known for its antimicrobial activities, shows potential as a natural food preservative due to its ability to suppress several foodborne pathogens. Studies indicate its efficacy in various food matrices, suggesting that it could extend shelf life and provide a safer alternative for food preservation (Carvalh et al., 2021). Similarly, phytochemical investigations of Styrax species have revealed lignans and triterpenoids with diverse biomedical properties, such as antifungal activity against Candida albicans and reduction of DNA damage in liver cells. These findings suggest that Styrax species could also be used as food additives (Son et al., 2021).

In addition, Elaeagnus seeds, which contain 50.87% oil and 36.27% protein, are rich in unsaturated fatty acids, particularly linoleic and oleic acids. Its proteins, especially alkaline protein, have essential amino acid profiles and functional properties comparable to those of soy protein isolate. On the other hand , *Echinops* setifer extract (ESE) is a bioactive source with health benefits. When added to yoghurt, it improved texture, mouth feel and functional properties. Extracts at concentrations of 30% and 40% maintained high viable counts and overall quality during 15 days of storage, highlighting their potential to improve both the nutritional value and functionality of yoghurt (Shirani et al., 2022).

## Conclusion

Studies have highlighted the significant potential of aromatic and medicinal plants such as *Melissa, Elaeagnus*, *Echinops* and *Styrax* in the improvement of food quality and safety. Their essential oils and extracts are valuable for improving shelf life and ensuring food safety, as they possess potent antibacterial, antioxidant and antifungal properties. These plants can act as functional ingredients in foods, providing natural preservatives that extend product life while inhibiting the growth of harmful microorganisms. When incorporated into functional foods such as bread, these plants offer not only nutritional benefits but also psychobiotic, therapeutic and protective properties, in line with advances in biotechnology to create versatile foods that contribute to overall health and well-being. Optimising the extraction and application of bioactive compounds from these plants, improving their scalability and ensuring their efficacy in food preservation, safety and quality will be the focus of future biotechnology developments. Medicinal and aromatic plants have the potential to revolutionize the food industry by providing safer, more sustainable, and natural alternatives to synthetic additives and promoting healthier, longer-lasting foods.

## Declarations

### *Ethical Approval Certificate*

The authors declare that ethical approval is not required for this research.

### *Author Contribution Statement*

Nurten Yılmaz.: Supervision, Data collection, investigation, formal analysis, and writing the original draft review and editing.

### *Fund Statement Conflict of Interest*

All authors declare that there is no conflict of interest related to this article.

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# The Effects of Carazolol Injection During Mating Following Progestagen Administration on Conception Rate in Lactating Lacaune Ewes

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ABSTRACT

The aim of the present study was to determine the the effects of carazolol injection during mating following progestagen + eCG administration on conception rate in breeding season Lacaune ewes. Sixty-two Lacaune ewes were used in the study. On day 0, an intravaginal sponge containing 20 mg flugestone acetate was inserted and left in place for 10 days. On day 10, the sponges were removed, and each ewe received an intramuscular injection of eCG at a dose of 480 IU. Ewes in estrus were mated with a proven rams (ewe:ram ratio of 5:1). Fifty-nine mated ewes were randomly assigned into two groups: control and treatment. In the carazolol group (n = 30), ewes received an intramuscular injection of carazolol (0.01 mg/kg; Simpanorm, Fatro, Italy) on the day of estrus, post-mating. In the control group (n = 29), ewes were not administered any treatment. Pregnancy diagnosis was conducted in all ewes on days 30 and 60 post-mating using transabdominal ultrasonography with a Hitachi EUB-405 device equipped with a 3.5 MHz convex probe. The estrus rate, conception rate, total pregnancy rate, and early fetal death rate were evaluated across all groups. The study results indicated no statistically significant differences between the control group and the carazolol group regarding conception rates (62.1% vs. 66.7%) and total pregnancy rates (79.3% vs. 83.3%). In addition, as a result of repeated ultrasound examinations, no early fetal death was found in the study. In conclusion, carazolol injection during mating following progestagen + eCG administration did not increase on fertility in breeding season Lacaune ewes.

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## Introduction

During the breeding season and outside the breeding season, estrus synchronization and estrus stimulation protocols are used to ensure that sheep become pregnant, allowing births to occur at the desired time. With these protocols, litter size can be increased, production can be scheduled according to periods of increased demand for meat and milk, and continuous production can be maintained throughout the year with three lambing in two years (Alaçam, 1993; Fındık, 2017; Takcı et., 2023; Uçar & Özyurtlu, 2012). Various hormone protocols are used in estrus synchronization/stimulation in order to control reproduction in ewes (Arıkan et., 2021). According to a meta-analysis study, it was reported that the progestagen +eCG protocols was the most effective method in ensuring estrus synchronization and desired pregnancy rates during the breeding season, and the pregnancy rate was 90.3% on average (Arıkan et., 2021).

The pregnancy rate can be increased by adding different hormones/drugs to these protocols. In order to prevent embryonic losses and increase reproductive performance in ewe, GnRH or hCG (Hashem et., 2015; Khan et., 2007; Kutlu & Dinç, 2021; Lashari & Tasawar, 2013; Olfati & Moghaddam, 2013; Sirjani et., 2012; Walker et., 1989) can be injected at various times after removal of the sponge. It is also reported that NSAIDs can be used after mating in farm animals to prevent luteolytic PGF2α release as a result of insufficient IFN-τ secretion (Aké-López et., 2005; Binelli et., 2001; Erdem & Guzeloglu, 2010). Successful results are not always obtained after additional hormone/drug application used in studies to increase reproductive efficiency. Mefepronic acid injection in addition to the progestagen + eCG protocol did not have an enhancing effect on reproductive parameters in ewes (Kutlu et., 2023). Further studies need to be continued in order to reach 100% pregnancy rate with estrus synchronization in ewes.

Carazolol (l-[carbazol-4-yloxy]-3-isopropylamino-2-propanol) is a beta-adrenergic antagonist that closely resembles adrenaline in structure. Following intramuscular administration, it is rapidly absorbed and exhibits a high clearance rate (Abshagen & Möllendorff, 1980). Carazolol has a strong affinity for both beta-1 and beta-2 adrenergic receptors (Bartsch et., 1981) and maintains its blocking effect for approximately 12 hours (Wengert, 1978). Carazolol has been reported to be used in many animal species, especially pigs, to treat stress-induced circulatory disorders; to reduce fetal deaths resulting from weak uterine contractions; to shorten the duration of parturition in multiple litters and thus reduce litter loss; to artificially inseminate females (Ballarini & Guizzardi, 1981; Enginler et., 2024; Retzer, 1987). Carazolol increases uterine contractions when used by iv injection in pigs (Rudloff & Bostedt, 1984).

Internal and external stress factors negatively affect many physiological events in ewes, and there is a close relationship between them and infertility (Gürbulak et., 2016). Intravaginal sponge application and estrus synchronization in ewes provide a significant advantage in grouping lambing periods. However, it should not be forgotten that these methods may create some stress factors. In particular, the presence of people in the barn during intravaginal sponge application and hand-mating method may cause stress on the animals (Acharya et., 2022).

During the process of hand-mating with rams, the animal responds to the noise, rough behavior or strain etc., in the barn with an increase in adrenaline secretion. Thus, as a result of the stimulation of beta 2-adrenoreceptors in the myometrium by adrenaline, the effect of oxytocin, which provides uterine contractions, is eliminated and the uterine tone weakens, as a result of which the passage time of spermatozoids through the genital canal is prolonged and they age. It has been determined that if fertilization occurs with old and fertile spermatozoids, the resulting zygote cannot continue its life and dies (Kırşan et., 1998). In addition, frequent ejaculation in rams leads to a significant decrease in volume density and number of sperm per ejaculate. Therefore, excessive use of rams in hand mating could negatively impact sperm volume and fertility outcomes (Jennings & McWeeney, 1976).

This study aimed to assess the impact of carazolol injection during mating on some reproductive parameters in Lacaune ewes following progestagen and eCG treatment during the breeding season.

## Materials and Methods

### *Animals and Management*

This study was conducted in a commercial sheep farm (Lat:37°49'15.31" N, Long: 34°016'25.49" E and Alt: 1.043 m) in Konya province in Türkiye during the breeding season (November) in 2024.

A total of sixty-two Lacaune lactating ewes, clinically healthy, 2‑5 years old with 50‑60-kg body weight having 120-130 days postpartum, were used. The ewes were milked twice a day, at 07:00 in the morning and 18:00 in the evening. The average milk yield was 1 kg per ewe per day. All ewes were fed a balanced diet consisting of 60% roughage and 40% concentrate feed. The roughage portion included equal parts wheat straw and alfalfa straw (50:50). Diets were formulated to meet the energy and protein requirements of the ewes using OptiTMR Pro 4.0.33, with *ad libitum* access to water.

### *Synchronization, Mating Protocols and Treatment Groups*

An intravaginal sponge containing 20 mg flugestone acetate (Chronogest® CR, MSD, France) was inserted for ten days (n= 62). On day 10, all ewes received an intramuscular injection of 480 IU eCG (Chronogest/PMSG, MSD, France). Estrus detection was carried out using a teaser ram, which was introduced to the ewes twice daily for 1-hour sessions over 24 hours following sponge removal. Ewes showing signs of estrus were hand-mated with one of the proven rams at a ewe-to-ram ratio of 5:1. Fifty-nine mated ewes were randomly assigned into two groups: control and treatment. In the carazolol group (n = 30), ewes received an intramuscular injection of carazolol (0.01 mg/kg; Simpanorm, Fatro, Italy) on the day of estrus, post-mating. In the control group (n = 29), ewes were not administered any treatment.

### *Ultrasonographic Examination*

Ewes were considered pregnant if the presence of gestational sac, embryo/fetus, offspring fluids and placentomes were observed in the transabdominal pregnancy examination performed on the 30th day and 60th day after mating. In ultrasonographic (USG) examinations, a real-time B-mode ultrasound device (Hitachi EUB-405, 3.5 MHz convex probe) was used by the same person.

### *Determination of Reproductive Performance*

Estrus rate (ER), conception rate (CR), total pregnancy rate (TPR) and early fetal death rate (EFDR) were calculated as reproductive parameters as follows;

$$\text{ER} = \frac{\text{NEB}}{\text{the number of total ewes}} \times 100$$

NEB: The number of ewes showing estrus behaviors

$$\text{CR} = \frac{\text{the number of pregnant ewes}}{\text{the number of mated ewes}} \times 100$$

$$\text{TPR} = \frac{\text{NP}}{\text{the number of mated ewes}} \times 100$$

NP: the number of pregnant ewes at 1st service, 2nd service and 3rd service

$$\text{EFDR} = \frac{\text{number of detected death fetus}}{\text{the number of pregnant ewes}} \times 100$$

### *Statistical Analysis*

For analyzing the calculated reproductive parameters, Chi-squared test, Fisher's exact test using the Proc GENMOD function of SAS 9.8 software. Results were reported as percentages. Statistical significance was defined as $P < 0.05$.

## Results

Results for estrus rate, conception rates, total pregnancy rate and early fetal death rate are presented in Table 1. Treatment did not affect any parameter ($P > 0.05$).

Table 1. Reproductive performance parameters of ewes

| | Control Group (n = 29) | Carazolol Group (n = 30) |
|---|---|---|
| Estrus rate (%) | 95.2 (59/62) | |
| Conception rate at 1st service on 30 post-mating day (%) | 62.1 (18/29) | 66.7 (20/30) |
| Conception rate at 1st service on 60 post-mating day (%) | 62.1 (18/29) | 66.7 (20/30) |
| Total pregnancy rate (%) | 79.3 (23/29) | 83.3 (25/30) |
| Early fetal death rate (%) | 0 (0/18) | 0 (0/20) |

* There were no significant differences between groups (P>0.05)

## Discussion

Intravaginal sponges containing progestagen with eCG are still the most widely used tools for initiating estrus stimulation in sheep flocks (Wildeus, 2000). In the presented study, the estrus rate was found to be 95.2% in ewes included in this study. Synchronization studies using different doses of progestagen+eCG report estrus rates between 64% and 100% in ewes (Hameed et., 2021). However, the success rate of this hormone protocol depends on many factors including breed, care and feeding conditions, season, lactation status, etc., and very different success rates are obtained in the studies carried out (Kutlu & Dinç, 2021).

Embryonic or fetal deaths in ewes cause major economic losses. According to research, embryonic and fetal death rates are approximately 30% in ewes (Dixon et., 2007; Fthenakis et., 2012). There is limited information on early fetal deaths in ewes, with rates ranging from 3.5% to 12% (Jones et., 2016). In the presented study, no early fetal death was observed in any animal during repeated ultrasonographic examinations on the 30th and 60th days after mating. Köse and Tekin (2022) reported the early fetal death rate as 6.1% in their study in which they performed two repeated examinations on the 35th day and the 50th day. In the presented study, it is thought that there was no early fetal death due to high animal welfare.

Pregnancy rate is a key indicator of reproductive performance and plays a crucial role in determining the economic profitability of farms. In the present study, it was found that conception rates and total pregnancy rate did not differ between in groups (P>0.05), indicating that carazolol had no effect on pregnancy. According to a meta-analysis study, with progestagen + eCG protocols, pregnancy rates in ewes in Turkey are 90.3% during the breeding season and 59.3% out of the breeding season (Arıkan et al., 2021). The conception rate and total pregnancy rate in the control group were acceptable. Carazolol is a β-adrenoceptor blocking drug that is structurally an analogue of adrenaline from the catecholamines (Borchard, 1998; Januszewski, 1985). Beta-adrenoreceptor blocking drugs interact with β-adrenoreceptors to form a drug-receptor complex, and it has been reported that they prevent endogenous catecholamines secreted especially under stress conditions from combining with β2-adrenoreceptors, and that this combination occurs in the form of competitive antagonism (Bademkıran & Horoz Kaya, 2006; Borchard, 1998; Costin et., 1983). Carazolol binds reversibly to beta receptors without causing adrenergic effects and blocks the action of catecholamines released during stress because their action sites are now saturated. Practically, when carazolol is applied, the negative effects caused by stress in the animal are reduced and the parameters are returned to normal physiological limits (Güneşli, 2018). In previous studies Hammer and Rüsse (1990) reported that a study involving 1.171 cows demonstrated that administering carazolol via injection into the cranial epigastric vein improved conception and pregnancy rates by approximately 10% in herds experiencing stress-related infertility. Kırşan et al (1998) reported that they applied carazolol administration via the vena coccygea to cows just before artificial insemination that uterine tonus increased in the carazolol group and the conception rate increased by 19% in the carazolol group compared to the control group. However, Pancarcı et al (2008) reported that although carazolol administration via the jugular vein five minute prior to timed artificial insemination increased uterine tonus in synchronized cows, it did not lead to any significant differences in conception rates. Gündüz et al (2010) in their study in which they inseminated Kıvırcık ewes with natural service, frozen or fresh semen, and in all three service methods, the lambing rate was 56% for control and 63% for carazolol, with no statistical difference between the groups. They reported that 0.5 mg carazolol intramuscular injection before natural mating or insemination did not improve the lambing rate. While these results obtained in the presented study are compatible with those reported in some studies (Gündüz et., 2010; Pancarcı et., 2008), they are also inconsistent with others (Hammerl & Rüsse, 1990; Kırşan et., 1998). These different results may be due to differences in species and breeds.

## Conclusion

In conclusion, the present study showed that carazolol injection during mating following progestagen + eCG administration did not increase pregnancy in breeding season Lacaune ewes. Further studies with different breeds, different dosages and different administration methods are necessary to evaluate this findings.

## Declarations

### *Ethical Approval Certificate*

The experimental procedures of this study were approved by the Local Animal Care and Ethics Committee of Selcuk University (November 2024 - 176/2024).

### *Author Contribution Statement*

Conceptualization: Metehan Kutlu, Neffel Kürşat Akbulut; data curation: Metehan Kutlu; formal analysis and investigation: Metehan Kutlu; funding acquisition: Metehan Kutlu; investigation: Metehan Kutlu; methodology: Metehan Kutlu, Neffel Kürşat Akbulut; project administration: Metehan Kutlu, Neffel Kürşat Akbulut; resources: Metehan Kutlu; Software: Metehan

Kutlu; supervision: Metehan Kutlu; Neffel Kürşat Akbulut; validation: Neffel Kürşat Akbulut, Metehan Kutlu; visualization: Metehan Kutlu; writing—original draft preparation: Metehan Kutlu; writing—review and editing: Metehan Kutlu, Neffel Kürşat Akbulut.

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***Conflict of Interest***

The authors declare no competing interests.

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# The Impact of PEG-induced Drought Stress on Seed Germination and Initial Seedling Growth of *Lupinus albus* L.

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ABSTRACT

Drought is regarded as one of the most significant abiotic constraints to agricultural crop output worldwide. Drought in the spring and early summer, which coincides with important reproductive stages, severely limits lupin yield in Mediterranean climate zones. The purpose of this study was to determine how different drought treatments affected seed germination and initial seedling growth in *Lupinus albus* L. (white or field lupin). Seed germination parameters and initial seedling growth traits were tested against five levels of drought stress induced with Polyethylene glycol-6000 ($PEG_{6000}$) at concentrations of 0, 4, 8, 12, and 16%. An experiment with four replications was conducted using a completely randomized design. The results revealed that the negative effect of drought stress started at 4% (-0.03 MPa or -0.3 bar) treatment for the initial seedling growth stage; whereas, it started at 12% (-0.2 MPa or -2 bar) treatment for the germination stage. Therefore, it was determined that *L. albus* was more sensitive to drought stress at the initial seedling growth stage than at the germination stage. However, it was observed that the growth parameters were more sensitive in shoot growth than in root growth to drought stress. There will be a sharp loss of yield in soils with levels of drought stress imposed by 12% $PEG_{6000}$ (-0.2 MPa-moderate drought-) and beyond. Therefore, it is likely that *L. albus* has low drought tolerance.

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## Introduction

Abiotic stresses such as drought, together with rising global population and per capita food consumption, pose a threat to global food supplies. Drought and other abiotic stressors reduce production and plant growth. They inhibit photosynthesis, limiting the supply of photosynthetic assimilates and energy to the plant. Plants must maximize their utilization of this limited supply of nutrients in order to live under stress (Bibi et al., 2012). Therefore, it is crucial to understand how plants react to drought in order to predict crop germination and establishment under conditions of limited water supply.

Chemical compounds such as polyethylene glycol (PEG), mannitol, sodium chloride (NaCl), sucrose, and glucose are frequently used to imitate osmotic stress during the germination stage (Kouighat et al., 2021). Polyethylene glycol (PEG) molecules with a molecular weight of 6000 are non-ionic, static, and cell-impermeable. They are small enough to impact osmotic pressure, but large enough that they are not absorbed by plants. As a result, they are commonly used. $PEG_{6000}$ is employed in the current work to induce osmotic stress in seedlings (Jamil et al., 2019).

*Lupinus albus* L. (also known as white or field lupin), a cool-season grain legume, is gaining popularity in European agriculture as a means of increasing dietary protein availability, addressing the severe lack of protein feedstocks, and improving agricultural sustainability (Annicchiarico et al., 2018). The adaptation of white lupin to extreme drought is crucial in Mediterranean climate zones, as this stress coincides with critical reproductive stages. Drought stress is expected to spread throughout the Mediterranean basin and into Europe as a result of reduced rainfall and higher evapotranspiration caused by climate change. The information available on the amount of white lupin genetic diversity for drought resistance is limited and mostly concerns landrace material (Pecetti et al., 2023). Despite all these important features, as in other plants, *Lupinus albus* L. is significantly affected by drought (Slabu et al., 2010). Despite its ability to recover quickly from severe water deficit (Pinheiro et al., 2004), *L. albus* suffers yield losses due to drought in the field, typically ranging from 30% to 90% (Hussain et al. 2019; Dietz et al. 2021). In addition, according to Annicchiarico et al. (2018),

drought causes a 79% loss in grain yield in *Lupinus albus* L. Moreover, drought, salt, and mineral fertilizer deficit are major limitations for lupin production (Yu & Rengel 1999). Therefore, it is important to determine the response of white lupine to drought. However, there are a limited number of reports available on the germination and initial seedling growth of *L. albus* under drought stress. Although many species respond to drought conditions, stress thresholds may change depending on genotype (Kayaçetin, 2022a). Studies generally focus on narrow-leafed lupin (*Lupinus angustifolius* L.). Therefore, this study was conducted to test the germination and initial growth parameters of L. albus in different $PEG_{6000}$-induced drought levels under laboratory conditions.

## Materials and Methods

### *Material*

Seeds of *Lupinus albus* L. acquired from RLP AgroScience GmbH (Germany) were used as plant material in the study. This research was conducted at Kırşehir Ahi Evran University (Türkiye), Faculty of Agriculture, Department of Soil Science and Plant Nutrition.

### *Germination Tests and Morphological Observations*

Test solutions were prepared with distilled water at concentrations of 0% (control), 4%, 8%, 12%, and 16% $PEG_{6000}$. According to Michel and Kaufmann (1973), this osmotic potential equivalent is -0.3 bar (-0.03 MPa), -1.03 bar (-0.1 MPa), -2.01 bar (-0.2 MPa), and -3.3 bar (-0.33 MPa). Four replicates of 25 seeds were germinated between three rolled filter sheets using 10 mL of the relevant test solutions. Before planting, seeds were given a fungicide treatment (Tetramethylthiuram disulphide -80%-), and the papers were changed every two days to minimize $PEG_{6000}$ buildup (Rehman et al., 1996). To prevent moisture loss, the rolled paper with seeds was placed in sealed, clear plastic bags. For 21 days, seeds were allowed to germinate at 20 ± 1ºC (Perisse et al., 2002) in the dark. The radicles were deemed to have germinated when they reached a length of approximately 2 mm. For ten days, the germination percentage was tracked every 24 hours (Şehirali and Yorgancılar 2011).

The equation Germination percentage (GP) was used to calculate the percentage of seeds that germinated after being subjected to drought stress (Al-Enezi et al., 2012).

$$GP = (NGS/TNS) \times 100 \quad (1)$$

NGS: Number of germinating seeds
TNS: Total number of seeds

Mean germination time (MGT) was calculated following Ellis and Roberts (1980) to assess the rate of germination.

$$MGT = \Sigma Dn/\Sigma D \quad (2)$$

Where n is the number of the seeds newly germinated on day D, and D is the number of days from the beginning.

The speed of germination serves as an index for the germination rate (GRI) determined using the following equation (Maguire 1962):

$$GRI = \Sigma NGS/ \Sigma ND \quad (3)$$

NGS : Number of Germinated Seeds
ND : Number of Days

Seedlings with stunted primary roots and short, thick, spiral-shaped hypocotyls were deemed to have aberrant germination. Initial seedling growth parameters (shoot and root length, shoot and root fresh weights, shoot and root dry weights, shoot and root dry matter, shoot and root water content, and seedling vigor index) were measured after the 21st day. Samples were dried in an oven at 70°C for 48 hours before dry weights were calculated (Beyaz et al., 2011).

$$SVI = (ARK+AHL) \times (GP) \quad (4)$$

SVI : Seedling vigor index
ARL : Average root length
AHL : Average hypocotyl length
GP : Germination percentage
(Abdul-Baki and Anderson, 1973)

Water content (WC) and dry matter (DM) were calculated formulas;

$$WC = (FW-DW)/FW \times 100 \quad (5)$$
(Zheng et al., 2008)

$$DM = (DW/FW) \times 100 \quad (6)$$
(Bres et al., 2022), respectively.

FW : Fresh weight
DW : Dry weight

### *Statistical Data Analysis*

The design completely randomized design with four replications. All recorded data pertaining to seed germination and seedling growth performance were statistically evaluated using IBM SPSS version 22.0 software (Bertsouklis et al. 2022). An analysis of variance (ANOVA) and Duncan's multiple range test were used to investigate potential treatment changes ($p \leq 0.05$). To ensure the homogeneity of the variance, the data of parameters that were calculated as percentages were arcsine transformed before the statistical analysis (Snedecor & Cochran, 1967). The standard error (SE) was computed for each treatment.

## Results and Discussion

### *Effects of Drought Stress on Germination*

The crucial stage of a plant's life cycle is germination, which is influenced by several variables, particularly hormones, light, temperature, and moisture availability. The biggest limiting factor for the growth of plants and the germination of seeds, however, is a lack of water.

Drought has a negative impact on the metabolic processes that affect seed germination and ultimately delay the establishment of seedlings. According to several studies, PEG6000 is frequently used to imitate the effects of drought, particularly on seed germination (Ahmed et al., 2022). In this study, the germination of *Lupinus albus* L. in different $PEG_{6000}$ percentage treatments was tested under laboratory conditions. The evolution of the early seed germination parameters according to different percentage treatments of $PEG_{6000}$ is shown in Table 1. The statistical data showed that the effect of increasing percentages of $PEG_{6000}$ on germination percentage (GP), mean germination time (MGT), and germination rate index (GRI) for L. albus seeds was significant ($p \leq 0.01$). Due to increasing percentages of $PEG_{6000}$ in treatments, GP and GRI values decreased while MGT values increased. At 12% $PEG_{6000}$, GP started to decrease by 13.4%. This decrease was sharper (46.7%) in the 16% $PEG_{6000}$concentration. A high germination index is beneficial as it predicts seed strength (Kayacetin, 2022b). Considering the results, the maximum GRI (6.47%) was obtained from the control group, while the lowest germination index (1.20%) was obtained with 16% $PEG_{6000}$ treatment. When the highest drought level (16% $PEG_{6000}$) was compared with the control group, it was determined that MGT increased by 28.09%, while GRI decreased by 81.45%. Overall, when these three germination-parameter data are considered, it was observed that the germination of *Lupinus albus* L. seeds was adversely affected after 12% $PEG_{6000}$, and this adverse effect was even more severe at 16% $PEG_{6000}$. The decline in germination due to the effect of high $PEG_{6000}$concentrations (-0.6 and -0.8 MPa) is in agreement with the previous studies of Perisse et al. (2002) on the $_{L. albus}$ local cultivar "Prima" in Argentina.

The present results agree with those reported by Kaya et al. (2006), who observed that an increase in drought conditions, such as exposure to -0.6 MPa $PEG_{6000}$, induces a reduction in germination parameters of sunflower. In addition, Kayaçetin (2022a) stated that $PEG_{6000}$ treatments (-0.2 and -0.4 MPa) inhibited seed germination in the safflower genotypes.

Table 1. The impact of different percentages of $PEG_{6000}$ on seed germination and growth traits of 21-day-old seedlings.

| T# | GP | MGT | GRI | SL | RL |
|---|---|---|---|---|---|
| | ---%--- | ---day--- | ---%--- | ----------cm---------- | |
| 0 | 100.0±0.00$^{a}$ | 5.98±0.04$^{b}$ | 6.47±0.72$^{a}$ | 8.38±2.20$^{a}$ | 6.16±0.76$^{a}$ |
| 4 | 100.0±0.00$^{a}$ | 6.37±0.58$^{b}$ | 5.34±1.97$^{a}$ | 4.78±1.71$^{b}$ | 5.53±0.92$^{ab}$ |
| 8 | 100.0±0.00$^{a}$ | 6.42±0.21$^{b}$ | 4.79±1.21$^{a}$ | 4.13±0.11$^{ab}$ | 5.60±0.60$^{ab}$ |
| 12 | 86.6±23.09$^{a}$ | 7.55±0.05$^{a}$ | 2.07±0.37$^{b}$ | 1.96±1.34$^{bc}$ | 4.55±0.22$^{bc}$ |
| 16 | 53.3±11.54$^{b}$ | 7.66±0.00$^{a}$ | 1.20±0.00$^{b}$ | 1.11±0.19$^{c}$ | 3.94±0.62$^{c}$ |
| Mean | 88.0 | 6.80 | 3.97 | 4.07 | 5.15 |
| Summary of one-way-ANOVA | | | | | |
| $PEG_{6000}$ | ** | ** | ** | ** | ** |
| T# | R/S | SFW | RFW | SDW | RDW |
| | ---%--- | ----------mg/plant---------- | | | |
| 0 | 0.71±0.14$^{c}$ | 1.89±0.23$^{a}$ | 0.22±0.04$^{abc}$ | 0.235±0.04$^{ab}$ | 0.018±0.001$^{b}$ |
| 4 | 1.14±0.12$^{c}$ | 1.39±0.22$^{b}$ | 0.34±0.03$^{a}$ | 0.194±0.00$^{b}$ | 0.039±0.012$^{a}$ |
| 8 | 1.35±0.11$^{bc}$ | 1.37±0.04$^{b}$ | 0.30±0.06$^{b}$ | 0.262±0.05$^{ab}$ | 0.041±0.012$^{a}$ |
| 12 | 3.24±2.26$^{ab}$ | 1.02±0.12$^{c}$ | 0.18±0.07$^{c}$ | 0.257±0.03$^{ab}$ | 0.033±0.000$^{ab}$ |
| 16 | 3.55±1.08$^{a}$ | 0.82±0.10$^{c}$ | 0.12±0.02$^{c}$ | 0.299±0.12$^{a}$ | 0.021±0.003$^{b}$ |
| Mean | 2.00 | 1.30 | 0.23 | 0.250 | 0.030 |
| Summary of one-way-ANOVA | | | | | |
| $PEG_{6000}$ | ** | ** | * | * | ** |

T: Treatments# (%); *Significant at $p \leq 0.05$, **significant at $p \leq 0.01$. Different letters in the same column signify substantial changes at the 0.05 level. #: 4% (-0.03MPa), 8% (-0.1 MPa), 12% (-0.2 MPa), and 16% (-0.3 MPa). Germination percentage (GP), mean germination time (MGT), germination rate index (GRI), shoot length (SL), root length (RL), root to shoot ratio (R/S), shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), and root dry weight (RDW)

Table 2. The impact of different percentages of $PEG_{6000}$ on growth traits of 21-day-old seedlings.

| T# | SDM | RDM | R/S DM | SWC | RWC | SVI |
|---|---|---|---|---|---|---|
| | ----------%---------- | | | | | |
| 0 | 12.53±2.74b | 8.21±0.90b | 0.66±0.03c | 87.46±2.74a | 91.78±0.90a | 1455±449a |
| 4 | 14.23±2.72b | 11.45±0.99ab | 0.82±0.03a | 85.76±2.72a | 88.54±0.99ab | 1031±394a |
| 8 | 19.06±3.48b | 13.44±2.35ab | 0.71±0.07bc | 80.93±3.48a | 86.55±2.35ab | 973±70a |
| 12 | 25.41±5.39ab | 21.48±11.42a | 0.81±0.03ab | 74.58±5.39b | 78.51±11.42b | 651±77ab |
| 16 | 37.79±19.15a | 17.59±3.68a | 0.53±0.08d | 62.20±19.15b | 82.40±3.68b | 441±146b |
| Mean | 21.80 | 14.43 | 0.70 | 78.19 | 85.56 | 910 |
| Summary of one-way-ANOVA | | | | | | |
| $PEG_{6000}$ | * | * | ** | * | * | ** |

T: Treatments# (%); *Significant at $p \leq 0.05$, **significant at $p \leq 0.01$. Different letters in the same column signify substantial changes at the 0.05 level. #: 4% (-0.03MPa), 8% (-0.1 MPa), 12% (-0.2 MPa), and 16% (-0.3 MPa). Shoot dry matter (SDM), root dry matter (RDM), root to shoot dry matter ratio (R/S DM), shoot water content (SWC), root water content (RWC), and seedling vigor index (SVI).


Figure 1. The morphology of 21-day-old seedlings is observed at different percentages of $PEG_{6000}$ treatments.

Pratap and Sharma (2010), Bagheri and Yeganeh (2011), and Baghizadeh and Hajmohammadrezaei (2011) also showed similar restriction of seed germination.

This could be an adaptation mechanism of seeds to limit germination in stressful environments and ensure adequate seedling establishment. It was stated that a decrease in the water potential gradient between seeds and their surrounding media could harm seed germination and subsequent metabolic events in seedling growth and development (Rohamare et al., 2014). Under drought conditions, there was a significant reduction in germination percentage and germination indices, which might be attributed to reduced metabolic processes, such as the synthesis of hydrolytic enzymes and the hydrolysis of dietary material and a delay in both cell division and elongation (Rogan and Simon 1975; Ahmed et al. 2022).

***Effects of Drought Stress on Growth Traits***

Water scarcity leads to a shortage in the plant tissues very quickly. Desiccation also has an impact on a crop's habitus, development, and metabolism. Dehydration causes substantial physiological changes that delay or even stop growth and jeopardize the stability of crop yield. The impact of drought on crop yield may be particularly significant during specific stages of crop development (Dietz et al., 2021). Initial seedling growth is crucial for plant establishment, and plants are susceptible to drought stress at this period (Jamil et al., 2019). In this study, initial growth parameters of 21-day-old seedlings (Figure 1) were tested under different levels of drought stress, using $PEG_{6000}$. SL, RL, R/S, SFW, RFW, SDW, RDW, SDM, RDM, R/S DM, SWC, RWC, and SVI are shown in Table 1 and Table 2. All growth parameters examined were found to be statistically significant. SL was reduced by 42.96%, 50.71%, 76.61%, and 86.75% under 4%, 8%, 12%, and 16% $PEG_{6000}$ treatments, respectively. Compared to the control, RL was reduced by 10.22% with 4%, 9.09% with 8%, 26.13% with 12%, and 36.03% with 16% $PEG_{6000}$ treatments.

The R/S was increased by 60.56% under 4% PEG treatment, 90.14% under 8% PEG, 356.34% under 12% PEG, and 400% under 16% PEG, in comparison with the control group. The results of this research showed that as the drought stress level increased, SL and RL decreased, while R/S increased significantly. Our results are consistent with those obtained by Kayaçetin (2022a), who indicated that drought stress imposed with different levels of $PEG_{6000}$, (-0.2 and -0.4 MPa) decreased SL and RL in sunflower genotypes, while R/S increased. Similarly, Robin et al. (2015) reported that $PEG_{6000}$-treated hydroponic culture significantly decreased the root length of wheat varieties. In addition, Li et al. (2011) stated that the shoot length of pyrethrum (*Tanacetum cinerariifolium*) was significantly affected at osmotic potentials in PEG solutions less than -0.3 MPa. The root-to-shoot ratio is an important metric for understanding how plants distribute biomass to adapt to stress conditions (Taraves et al., 2021). However, a healthy root system not only helps the plant acquire water, which is a key drought adaptation technique, but also helps the plant's above-ground portions establish (Keresa et al., 2008). Root length at the seedling stage provides an accurate prediction of root growth in the field (Ali et al. 2011a,b; Rajendran et al. 2011). According to our results, in terms of elongation, shoots were more affected than roots by increasing drought levels. Drought stress decreased shoot growth more than root growth and, in some situations, enhanced root growth (Salih et al. 1999; Younis et al. 2000; Okçu et al. 2005; Bibi et al. 2010). Shoot length may be reduced due to reduced water absorption and a decrease in external osmotic potential caused by PEG (Kaydan and Yagmur, 2008). However, considering the $PEG_{6000}$ treatment concentrations, the adverse effect on SL and RL was observed to sharply begin at 12%. Reduced seedling growth is caused by restricted cell division and enlargement due to drought stress (Bibi et al., 2012). The reduced root and shoot length might be attributed to decreased cell division and elongation, which resulted in tuberization and lignification, and subsequently inhibited the plant growth process in the stressed environment (Ahmed et al., 2022).

Under drought stress, SFW decreased and RDW increased, while RFW and SDW showed variability (Table 1). It was observed that RFW increased under 8% $PEG_{6000}$ treatment, compared to the control group, and decreased with 12% and 16% $PEG_{6000}$. However, it was recorded that RDW increased with 8% $PEG_{6000}$ treatment, compared to the control group, but then decreased with 12% and 16% $PEG_{6000}$ treatments while remaining higher than the control group. In general, considering these results, the shoots of initial growth stage seedlings of *L. albus* were adversely affected after 4% $PEG_{6000}$ treatment (-0.03 MPa -low drought-), while the roots began to be adversely affected after 12% $PEG_{6000}$ treatment (-0.2 MPa -moderate drought-). These results are also supported by the findings of Kaya et al. (2006), who indicated that seedling fresh weight significantly decreased as the osmotic potential of $PEG_{6000}$ solutions decreased, resulting in shorter shoot and root lengths. Similarly, Toscano et al. (2017), Jamil et al. (2019), and Kayaçetin (2022a) observed a decrease in seedling fresh weights of ornamental sunflowers, rapeseed, and safflower genotypes with increasing $PEG_{6000}$ concentrations or decreasing water potential. Contrary to our expectations, seedling dry weights decreased. Bibi et al. (2012) reported that morphological characters such as SFW, RFW, and RDW at the seedling stage are reduced by water stress in sorghum. In addition, Ahmed et al. (2022) stated that increased $PEG_{6000}$ concentrations (10% and 20%) resulted in decreased SFW and RDW in sesame. Robin et al. (2021) reported that, in support of our results,

that root dry weight increased in wheat genotypes, which they assumed to be drought tolerant with increasing $PEG_{6000}$ concentrations. The ability of shoots to absorb assimilates as drought stress intensifies may contribute to plant survival and seed filling under stress via retranslocation (Annicchiarico et al., 2018). Therefore, contrary to the information in the literature, the increase in drought severity and shoot dry weight (SDW) detected in our research may indicate that L. albus is engaging its adaptation mechanism against drought. Our results show that shoot growth is more negatively affected than the root growth of *L. albus* under drought stress. Supporting our results, Perisse et al. (2002) reported that the shoot-root dry weight was reduced with decreasing osmotic potentials (0.0, -0.4, -0.6, and -0.8 MPa) in *L. albus* cultivar "Prima," and root growth was dominant as well. In addition, Hessini et al. (2009) found that the shoot-to-root ratio, whether based on fresh or dry weight, diminishes due to water stress, suggesting that shoot growth is more susceptible to this stress than root growth in Spartina alterniflora. Moreover, a similar statement was made by Ashraf and Foolad (2007).

$PEG_{6000}$ solution caused a growth reduction in the SL and RL of *L. albus* seedlings. However, the SDM, RDM, and root to shoot dry matter ratio, (R/S DM) increased with rising drought stress (Table 2). Compared with the control plants, all the $PEG_{6000}$ treatments significantly increased SDM by 13.56%, 52.11%, 102.79%, and 201.59%, and RDM by 39.46%, 63.70%, 161.63%, and 114.25%. However, R/S DM increased by 24.24%, 7.57%, and 22.72% under 4%, 8%, and 12% $PEG_{6000}$ treatments, respectively, while it decreased by 19.69% under 16% $PEG_{6000}$. Considering R/S DM, it was observed that there was more dry matter accumulation in the root until 12% $PEG_{6000}$ treatment, however, the dry matter ratio decreased with 16% $PEG_{6000}$ (-0.3 MPa). Therefore, according to these results, there is more accumulation in the root under drought stress for L. albus, and the threshold value of this accumulation is 12% $PEG_{6000}$ (-0.2 MPa). Similarly, Atak et al. (2006) reported that increased osmotic stress (-0.45 MPa) caused a decrease in SL and RL in triticale seedlings and an increase in SDM.

SWC and RWC decreased with rising osmotic stress (Table 2). Compared with the control plants, drought stress decreased SWC by 1.94%, 7.46%, 14.72%, and 28.88%, respectively, and RWC by 3.53%, 5.69%, 14.45%, and 10.22%, respectively, under 4%, 8%, 12%, and 16% $PEG_{6000}$ treatments. Considering these cumulative reduction values in treatments, it is observed that water loss in the shoot is greater than in other parts of the plant. A decrease in water contents of shoots could result from a decrease in water flow from the roots to the respective shoots (Kayaçetin, 2022a). The decrease in the amount of water in roots and shoots can be explained by the decrease in root length, which is due to low water potential, resulting in reduced water uptake capacity of the root. Similarly, Bajji et al. (2000) reported that water contents of the roots and shoots of wheat cultivar seedlings were reduced by water stress.

Increasing concentrations of $PEG_{6000}$ solutions induced a marked reduction in SVI of *L. albus* (Table 2). Compared with the control application, drought stress decreased SVI by 29.14%, 33.12%, 55.25%, and 69.69%, under 4%, 8%, 12%, and 16% $PEG_{6000}$ treatments, respectively. These results showed that SVI decreased drastically at 12% $PEG_{6000}$. Therefore, in terms of SVI, it is understood that *L. albus* will suffer significant yield loss at 12% $PEG_{6000}$ (-0.2 MPa) and concentrations more negative than -0.2 MPa. The findings of Kayaçetin (2022a) confirmed our results, and as the water potential decreased with increasing $PEG_{6000}$ concentrations, a significant decrease in the seedling vigor index adversely affected the vegetative growth of safflower genotypes. In addition, this result was consistent with the findings of Ahmed et al. (2022), which showed the $PEG_{6000}$ induced osmotic impact decreased the vigor index of sesame seedlings. Furthermore, our findings showed a diminution in the SVI after seedlings' exposure to drought, which is in agreement with reports of Kouighat et al. (2021) in mutant sesame, Spielmeyer et al. (2007) in wheat, and Koskosidis et al. (2020) in chickpeas.

## Conclusion

Spring and early summer drought, which coincides with critical reproductive stages, severely limits *L. albus* output in Mediterranean climate zones. Therefore, the response of *L. albus* to drought should be well understood. In this study, germination and initial seedling growth, which are very critical in drought, were examined, as in other abiotic stress factors. As a result of the study, it was determined that the negative effect of drought stress begins at 12% $PEG_{6000}$, (-0.2 MPa) treatment for germination. However, initial seedling growth was adversely affected after 4% $PEG_{6000}$ (-0.03 MPa), and this adverse effect was exacerbated by higher $PEG_{6000}$ treatments (12% and 16%). Therefore, in light of these data, we conclude that the initial seedling growth period in *L. albus* is more sensitive than the germination period under drought stress. However, when looking at shoot and root growth parameters, it was seen that the responses to drought stress were different, and shoot growth was more negatively affected than root growth. Overall, according to our results, *L. albus* would be classified as a species susceptible to drought, especially in the initial growth stage.

## Declarations

### *Author Contribution Statement*

R.B.: Data collection, investigation, conceptualization, methodology and writing the original draft

V.V.U.: Review and editing

### *Conflict of Interest*

The authors declare no conflict of interest.

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# Effect of NaCl-induced Salt Stress at Germination and Early Seedling Growth Stage in *Lupinus albus* L.

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ABSTRACT

Salinity is a primary abiotic factor affecting agricultural productivity in arid and semiarid environments. The stages that are most vulnerable to salinity are germination and early seedling growth. There are limited reports on the responses of *Lupinus albus* L. to salinity. Therefore, in the study, we aim to test germination and early seedling growth of *L. albus* under different salinity levels. To this end, seeds of *L. albus* were treated with different concentrations (0, 50, 100, 150, and 200 mM) of NaCl under laboratory conditions. A total of 16 parameters, including germination and growth, were examined. The results showed that under 200 mM NaCl, germination percentage (GP) decreased by 13.4% and germination rate index (GRI) decreased by 69.24%, while mean germination time (MGT) increased by 30.02%. In addition, the shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RWF), and root dry weight (RDW), root to shoot dry matter (R/S DM), shoot water content (SWC), root water content (RWC), and seedling vigor index (SVI) were reduced respectively by 82.69%, 75.65%, 53.30%, 70%, 66.66%, 70.86%, 23.47%, 0.35% and 82.57% under 200 mM NaCl, compared to the control condition. However, root to shoot ratio (R/S), shoot dry weight (SDW), shoot dry matter (SDM), root dry matter (RDM) were increased by 43.33%, 65.07%, 249.68%, and 3.22% under 200 mM NaCl. Overall, the study results showed that the critical level to mitigate the negative effect of salinity is 150 mM NaCl (-0.6 MPa osmotic potential) for germination and 50 mM NaCl (-0.2 MPa osmotic potential) for growth. Therefore, *L. albus* has a low tolerance to salinity.

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## Introduction

Salinity restricts the dispersal of plants throughout their natural habitats and poses a serious agricultural challenge in many parts of the world (Fernandes et al., 2004). An important environmental issue impacts about 20% (954 million hectares) of agricultural land. The Global Map of Salinity-affected Areas The spatial distribution of salt-affected soils indicates that more than 424 million hectares of topsoil and 833 million hectares of subsoil are affected by salinity. Most salt-affected soils are saline, including 85% of topsoils and 62% of subsoils, while sodic soils account for 10% of topsoils and 24% of subsoils. Significantly almost two-thirds of these salt-affected soils are located in arid and semi-arid regions, comprising 37% in deserts and 27% in arid steppe environments (Singh 2021; FAO 2022; Farooq et al. 2024). The principal salt responsible for salt stress is sodium chloride (NaCl). A diverse array of wild plants is adversely affected by sodium ions, while specific species suffer from elevated chloride concentrations. The germination of seeds and the growth of seedlings are the stages most susceptible to salinity. Salt stress induces detrimental physiological and biochemical alterations that impede seed germination. Seed germination and stand establishment can be affected by oxidative stress, osmotic stress, and ion-specific effects. Salinity hinders seed germination by affecting the mobilization of stored reserves, diminishing water availability, and changing protein structural order (Ibrahim, 2016).

In agriculture, the Leguminosae family is the second most significant family behind the Gramineae (Ozaktan et al. 2023). White or field lupin (*Lupinus albus* L.) is a plant of the Leguminosae family that was domesticated as a pulse crop for human consumption and livestock feed before 2000 BC (Ihsan et al. 2024). Over 400 species are recognized within the genus *Lupinus*, of which only four hold agronomic significance: *L. albus* (white lupin), *L. angustifolius* (blue or narrow-leafed lupin), *L. luteus*

(yellow lupin), and *L. mutabilis* (pearl or Tarrwi lupin). The initial three species are native to the Mediterranean region, encompassing Türkiye (Al-harbi et al. 2014). *Lupins* are grown on an area of 1 million hectares globally, yielding a total production of 1.385 million metric tons (Ihsan et al. 2024). The elevated protein concentration in their seeds renders them a potential alternative to soybean [*Glycine max* (L.) Merr.] in cattle and poultry feed. White lupin is a versatile, climate-resilient pulse crop with an extraordinarily high protein content, rendering it a viable substitute for soybean in livestock feed. The alkaloid concentration in bitter cultivars varies from 5 to 40 g/kg, whereas low-alkaloid cultivars exhibit levels between 0.08 and 0.12 g/kg. Alkaloid-free cultivars of white lupins exist, and the creation of these mutants has facilitated the use of white lupins as a protein source for animals. White lupins possess moderate to high levels of crude protein (202–424 g/kg), crude fat (60–130 g/kg), and fiber (105–162 g/kg) (David et al. 2024). It can grow very well in marginal areas, but despite all these important features, it is reported that its tolerance to salinity is low (Ihsan et al. 2024). However, there are limited reports (Yu and Rengel, 1999; Fernandes et al., 2004; Slabu et al., 2010; Hussien, 2022) on the effects of salinity on species of *Lupinus* genus, including *L. albus*. Therefore, this study aimed to reduce the knowledge gap on early responses of *L. albus* to salt stress. This study aimed to evaluate the germination and early seedling growth of *L. albus* under different salt stress levels in a laboratory setting.

## Materials and Methods

### *Material*

Seeds of *Lupinus albus* L. acquired from RLP AgroScience GmbH (Germany) were used as plant material in the study. This research was conducted at Kırşehir Ahi Evran University (Türkiye), Faculty of Agriculture, Department of Soil Science and Plant Nutrition.

### *Methods*

*Germination tests and morphological observations*

Test solutions were formulated using distilled water at concentrations of 0 (control), 50, 100, 150, and 200 mM NaCl (Sigma-Aldrich). Four replicates of 25 seeds were germinated between three rolled filter sheets using 10 mL of the corresponding test solutions. Before planting, seeds were given a fungicide treatment (Thiram 80%), and papers were changed every two days to minimize salt buildup (Beyaz et al., 2011). To prevent moisture loss, the rolled paper with seeds was placed in sealed, clear plastic bags. For 21 days, seeds were allowed to germinate at 20 ± 1ºC (Perisse et al., 2002) in an incubator in the dark. The radicles were deemed to have germinated when they reached a length of ~ 2 mm. For ten days, the germination percentage was tracked every 24 hours (Şehirali and Yorgancılar 2011).

Germination percentage (GP) was calculated according to Al-Enezi et al. (2012). (GP) = (Number of germinating seeds/ Total number of seeds) × 100 (Eq.1). The mean time to germination (MGT) was determined by Ellis and Roberts in 1980 MGT=∑Dn/∑D (Eq. 2), where 'n' represents the quantity of germinated seeds on day T, and Σn denotes the cumulative sum of germinated seeds. The speed of germination serves as an index for the germination rate (GRI), determined using the following equation (Maguire 1962): GRI = Σ No of Germinated Seeds/ Σ No of Days (Eq. 3). Seedlings with stunted primary roots and short, thick, spiral-shaped hypocotyls were deemed to have aberrant germination.

Early seedling growth parameters (shoot and root length, shoot and root fresh weights, shoot and root dry weights, shoot and root dry matter, shoot and root water content, and seedling vigor index) were measured after the 21st day. Samples were dried in an oven at 70°C for 48 hours before dry weights were calculated (Beyaz et al., 2011). The Seedling Vigor Index (SVI) was computed using the method of Abdul-Baki and Anderson (1973). (SVI) = (average root length + average hypocotyl length) × germination percentage (GP) (Eq. 4). TheWC = (Fresh weight − Dry weight) / Fresh weight × 100 (Eq. 5) (Zheng et al., 2008); as well as dry matter (DM) = (Dry weight / Fresh weight) × 100 (Eq. 6) (Bres et al., 2022), were calculated using these formulas.

*Statistical Data Analysis*

The design used was a completely randomized design with four replications. All recorded data pertaining to seed germination and seedling growth performance were statistically evaluated using IBM SPSS version 22.0 software. An analysis of variance (ANOVA) and Duncan's multiple range test procedure were used to investigate potential treatment differences ($P \leq 0.05$). Data expressed as percentages, underwent arcsine transformation of the square root of X prior to statistical analysis (Snedecor and Cochran, 1967). The standard error was computed for each treatment.

## Results and Discussion

### *Effects of Salt Stress on Germination*

A crucial step in the establishment of seedlings and subsequent plant health and vigor is seed germination (Cokkızgın, 2012). Rapid seed germination and stand establishment are key factors influencing crop productivity in stressful situations (Ibrahim, 2016; Benlioğlu et al., 2024). One of the most significant variables contributing to delayed seed germination and limiting final germination percentage is salinity (Cokkızgın, 2012). In salty soils, most crops will not germinate. Seed germination is the most crucial stage in the plant life cycle and is sensitive to salinity (Zhang et al. 2024). In this study, the effect of five different levels of NaCl-induced salt stress on the germination parameters of *L. albus* L. seed was investigated under laboratory conditions. The evolution of the early seed germination parameters according to different NaCl treatments is shown in Table 1.

The effect of increasing concentrations of NaCl on germination percentage (GP), mean germination time (MGT), and germination rate index (GRI) of lupin seeds is statistically significant (Table 1). Due to increasing NaCl concentrations, GP and GRI values decreased while MGT values increased. GP values (100%) did not change from 0 to 150 mM, but decreased at 200 mM (86.6%). When the control group and the highest salt stress level (200 mM NaCl) were compared, it was determined that MGT increased by 30.02% and GRI decreased by 69.24%.

Table 1. Impact of different NaCl concentrations on germination

| Treatments (mM) | GP | MGT | GRI |
|---|---|---|---|
| | ---%--- | ---day--- | ---%--- |
| 0 | 100.0±0.0a | 4.13±0.13c | 6.47±0.75a |
| 50 | 100.0±0.0a | 4.27±0.17c | 5.39±1.28a |
| 100 | 100.0±0.0a | 4.43±0.18bc | 4.82±0.99b |
| 150 | 100.0±0.0a | 5.05±0.44ab | 2.62±1.16b |
| 200 | 86.6±11.5b | 5.37±0.55a | 1.99±1.07b |
| Means | 97.32 | 4.65 | 4.25 |
| Summary of one-way-ANOVA | | | |
| NaCl | * | ** | ** |

*significant at p≤0.05, **significant at p≤0.01. Different letters in the same column signify substantial changes at the 0.05 level. GP: Germination percentage, MGT: mean germination time, GRI: germination rate index

Table 2. Impact of different NaCl concentrations on seedling growth of 21-day-old *L. albus* seedlings.

| T | SL | RL | R/S | SFW | RFW | SDW | RDW |
|---|---|---|---|---|---|---|---|
| | ----------cm---------- | | ---%--- | ----------mg/plant---------- | | | |
| 0 | 13.35±1.67a | 8.05±1.82a | 0.60±0.09c | 2.42±0.24a | 0.30±0.03a | 0.189±0.06c | 0.030±0.004a |
| 50 | 7.75±2.41b | 4.83±1.25b | 0.64±0.21bc | 1.56±0.30b | 0.22±0.05ab | 0.224±0.02bc | 0.026±0.008ab |
| 100 | 5.40±0.80ab | 3.93±0.90ab | 0.75±0.11abc | 1.30±0.08bc | 0.15±0.03bc | 0.237±0.01b | 0.017±0.003bc |
| 150 | 4.13±1.81bc | 3.60±1.05ab | 0.91±0.07a | 1.14±0.10c | 0.12±0.04c | 0.257±0.01b | 0.013±0.007c |
| 200 | 2.31±0.38c | 1.96±0.37c | 0.86±0.28ab | 1.13±0.07c | 0.09±0.02c | 0.312±0.02a | 0.010±0.003c |
| Means | 6.59 | 4.47 | 0.75 | 1.51 | 0.18 | 0.233 | 0.019 |
| Summary of one-way-ANOVA | | | | | | | |
| NaCl | ** | ** | * | ** | ** | ** | ** |

T: Treatments (mM); *significant at p≤0.05, **significant at p≤0.01. Different letters in the same column signify substantial changes at the 0.05 level. SL: shoot length, RL: root length, R/S: root to shoot ratio, SFW: shoot fresh weight, RFW: root fresh weight, SDW: shoot dry weight, RDW: root dry weight

Table 3. Impact of different NaCl concentrations on seedling growth of 21-day-old *L. albus* seedlings.

| T | SDM | RDM | R/S DM | SWC | RWC | SVI |
|---|---|---|---|---|---|---|
| | ----------%---------- | | | | | |
| 0 | 7.87±0.92d | 9.92±0.20b | 1.27±0.15a | 94.70±3.64a | 90.07±0.20a | 2140±326a |
| 50 | 14.74±3.44c | 11.50±0.84a | 0.81±0.17b | 85.25±3.44b | 88.49±0.27b | 1258±300b |
| 100 | 18.31±0.75bc | 11.33±0.24a | 0.62±0.03bc | 81.68±0.75bc | 88.66±0.24b | 933±64bc |
| 150 | 22.77±2.97ab | 10.42±0.21b | 0.47±0.17cd | 77.22±2.9cd | 89.57±0.21a | 773±271cd |
| 200 | 27.52±2.39a | 10.24±0.59b | 0.37±0.00d | 72.47±2.39d | 89.752±0.43a | 373±80d |
| Means | 18.24 | 10.68 | 0.71 | 82.26 | 89.31 | 1095 |
| Summary of one-way-ANOVA | | | | | | |
| NaCl | ** | ** | ** | ** | ** | ** |

T: Treatments (mM); **significant at p≤0.01. Different letters in the same column signify substantial changes at the 0.05 level. SDM: shoot dry matter, RDM: root dry matter, R/S DM: root to shoot dry matter ratio, SWC: shoot water content, RWC: root water content, SVI: seedling vigor index

It was observed that the germination parameters for Lupinus albus L. were sharply affected by 200 mM NaCl. The present findings are consistent with those made by Cokkızgın (2012), who noted that an increase in salt causes a decrease in the proportion of germinating seeds and a delay in the beginning of the germination process in significant legumes like *Phaseolus vulgaris* L.

Reports indicate that elevated salt content diminishes germination percentage and prolongs germination duration (Jamil et al. 2005; Patade et al. 2011; Rouhi et al. 2011; Ansari and Sharif-Zadeh 2012; Beyaz et al. 2018). The reduction in germination rates under elevated salt concentrations may result from impaired water absorption, the toxic impact of certain ions, or the inactivation of essential enzymes required for germination (Aydinşakir et al. 2013). Salt stress impedes seed germination in plants by somehow leading to or enhancing the hydrolysis of urea produced by arginine (Liang and Jiang 2024).

***Effects of salt stress on morphological characters***

Another stage that is most sensitive to salinity after germination in plants is the early seedling growth stage. The statistical data showed that the effect of increasing concentrations of NaCl on growth parameters such as SL, RL, R/S, SFW, RFW, SDW, RDW, SDM, RDM, R/S DM, SWC, RWC, and SVI is statistically significant (P≤0.01) (Table 2 and Table 3). The SL, RL, SFW, RFW, and RDW were reduced, respectively, by 82.69%, 75.65%, 53.30%, 70%, and 66.66% when treated with 200 mM NaCl, compared to the control. Increasing salt levels had a significant negative effect on early seedling growth of *L. albus* (Figure 1). Similarly, Akladious and Hanafy (2018) reported that 150 and 300 mM NaCl salinity levels caused significant reductions in all growth parameters such as SL, RL, SFW, RFW, SDW, and RDW of *Lupinus termis* L.

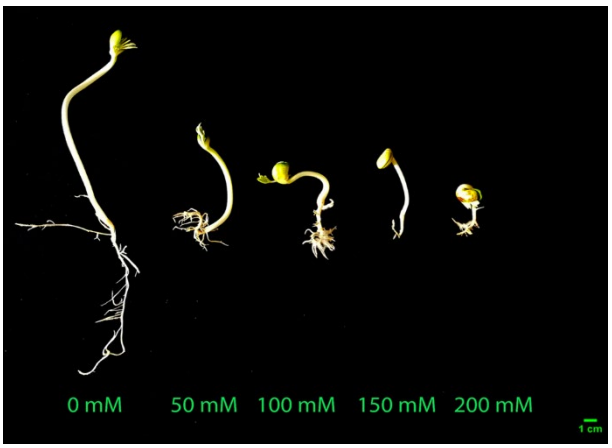


Figure 1. The morphology of 21-day-old seedlings at different NaCl treatments.

Also, Hussien (2022) stated that different growing parameters such as total length of plant, seedling fresh weight, and number of lateral roots were significantly affected by salinity stress (sodium bicarbonate) in *L. albus*. In addition, Slabu et al. (2010) concluded that too much NaCl in the soil affects the vegetative growth of *L. albus* cultivars by decreasing the amount of stored biomass.

Meanwhile, Akladious and Hanafy (2018) stated that the lowest concentration of NaCl (75 mM) caused significant increases in all growth parameters (SL, RL, SFW, RFW, SDW) of *L. termis* as compared with control plants. But the results of the study showed a decrease in these growth parameters (except SDW), beginning with the lowest concentration of NaCl (50 mM) applied to *L. albus*. However, SDW increased with increased salinity levels. Therefore, the results indicated that increased salinity does not always have a negative effect on growth, of different plant organs. Similarly, this was previously approved by Hussien (2022) who concluded that 0.02 M salinity (sodium bicarbonate) increased the fresh weight of 14-day-old *L. albus* seedlings. Also, this agrees with Delgado and Sanchez-Raya (2007), who proved that increasing the salt content does not necessarily harm certain plant organs in sunflower. In addition, according to Yu and Rengel (1999), NaCl concentrations (12.5, 25, 50, and 100 mM) had no effect on SFW and RWF of *L. angustifolius* seedlings; however, increasing NaCl concentrations had an effect on RWF and RDW, causing a decrease in their values. Considering the above parameters, overall shoot growth appears to be more affected by salinity than root growth. However, contrary to these results, Yu and Rengel (1999) in *Lupinus angustifolius* L., Van Steveninck et al. (1982) in *Lupinus luteus* and *Lupinus angustifolius*, and Jeschke et al. (1986) in Lupinus albus L. cv 'Ultra' reported that salinity has a more negative effect on root growth, than in many other plants. Responses to salt stress can vary significantly among plants, within the same species, and even among varieties/cultivars and genotypes of depending on their genetic structure or habitats.

In the current study, the results show that salinity caused an increase in SDM and RDM values, and a decrease in SWC and RWC values of *L. albus* seedlings (Table 2). Similar to these data, Beyaz et al. (2011) reported that salinity (NaCl) caused an increase in dry matter in sainfoin (Onobrychis sativa) seedlings. In addition, Bres et al. (2022) stated that due to salinity, the water content in the *Lactuca sativa* L seedlings was decreasing gradually, whereas the dry matter content was increasing. However, when the DM ratios under salt stress were examined, it was observed that the increase in the shoot was higher than in the root. On the other hand, with respect to the WC, it was determined that the decrease in values was more in shoots (Table 2). However, the R/S DM was significantly ($P \leq 0.01$) reduced with increasing salt levels. According to Beyaz et al. (2016), the decrease in plant tissue water content increases the dry matter ratio in the tissues. Therefore, more dry matter accumulation in the shoots of seedlings may be explained in this way. Salinity typically diminishes the growth of crop shoots more significantly than root growth, as assessed by dry weight rather than length measurements (Shalhevet et al. 1995). These study data showed that salinity affected roots more adversely than shoots in terms of dry weight in seedlings. It has also been reported in previous studies, that salinity affects the roots more negatively than shoots in plants (Kaya et al. 2003; Bandeoğlu et al. 2004).

Salinity significantly ($P \leq 0.01$) decreased SVI of L. albus, which measures the health of seedlings. This decrease in SVI ranged from 2140 to 373 when compared to control and 200 mM (Table 2). The fact that the seedling vigor index decreased as salinity increased suggests that seeds are negatively impacted by salt content (Khan et al., 2022). In addition, the absorption of essential nutrients like phosphorus (P) and potassium (K) is inhibited by salt, which has been observed to have an adverse effect on seedling growth and vigor index (Nasim et al., 2008). Because the seedling vigor index can be determined from root and shoot length, it is the most accurate predictor of a plant's resistance to salt. Consequently, the seedling vigor index can be used to gauge how well plants tolerate salt (Zhang et al. 2021). It has also been reported in previous studies that salinity affects and reduces SVI in other plants as well (Chakraborty et al. 2019; Rajabi Dehnavi et al. 2020).

## Conclusion

In conclusion, this study demonstrated that the early seedling growth stage of *L. albus* is more sensitive to salt stress than the germination stage. This sensitivity started at 50 mM NaCl (-0.2 MPa) for the early seedling growth stage, and 150 mM NaCl (-0.6 MPa osmotic potential) for germination. Therefore, in the light of these data, it is concluded that growing *L. albus* in areas where it would be exposed to 50 mM and above salt (NaCl) stress would result in significant yield losses.

## Declarations

### *Author Contribution Statement*

R.B.: Data collection, investigation, conceptualization, methodology and writing the original draft

V.V.U.: Review and editing

### *Conflict of Interest*

The authors declare no conflict of interest.

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# Managing Heat Stress in Tomato (*Lycopersicon esculentum* L.) Plants through Proline Foliar Application

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ABSTRACT

The tomato is a significant vegetable worldwide in terms of consumption, nutrition, and extensive use in processed foods. During plant growth and development, amino acids especially exogenous application of proline (Pro), plays a crucial role in enhancing stress tolerance under various abiotic stresses. Among these stressors, temperature is considered as a critical and alarming factor affecting plant growth and development and even often a substantial drop in crop productivity results from a significant increase in temperature. The present investigations was conducted at the Horticulture Lab, College of Agriculture, University of Sargodha, during 2021-22 to examine the role of foliar application of 'Proline' under heat stress in tomato plants. Tomato seedlings with true leaves were exposed to high temperatures (25°C [control], 40°C, and 45°C) with exogenous 'Pro' sprays at concentrations of 0, 0.5, 1 and 1.5 mMmL$^{-1}$). Various growth attributes were studied, including morphological traits i-e number of leaves, leaf area cm$^2$, shoot fresh weight (mg), shoot dry weight (mg), root fresh weight (mg), and root dry weight (mg). Along this physiological parameters like photosynthetic rate (µ mol/m$^{-2}$s$^{-1}$), chlorophyll contents (spad), stomatal conductance (µ mols m$^{-2}$ s$^{-1}$), transpiration rate (µ mol/m$^{-2}$ s$^{-1}$), were also studied. The findings indicated that foliar application of 'Pro' at 1.5 mML$^{-1}$ under heat stress at 40°C and 45°C was particularly beneficial in improving growth attributes such as the number of leaves (12.2), leaf area (8.3 cm$^2$), shoot length (10.39 cm), shoot fresh weight (1.88 mg), shoot dry weight (0.28 mg), and root dry weight (0.20 mg), thereby mitigating the adverse effects of heat stress in tomato plants. The variation between control and Proline treated heat-stressed plants suggests that proline may play a role in alleviating heat stress in tomato plants.

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## Introduction

Tomato (*Lycopersicon esculentum* L.) is globally recognized as a significant vegetable both commercially and as a food item. A member of the Solanaceae family, the tomato originates from Peru and Mexico (Ali et al., 2020). Globally, and even in Pakistan, tomatoes can grow in a variety of soil types and climates. In Pakistan, tomatoes are cultivated on 55,258 hectares yielding an average of 10.15 tons per hectare and an annual production of 561,293 tons (FAOSTAT, 2022). Tomato production is significantly affected by three main constraints: heat stress, drought, and salinity. In response to high temperatures, most plants produce a range of heat shock proteins to some extent (Meena et al., 2018). Among them, chitin-binding proteins (CBP) family member CaChiVI2 plays a crucial role in eliminating the impact of adverse environmental conditions, such as cold and salt stress (Ali et al. 2020).

Heat stress is a phenomenon where increased temperature leads to morpho-physiological and metabolic changes in plants, potentially limiting growth and potentially reducing economic yield (Sher et al., 2022). Heat stress has adverse effect on morpho-physiological and biochemical attributes of tomato plants. Air temperatures higher than 35°C inhibited fruit development, plant growth and diminished flowering (Haque et al., 2021). High temperature stress impacts plant development, including germination, expansion, and reproduction, particularly in early growth phases of tomatoes and vegetables. It disrupts chloroplasts' photosynthetic machinery, affecting carbon

metabolism and chemical processes (Alsamir et al., 2020). (Ali et al., 2020) testified that high temperature reduces seed germination because it disrupts the task of enzymes necessary for the digestion of starch and accumulation of abscisic acid, as well as hampers protein synthesis. One of the severe and troubling threats to the environment that restricts plant development and production is heat stress.

Proline is a notable osmolyte and non-essential amino acid that is crucial to plants for fundamental metabolism. It helps maintain cellular turgidity, lessen oxidative harm, and ease the stress on living membranes and intracellular structure (Zulfiqar et al., 2023). Proline has been found to be applied exogenously under various abiotic stressors like water deficiency, salt toxicity, and high temperature. Numerous studies indicated that as proline accumulated, plants exhibit enhanced tolerance to stress (Sadeghipour et al., 2020). According to studies, fruiting becomes more difficult when high temperatures stress the flow of photosynthates to the reproductive structures (Hassan et al., 2021). Additional effects of heat stress include sunburn, denaturation of proteins, early flowering of the plant, twisting, increased membrane fluidity, and stunted plant growth development. (Hussain et al., 2021) found that the exogenous application of 2.5 mM proline resulted in elevated levels of proline, glycine betaine, free amino acids, and chlorophyll in the leaves of stressed plants. Higher concentrations of antioxidant enzymes (SOD, CAT, and POX) were found in heat-tolerant genotypes of okra when subjected to high temperatures, compared to heat-sensitive genotypes. (Priya et al., 2019) and (Ullah et al., 2012) studied the effects of exogenous proline treatment on mungbean (*Vigna radiata* L.) plants during flowering stage under high temperature environment and found that proline application and management improved stigma and pollen fertility, as well as increased internal proline in both reproductive and vegetative parts. It also reduced photosynthetic damage and improved leaf hydration status, which significantly affected mung bean yield (Ullah et al., 2012). The investigations of (Orsini et al., 2018) exhibited that effects of foliar application (5 µM) under high temperature and saline conditions on lettuce and found improved growth and yield traits. Additionally, applying proline topically to different crops under varying stress scenarios strengthened plant tolerance and growth (Hosseinifard et al., 2022).

Based on the previous findings and problems associated with tomato plants, the significance of the study were: to judge the role of proline in moderating stress due to extreme temperature in tomato plants, to control the biological and morphological characteristics that enable tomatoes tolerate high temperatures and figure out the optimal proline concentration that provides the optimum resilience towards thermal stress.

## Materials and Methods

This experiment was conducted at Horticulture Lab, College of Agriculture, University of Sargodha, Pakistan during 2021-22 aimed to alleviate the effect of high temperature in tomatoes *(Lycopersicon esculentum* L.) by applying proline through foliar spray. Tomato seeds (cv. Roma) were obtained from the Ayub Agricultural Research Institute (AARI) Faisalabad.

### *Growth Conditions*

The planned study was done to reveal the foliar application of proline at the seedling stage in tomatoes under temperature stress. The seeds were cleaned with a 5% sodium hypochlorite solution. Three seeds of tomatoes were sown in white plastic pots (10 × 4 cm) filled with fine sandy soil (1:1) as a medium of growth. The growth process was carried out at 25°C ambient temperature. Seeds were irrigated daily with tap water until germination was completed. The pots were closed at one end with a small hole to improve drainage. After complete germination, thinning was practiced to maintain one vigorous plant in each pot. The plants were permitted to grow under controlled conditions from germination until the emergence of true leaves. After true leaf emergence, the plants were exposed to high-temperature strain. The temperature was incrementally raised by 2°C each day to elude any abrupt shock while waiting for the targeted high temperature level (25, 40, and 45°C) was attained. Proline was applied at different levels (0, 0.5, 1, and 1.5 mM $ml^{-1}$ via spray respectively).

### *Growth Attributes*

In the present research, the data were taken on the following parameters at their proper time and methods i-e number of leaves, leaf area ($cm^2$), shoot fresh weight (mg), shoot dry weight (mg), root fresh weight (mg), root dry weight (mg), and physiological parameter; photosynthetic rate the rate of $CO_2$ assimilation (µmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) measured under steady-state conditions, chlorophyll contents (spad) measured using a SPAD meter to assess chlorophyll content as an indicator of leaf health, stomatal conductance (µmol$m^{-2}$ $s^{-1}$ Measured in mol $H_2O \cdot m^{-2} \cdot s^{-1}$) and on transpiration rate (µmole$m^{-2}$ $s^{-1}$),) the rate of water vapor loss (mol $H_2O \cdot m^{-2} \cdot s^{-1}$).

### *Statistical Analysis*

A completely randomized design (CRD) which incorporates two factorial configurations and three replications was used to set up the experiment. The data for all parameters were examined by the analysis of variance (ANOVA) technique. The least significant differences test was implemented to evaluate the treatment means at the 0.05% probability level (Steel et al., 1980).

## Results

From the current assessment, it was determined that all parameters were affected considerably under high temperature strain. Application of proline not only help to alleviate the heat stress, but it also helped to increase the highest number of leaves (12.2) recorded at 25°C + 1.5 $mML^{-1}$ proline level, while the minimum number of leaves (8.4) was produced at 45°C + 1.5 $mML^{-1}$ proline in tomato plants (Table 1). The interactions of foliar applied proline under heat stress for the number of leaves was found non-significant which is mentioned in (Table 3).

The leaf area of tomato plants under heat stress showed a significant decline. Foliar application of proline illustrates a positive relationship and increases the leaf area (8.3 $cm^2$) at 25°C + 1.5 mM $L^{-1}$ pro. Among several foliar proline levels, 1.5 mM $L^{-1}$ was found to be more rewarding to enhance leaf area (7.1 $cm^2$) at 40°C + 1.5 mM $L^{-1}$ pro (Table 1).

Table1. Number of leaves, leaf area ($cm^2$), shoot length (cm), shoot fresh weight (mg), shoot dry weight (mg), root fresh weight (mg) and root dry weight (mg) of tomato as affected by temperature, and exogenous application of proline at 0.5, 1.0, and 1.5 $MmolL^{-1}$.

| T | Proline sprays | No. of leaves | Leaf area ($cm^2$) | shoot fresh weight (mg | shoot dry weight (mg) | root fresh weight (mg) | root dry weight (mg) |
|---|---|---|---|---|---|---|---|
| 25°C | Control | 8.2 | 6.1 | 1.43 | 0.22 | 0.5 | 0.13 |
| | 0.5 | 10.4 | 6.4 | 1.75 | 0.24 | 0.57 | 0.14 |
| | 1 | 11.3 | 7.4 | 1.82 | 0.26 | 0.58 | 0.15 |
| | 1.5 | 12.2 | 8.3 | 1.88 | 0.28 | 0.59 | 0.16 |
| 40°C | Control | 7.2 | 6.4 | 1.58 | 0.18 | 0.47 | 0.11 |
| | 0.5 | 8.4 | 6.9 | 1.62 | 0.17 | 0.52 | 0.12 |
| | 1 | 9.6 | 6.6 | 1.68 | 0.23 | 0.46 | 0.13 |
| | 1.5 | 10.3 | 7.1 | 1.72 | 0.24 | 0.51 | 0.14 |
| 45°C | Control | 5.7 | 5.5 | 1.18 | 0.16 | 0.37 | 0.11 |
| | 0.5 | 6.5 | 6.1 | 1.39 | 0.17 | 0.39 | 0.11 |
| | 1 | 7.6 | 6.4 | 1.48 | 0.19 | 0.42 | 0.1 |
| | 1.5 | 8.4 | 6.7 | 1.58 | 0.22 | 0.47 | 0.12 |

Y: Temperature

Table 2. Physiological parameters: Photosynthetic rate (µmolm-2 s-1[1]), chlorophyll contents (spad), stomatal conductance (µmol m-2s-1)] and transpiration rate (µ $mol/m^{-2}s^{-1}$), of tomato as affected by temperature, and exogenous application of proline.

| T | Proline sprays $Mmml^{-1}$ | Photosynthetic rate ($\mu molm^{-2}\ s^{-1}$) | Chlorophyll contents (spad), | Stomatal conductance ($\mu mol\ m^{-2}s^{-1}$ | Transpiration rate (µ $molm^{-2}s^{-1}$ |
|---|---|---|---|---|---|
| 25°C | Control | 2.49 | 10.53 | 0.019 | 2.13 |
| | 0.5 | 3.09 | 11.42 | 0.021 | 2.78 |
| | 1 | 3.23 | 12.45 | 0.024 | 2.13 |
| | 1.5 | 3.52 | 12.87 | 0.028 | 2.56 |
| 40°C | Control | 2.32 | 9.03 | 0.015 | 1.85 |
| | 0.5 | 2.91 | 10.45 | 0.017 | 2.11 |
| | 1 | 2.97 | 11.78 | 0.019 | 2.22 |
| | 1.5 | 3.23 | 12.27 | 0.020 | 2.38 |
| 45°C | Control | 1.02 | 6.54 | 0.014 | 1.36 |
| | 0.5 | 1.68 | 7.82 | 0.012 | 1.38 |
| | 1 | 2.26 | 8.58 | 0.013 | 1.61 |
| | 1.5 | 3.06 | 9.25 | 0.014 | 1.96 |

Y: Temperature; LSD test was non-significant NS, significant *, and highly significant** at 1 and 5%, probability level respectively.

Table 3. ANOVA about no of leaves, leaf area, Shoot fresh weight, Shoot dry weight, Root fresh weight and Root dry weight.

| Treatments\Traits | No of leaves | leaf area | Shoot fresh weight | Shoot dry weight | Root fresh weight | Root dry weight |
|---|---|---|---|---|---|---|
| Temp. | 1883.614* | 7.46** | 4.27* | 159.32* | 6.53* | 6.98** |
| Proline | 1249.824* | 14.66* | 5.54* | 13.51* | 16.50** | 4.42* |
| Temp x Pro | $616.702^{NS}$ | 0.33** | 6.86** | 0.22* | 0.12 NS | 2.78* |

LSD test with the significance level of $P < 0.05$; Marked * as Significant, ** Highly Significant, and NS for Non-Significant respectively

The interaction between foliar applied proline and high temperature stress in tomato plants showed a progressive response for shoot fresh weight (Table 3).

Maximum shoot fresh weight (1.88 mg) was observed at 25°C + 1.5 mM $L^{-1}$ pro followed by (1.72 mg) at 40°C + 1.5 mM $L^{-1}$ pro (Table 1). Non-significant trends were observed among the interactions of foliar applied proline under heat stress for root fresh weight. Statistically significant results were observed for shoot dry weight. The maximum shoot dry weight (0.28 mg) was noted at 25°C + 1.5 mM $L^{-1}$ pro, while the lowest shoot dry weight (0.22 mg) was noted at 45°C + 1.5 mM $L^{-1}$ pro. Moreover, improvement in root dry weight was recorded at its maximum (0.25 mg) at 25°C + 1.5 mM $L^{-1}$ pro), trailed by 0.12 at 40°C + 1.5 mM $L^{-1}$ pro (Table 3).

Among various concentrations of proline and different temperature strains the highest upturn in root fresh weight (0.59 mg) was observed for treatment (25°C +1.5 mM $L^{-1}$ pro) while intermediate response regarding root fresh weight (0.51 mg) was recorded for treatment 40°C +1.5 mM $L^{-1}$ pro. The lowest root fresh weight (0.47) was perceived by treatment 45°C +1.5 mM $L^{-1}$ pro. The interaction among different temperature strain and exogenous applied proline were found non-significant for root fresh weight (Table 3).

The supreme root dry weight (0.25 mg) was recorded in treatment (25°C + 15 mM $L^{-1}$ pro) while lowest root dry weight (0.12 mg) was observed for the treatment (45°C +1.5 mM $L^{-1}$ pro).

Table 4. ANOVA for Photosynthetic rate, Chlorophyll Content, Transpiration rate and Stomatal conductance.

| Treatments\Traits | Photosynthetic rate | Chlorophyll Content | Transpiration rate | Stomatal conductance |
|---|---|---|---|---|
| Temp. | 4.78* | 3.58* | 13.65** | 3.68* |
| Proline | 3.28* | 6.50** | 3.62* | 3.44* |
| Temp x Pro | $1.34^{NS}$ | $0.120^{NS}$ | $0.46^{NS}$ | 2.91* |

Marked * as Significant, ** Highly Significant, and NS for Non-Significant respectively LSD test with the significance level of $P < 0.05$

However, intermediate response (0.11 mg) was observed in treatment (40°C + 1.5 mM $L^{-1}$ pro). Results concerning interaction among different temperature strains and exogenous applied proline for this parameter were found significant (Table 3). The maximum photosynthesis (3.52 µmol$m^{-2}$ $s^{-1}$) was noted in (25°C + 1.5 mM pro) followed by (3.23 µmol$m^{-2}$ $s^{-1}$) in treatment (40°C +1.5 mM $L^{-1}$ pro), while treatment (45°C + 1.5 mM $L^{-1}$ pro) revealed minimum rate of photosynthesis (3.06 µmolm-2 s-1[1]) in tomato plants. Moreover, the interaction of high temperature strain and foliar application of proline showed non-significant variations for photosynthetic rate (Table 4).

The highest chlorophyll contents (12.87 spad) was observed in (25°C 1.5 mM $L^{-1}$pro) followed by (12.27 spad) in treatment (40°C + 1.5 mM $L^{-1}$ pro), while treatment (45°C +1.5 mM pro) showed lowest chlorophyll contents (9.25 spad) in tomato plants. On the other hand, the cumulative influence of heat stress and foliar applied proline was noted non-significant for this character.

The highest stomatal conductance (0.028 µmol $m^{-2}s^{-1}$) was noted for treatment (25°C + 1.5 mM $L^{-1}$ pro) followed by (0.020 µmol $m^{-2}$ $s^{-1}$) in treatment (40°C +1.5 mM $L^{-1}$ pro), while treatment (45°C + 1.5 mM pro) indicated least amount of stomatal conductance (0.014 µmol $m^{-2}s^{-1}$). The investigations about interaction between foliar application of proline and high temperature stress were found significant for stomatal conductance.

The maximum transpiration rate (2.56 µ mol/$m^{-2}s^{-1}$) was noted in treatment (25°C with 1.5 mM $L^{-1}$ pro) followed by (2.38 µ mol/$m^{-2}s^{-1}$) in treatment (40°C +1.5 mM pro), while treatment (45°C +1.5 mM $L^{-1}$ pro) exhibited lowest transpiration rate (1.96 µ mol/$m^{-2}s^{-1}$). Results concerning interaction between foliar application of proline and high temperature stress were found non-significant for this trait

## Discussion

Recently, crop production faces substantial challenges such as ups and downs in climate, rising temperatures, irregular and abrupt rainfall, and harsh meteorological environments, leading to reduced revenues (Clay and King, 2019; Ullah et al., 2014). Agricultural experts have considered heat stress as the primary factor significantly affecting the yield potential of crop species (Ali et al., 2020). The current study observed the performance of tomato seedling under heat stress condition, with exogenous application of proline. The results revealed that heat stress significantly affected all tested parameters such as; total number of leaves per plant, leaf area, fresh shoot mass, dried shoot mass, fresh root weight as well as physiological characteristics such as photosynthetic rate, transpiration rate, chlorophyll and stomatal conductance in tomato plants (Wang et al., 2020). In many countries worldwide, including Pakistan, heat stress severely restricts sustainable crop production, and high temperatures have been found to damage crops and cause significant losses (Fahad et al., 2019). Increase in normal temperature can cause pretty recognizable deviations, involving scorching of foliage and stems, and other issues like sunscald on leaves, twigs, and stems, premature leaf senescence, fruits drop, and a decline in yield (Santos et al., 2022).

In many countries worldwide, including Pakistan, heat stress severely restricts sustainable crop production, and high temperatures have been found to damage crops and cause significant losses

High temperature stress can be alleviated by several strategies, including thermotolerance, genetic approaches, and utilising osmolytes protectants (Oyebamiji et al., 2023). Some research works have supported that external proline supplementation on plants enhanced their growth and productivity during abiotic stress like salinity, high temperature and drought (Santos et al.,2022). Numerous researchers demonstrated that introducing exogenous proline can decline such negative impacts on tomato plants.

Some growth parameters were improved in chillies by applying (5 and 10 mM $L^{-1}$) proline which also assisted in heat stress-tolerance up to some extent under high temperature stress of 40°C for thirty days (Akram et al., 2021)**.** The outcomes issued by (Kahlaoui et al., 2013) on tomato plants indicated that 10 mg $L^{-1}$ Proline induced growth more efficiently in both tomato cultivars; Rio Grande and Heinz-2274. (Posmyk et al., 2007) scrutinized that external proline utilisation has upgraded growth and catalysed germination of *Vigna radiata* L. seeds when treated with proline. Hare et al., (2003) described that exogenously proline spraying enhances the seed germination of *Arabidopsis thaliana*. According to the conclusions obtained by (Fedina et al., 1993) the execution of proline foliage spray boosted plant growth as well as productivity of peas plants under chilling stress. Under different stress regimes, plant growth was improved by exogenous use of proline in several crops (Ashraf & Foolad, 2007). Hence, selecting tomatoes as a versatile crop, an experiment was steered to improve tomato plants growth and heat tolerance by applying proline as a foliar spray under high temperature stress conditions, aiming to identify the optimal concentration for optimal response.

### *Phenological Parameters*

*Number of Leaves Plant$^{-1}$*

The stress caused by heat has an impact on a plant's leaf quantity in tomato seedlings. The results revealed deviations between the various concentrations of proline application on tomato plants. Statistically significant results concerning leaves plant$^{-1}$ mentioned in (Table 1) revealed that the highest number of leaves (12.2) was documented from treatment (25°C + 1.5 mM $L^{-1}$ pro), an intermediate response regarding the number of leaves (10.3) was recorded from treatment (40°C + 1.5 mM $L^{-1}$

pro), however, the lowest number of leaves (8.4) was observed by treatment (45°C with 1.5 mM pro). Hence, non-significant interaction between heat stress and the exogenous application of proline on tomatoes was noted (Table 3). Tomato seedlings under heat stress indicated progressive reaction to externally driven proline operations and an increase in the quantity of leaves. Our results were with same channel with the findings of (Kahlaoui et al., 2018), who had reported that under abiotic stress (Salinity) stressed tomato plants that were not treated with proline had fewer leaves than those that had received proline externally at 10 $mgL^{-1}$ concentration and subsequently increased the amount of leaves in tomatoes.

*Leaf area ($cm^2$)*

Results regarding leaf area in (Table 1) displayed that under heat stress leaf area was significantly affected. With the various exogenous applications of proline, variation was found among leaf areas. The maximum increment in leaf area (8.3 $cm^2$) was recorded in treatment (25°C with 1.5 mM $L^{-1}$ pro), followed by 7.1 $cm^2$ in (treatment 40°C with 1.5 mM $L^{-1}$ pro). Treatment (45°C with 1.5 mM $L^{-1}$ pro) indicated a minimum increase (6.7 $cm^2$) in leaf area. Hence, interaction between high temperature and the application of proline as a foliar spray on tomatoes for this parameter revealed highly significant. Heat stress initiated sizable damages like reduction and the burning of leaves (Santos et al., 2022). Our outcomes are in agreement with the former conclusions of (Gupta et al., 2022), who documented that high temperature decreased leaf area and reduced photosynthetic activity.

*Shoot fresh weight*

The results concerning shoot fresh weight in (Table 1) indicated that rise in temperature have adversely affected shoot fresh weight. A significant relationship in temperature stress with various doses of proline applied on tomato seedling was noted. Maximum enhancement in shoot fresh weight (1.88 mg) was observed for treatment (25°C with 1.5 mM $L^{-1}$ pro) and trailed by 1.72 mg for treatment (40°C with 1.5 mM $L^{-1}$ pro). Treatment (45°C with 1.5 mM $L^{-1}$ pro) showed the lowest increase (1.58 mg) in shoot fresh weight. Here, the interaction between foliar-applied proline and high temperature stress on tomatoes showed a highly significant response for shoot fresh weight. A similar limiting effect of heat stress on this character of plants has been observed, and it was determined that heat stress severely declines the normal shoot growth (Bonsu et al., 2022). Our findings correspond with (Khan et al., 2015) who concluded that high temperature had reduced the shoot fresh weight of okra plants, but exogenous use of proline had expressed improvement in shoot weight.

*Shoot dry weight (mg)*

Significant results pertaining in (Table 1) showed that shoot dry weight in tomato were highly affected by high temperature stress. Variations among various concentrations of proline application were noted in the shoot dry weight of tomato plants. The highest increase in shoot dry weight (0.28 mg) was found from treatment (25°C with 1.5 mM $L^{-1}$ pro), while an intermediate response regarding shoot dry weight (0.24 mg) was recorded from treatment (40°C with 1.5 mM $L^{-1}$ pro). The lowest shoot dry weight (0.22) was perceived by treatment (45°C with 1.5 mM pro). In addition, the interaction between foliar-applied proline and high temperature stress on tomatoes for this trait showed a positive response. Related conclusions were presented by (Guo et al., 2022), who observed the decrease in shoot dry weight under high temperature stress in tomato plants but spray of $GA_3$ has bring improvement in shoot dry weight.

*Root fresh weight (mg)*

Significant results concerning (Table 1) revealed that temperature strains have a harmful influence on root fresh weight of tomato seedlings. All the treatment was significantly different when proline was sprayed on the tomato. Among various concentrations of proline, the highest upturn in root fresh weight (0.59 mg) was observed in treatment (25°C with 1.5 mM $L^{-1}$ pro), while an intermediate response regarding root fresh weight (0.51 mg) was recorded in treatment (40°C with 1.5 mM $L^{-1}$ pro). The lowest root fresh weight (0.47) was perceived by treatment (45°C with 1.5 mM $L^{-1}$ pro). Afterward, the interaction among different temperature strains and exogenous applied proline was found to be non-significant for root fresh weight. High temperatures harshly decline the root fresh weight in many vegetables. This decline in root fresh weight may be due to the inadequate stock of metabolites in undeveloped emergent tissues because metabolite production is considerably agitated by heat stress, whichever is due to a limited supply of water uptake or the detrimental effect of $Na\ Cl^{+}$. Our findings favour the results found by (Giri et al., 2017) who identified that high temperature decreased root weight and production of tomato.

*Root dry weight (mg)*

Statistical assessment from (Table 1) concluded that root dry weight in tomatoes was reduced under heat stress. With different levels of proline applied indicated highly significant results. The maximal root dry weight (0.25 mg) was noted at 25°C with 1.5 mM $L^{-1}$ pro, while the lowest root dry weight (0.12 mg) was observed at 45°C with 1.5 mM $L^{-1}$ pro. However, an intermediate response (0.11 mg) was observed in treatment (40°C with 1.5 mM $L^{-1}$ pro). Results concerning the interaction among different temperature strains and exogenous applied proline for this character were found to be significant. Our observation confirmed the results obtained by (Alsamir et al., 2017) that Dry matter content was considerably provoked by high-temperature strain, and heat stress encouraged alterations in the physiology of tomato plants or might modify the configuration of growth.

***Physiological Attributes***

*Photosynthetic rate*

Statistically significant results in (Table 2) revealed that under heat stress photosynthetic rate was decreased. Maximum photosynthesis (3.52 $\mu molm^{-2}s^{-1}$) was noted in treatment (25°C with 1.5 mM $L^{-1}$ pro), followed by (3.23 $\mu molm\ ^{2}s^{-1}\ ^{-}$) at treatment (40°C with 1.5 mM $L^{-1}$ pro), while treatment (45°C with 1.5 mM $L^{-1}$ pro) revealed the minimum rate of photosynthesis (3.06 $\mu molm^{-2}s^{-1}$) in tomato plants. The interaction of high-temperature strain and foliar application of proline showed non-significant variations in photosynthetic rate. Our results hence approve the conclusions of (Inayat et al., 2024), that proline application in radish has improved photosynthetic activities by maintaining cell turgor, stabilizing membranes under abiotic salt stresses.

*Chlorophyll contents*

Statistically significant analysis from (Table 2) argued that under heat stress chlorophyll contents were vastly affected in tomato plants. The highest chlorophyll contents (12.87 spad) were observed by treatment (25°C + 1.5 mM $L^{-1}$ pro), followed by 12.27 spad at treatment (40°C + 1.5 mM $L^{-1}$ pro), while treatment (45°C + 1.5 mM $L^{-1}$ pro) showed the lowest chlorophyll contents (9.25 spad) in tomato plants. Moreover, the cumulative influence of heat stress and foliar-applied proline was noted as non-significant for this parameter. Chlorophyll contents were reduced when plants were subjected to an extraordinary temperature strain. These outcomes align with the observations of several past researchers (Jahan et al., 2019 and Zhou et al., 2019) who explored that the drastic rise in temperature may lead to the decline in chlorophyll contents in plants that hamper plant growth. However, (Inayat et al., 2024) demonstrated that under salinity in radish chlorophyll contents showed positive relationship when proline was applied.

*Stomatal Conductance*

The findings assessed in (Table 2) indicated that stomatal conductance was boosted under the influence of heat stress. The stomatal conductance was substantially affected by the proline concentrations.

Stomatal conductance decreased with the use of proline on tomato plants. The highest stomatal conductance (0.028 µmol m-2s-1) was noted from treatment (25°C + 1.5 mM $L^{-1}$ pro), followed by (0.020 µmol m-2s-1) treatment (40°C + 1.5 mM $L^{-1}$ pro), while treatment (45°C + 1.5 mM $L^{-1}$ pro) indicated the least amount of stomatal conductance (0.014 µmol m-2s-1). The investigations about the interaction between foliar application of proline and high temperature stress were found significant for stomatal conductance. Stomatal conductance was increased under heat stress which directly alters plant water relations and photosynthesis (Urban et al., 2017). Our observations are in agreement with the results of (Gadallah et al., 1999) who published that in Vicia faba, the upper and lower stomata reacted in different ways with several concentrations of exogenously applied proline, showing that the stomata on abaxial surfaces were more resistive than those on axial surfaces when treated with proline. It is thus evident that the control of stomatal behaviour can be influenced by the application of proline during stress.

*Transpiration rate*

The outcomes that were displayed in (Table 2) sponsored that transpiration rate was significantly affected under the influence of heat stress. A decline was recorded in the status of transpiration rate in response to high temperature strain. The maximum transpiration rate (2.56 µ mol/$m^{-2}s^{-1}$) was noted at treatment (25°C + 1.5 mM $L^{-1}$ pro), followed by (2.38 µ mol/$m^{-2}s^{-1}$) in treatment (40°C + 1.5 mM $L^{-1}$ pro), while treatment (45°C + 1.5 mM pro) exhibited the lowest transpiration rate (1.96 µ mol/$m^{-2}s^{-1}$). The interaction between foliar application of proline and high temperature stress were found to be non-significant for this trait. However, among various concentrations of exogenous applied proline, a 1.5 mM $L^{-1}$ pro level was found to be best under heat stress. Our results are matching with (Rajametov et al., 2021) who investigated that under heat stress high proline content combined with increased transpiration rate maintain a constant level of photosynthetic rate which allows rapid recovery of heat damage to heat-tolerant cultivars.

## Conclusion

Tomatoes cultivation is severely hindered by heat stress therefore; it is need of the day to recognise stress-tolerant characteristics of tomatoes that can thrive in high-temperature conditions. Exogenously application of proline has a positive role in alleviating the waves of heat stress in tomato seedlings. The results of this study suggested that foliar spraying on tomato plants with proline could mitigate the negative impacts of heat stress and promote both the plant's phenological and physiological responses. The recommended concentration of proline (1.5 mM $L^{-1}$) may help to reach certain goals of thermo-tolerance, since foliar applications of proline in this experiment amended different aspects of plant growth and other growth attributes like no of leaves, shoots fresh weight, chlorophyll contents etc. Under heat stress (40°C and 45°C), and an exogenous foliar application strategy of 1.5 mM $L^{-1}$ proline could be advantageous for improving the plant physiological changes during the early growth stage of tomato plants.

## Declarations

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***Conflicts of Interest***

The authors declare no conflict of interest.

***Authors Contribution Statement***

Performed the experiments: S.U.R., Analysed the data: S.U.R. & A.A., Contributed materials/ analysis/ tools: A.H., S.N. Wrote the paper: S.U.R., A.A., & S.N., Revision M.U.S., & W.A.

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# Optimizing Artificial Shading for Microclimate, Yield, Leaf Nutrient and Economic Benefits in Sinceri (*V. vinifera* L.) Grape Cultivation

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ABSTRACT

This study was conducted on the Sinceri grape cultivar grown for both table and raisin (drying) purposes, in the 2021 growing season in Siirt/Türkiye. The primary objective was to create a microclimate within the vine canopy by installing net covers with different shading rates (35%, 55%, and 75%) during the veraison period, thereby delaying the harvest and obtaining high-quality, high-yield grapes. Regarding phenological development, the period between full bloom and harvest was the shortest under the 55% shading treatment, which also recorded the lowest mean temperature (28.54 °C) and the lowest Effective Heat Summation (EHS) value (1965.70 dd). The highest mean temperature (26.28 °C) was observed under the 75% shading treatment, while the highest EHS value (2401.05 dd) was recorded under the 35% shading treatment. In terms of yield compared to the control, the 35% shading treatment provided a 21.75% increase, the 55% treatment yielded a 57.44% increase, and the 75% treatment led to a 37.45% increase. Furthermore, it was determined that all shading treatments increased the macro- and micronutrient contents in grapevine leaves. Economic analyses for the Sinceri grape cultivar revealed that the shading treatments had a statistically significant effect on yield. In conclusion, the net cover with a 55% shading rate proved to be the most effective treatment.

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## Introduction

Shaped by thousands of years of meticulous cultivation and cultural integration, grapevine stands as one of the most economically profitable fruit species globally (Vivier & Pretorius, 2002). As a highly propagative species with exceptional regeneration capability, the grapevine has successfully disseminated and adapted across diverse regions of the world (Vivier & Pretorius, 2000). Throughout history, Anatolia has been regarded as the cradle of viticulture, offering an ideal environment for grape cultivation, where wild grapevines still thrive naturally (Vouillamoz et al., 2006). Türkiye, as a genetic center of viticulture is recognized for hosting approximately 1 500 cultivars (Hizarci et al., 2012). It is evident that grape cultivation holds not only a historic and genetic significance but also substantial economic value. As a leading grape producer, the Republic of Türkiye ranked as the 6th largest globally, producing nearly 3 670 000 tons of fresh grapes in 2021, of which 264 505 tons were exported as fresh grapes and 257 165 tons in processed forms (Country Statistics OIV, 2024). The economic significance of grape cultivation is further reinforced by its role in supporting both local and international markets as diverse consumer products like wine, table grape, raisins, juice and various industrial uses (Alston & Sambucci, 2019). To achieve sustainable and high-quality grape production, it is crucial to consider various factors influencing grape yield and quality. These factors include genetic diversity (Bigard et al., 2020), climatic conditions (Ponti et al., 2018), soil characteristics (Arnó et al., 2012; Li et al., 2024), and vineyard management practices (Tesic et al., 2007). Among these, cultural practices are particularly significant, as they directly affect vine growth, fruit development, and overall yield (Reynolds, 2022).

Cultural practices in viticulture encompass a wide range of interventions, such as pruning (Main & Morris, 2008), irrigation (Balint & Reynolds, 2017), canopy management (Smart et al., 1990), fertilization (James et al., 2023; Peuke, 2009), and pest control (Cabras & Angioni, 2000; Steenwerth & Guerra, 2012; Yilmaz et al., 2015). Also, the use of shading

material has been reported to have a strong effect on reducing the severity of Botrytis cinerea (Cangi et al., 2011b; Kesgin, 2011). In addition to these, innovative techniques, such as the use of shading materials have emerged as effective tools for mitigating the effects of environmental stressors like excessive sunlight and heat (Lu et al., 2021). Türkiye's climate is predominantly Mediterranean, featuring hot, dry summers and mild wet winters. The country's varied topography and diverse land-use patterns significantly influence large-scale atmospheric dynamics due to the complexity of surface conditions. The mountain ranges that stretch parallel to the Mediterranean and Black Sea coasts create pronounced climatic differences between the northern and southern slopes of these mountains (Önol & Unal, 2014). Climate change is a major concern affecting agricultural production in mediterranean climate, furthermore grapevine production is highly prone to water deficits, mediately high temperatures (Santillán et al., 2020). Plant physiological activities are profoundly influenced by the amount of solar radiation reaching the leaves, as it directly impacts key processes such as stomatal regulation and leaf temperature. The intensity of solar radiation affects stomatal aperture, influencing gas exchange, transpiration, and photosynthesis rates. Additionally, excessive radiation can elevate leaf temperatures, potentially leading to heat stress, reduced enzymatic activity, and disruption of cellular functions. Balancing solar radiation exposure is thus crucial for optimizing plant health and productivity (Urban et al., 2017). To mitigate the adverse effects of heat and light-induced stress on grapevines, artificial shading is a promising and effective solution in viticulture. Shading, primarily impacts the vine microclimate, influencing temperature, humidity, and photosynthetically active radiation (PAR). These changes subsequently affect key grape characteristics, including phenology, anthocyanin levels, pH, acidity, and total soluble solids content (TSSC) (Kesgin et al., 2020). The effects of shading appear to vary depending on the cultivar and environmental conditions. The primary parameter is the shading percentage and net characteristics of the shading material (Miccichè et al., 2023).

This study aimed to evaluate the effects of different shading ratios on the canopy of the Sinceri grape cultivar by comparing shading treatments at 25%, 50%, and 75% with a control group. The comparisons focused on parameters such as Effective Heat Summation (EHS) values during phenological development stages, average canopy temperature, leaf nutrient element contents, and grape yield.

## Material and Method

### *Material*

*Geographical location of the experimental vineyard*

The experiment was conducted in a 5 decare producer vineyard established with the Sinceri grape cultivar in Bağtepe Village, Siirt province. The geographical coordinates of the vineyard are 37° 57' 33.5" N latitude and 41° 58' 36.2" E longitude, at an altitude of 1117 meters above sea level (Figure 1). The vineyard was established with a pergola-type wire training system. The vines used in the experiment were systematically identified and marked using a standardized labeling method to ensure precision and consistency throughout the study.

*Plant material*

Sinceri is a white grape cultivar widely cultivated in the Southeastern Anatolia Region. It is a local cultivar that has been grown for centuries in Siirt and its surrounding areas. The vines are typically pruned short or mixed, and the cultivar is considered mid-to-late in terms of maturation. Sinceri grapes are valued for their distinct aroma and are utilized both as table grapes and for drying, as well as in the production of fruit leather (pestil) (Ünal et al., 2019).

*Shading material*

In the experimental vineyard, polyethylene nets with three different shading densities (35%, 55% and 75%) were covered on the vines on June 19, 2021.

### *Method*

Annual cultural maintenance operations such as pruning, soil cultivation, fertilization, and plant protection were carried out regularly. Winter pruning was performed on March 1, 2021, with the vines evenly pruned to 20±2 buds per vine.

*Climate data of the experimental vineyard*

The climate data for the experimental vineyard, including temperature, humidity were collected throughout the growing season, from budburst to leaf fall. Measurements were taken using a HOBO U12-013 Onset device installed at the level of the vine training wires, recording data at 60-minute intervals. The recorded data were processed into monthly averages to analyze their relationship with climatic parameters and treatments (Figure 2). The collected climate data were also used to calculate the Effective Heat Summation (EHS) to support further analysis of the growing conditions in the experimental vineyard.

Figure 1. Geographical location of experiment vineyard


Figure 2. HOBO U12-013 installed in the vineyard

*Soil analysis*

Soil samples were collected from a depth of 30–60 cm. The physical properties and macro and micro nutrient contents of the soil were evaluated according to the criteria set by Aksu (2008). The soil analysis was conducted through laboratory service procured from the Siirt University Science and Technology Application and Research Center. The soil analysis included the measurement of various physical and chemical parameters. Micro nutrient concentrations, including copper (Cu), manganese (Mn), iron (Fe), zinc (Zn), and boron (B), were determined. Macro nutrient levels, such as potassium ($K_2O$), phosphorus ($P_2O_5$), calcium (Ca), and magnesium (Mg), were analyzed. Additionally, the soil texture was assessed by determining the proportions of clay, silt, and sand, and the texture class was identified. Other measured parameters included pH, electrical conductivity (EC), organic matter content, and lime ($CaCO_3$) percentage.

*Phenological observations of the applications in the experimental vineyard*

Phenological observations of the applications in the experimental vineyard were conducted based on the classification of grapevine phenological stages proposed by (Eichorn & Lorenz, 1977).

*Data collected from the vines*

Harvesting was performed when the grapes reached 22% Total Soluble Solids Contents (TSSC) or when biotic and abiotic factors necessitated harvest. The number of clusters was determined by counting all clusters separately for each application and each repetition. The average weight of cluster was carried out on 10 randomly selected clusters from 5 vines in each replication. Grape yield was determined by weighing the total amount of harvested grapes and calculating yields per vine.

*Leaf analysis*

On September 1, 2021, 80 leaf samples taken randomly (only in the leaf blade) from each application and each repetition of after harvest leaves were analyzed for macro and micro nutrient content by the Siirt University Science and Technology Application and Research Center.

*Economic analysis*

In order to reveal the economic impact of different shade material applications and to find out which shade material application provides a higher net income, the partial budget analysis method was used. Partial budget analysis is a widely used method to determine whether different applications have an effect on gross income. Because only the inputs resulting from application differences are included in the yield calculations. Since other inputs are fixed or the same in all applications, it is possible to explain the relationship between yield and production cost directly with application differences. Thus, the formula for reaching the gross income per vine can be explained as follows;

$$\text{Gross Production Value} = \text{Price} \times \text{Yield}$$

The product prices in the formula represent the 2021 retail sales price (TL/kg), and the yield represents the yield obtained per vine (kg/vine).

$$\text{TVC} = (\text{SMP} \times \text{AU}) + \text{TL}$$

TVC : Total variable costs
SMP : Shading material price
AU : Amount used
TL : temporary Labor

The total variable costs in the formula represent the 2021 purchase cost of the shading material used for each application (TL/$m^2$) and the amount used per vine ($m^2$/vine). Labor represents the labor cost of applying shading material per vine for each application (TL/vine).

$$\text{GP} = \text{GPV} - \text{TVC}$$

GP : Gross Profit
GPV : Gross production value
TVC : Total variable costs

As a result, in order to obtain the gross profit in the partial budget analysis, the difference between the gross production value obtained from each application and the total variable costs was taken to reveal the level of profitability between the applications.

***Statistical Analysis***

The experiment was conducted using a randomized plot design with three replications (5 vines in each replication). After the data were analyzed with variance analysis, the difference between the means was examined with the Tukey multiple comparison test at the 5% level. The JMP package program was used in the analysis (Morris et al., 2001). Statistical differences are shown in lower case letters on the right side of the data.

In addition to basic descriptive statistics, the suitability of variables related to technical and economic data for normal distribution was tested. For variables that showed a normal distribution, the T-test was applied. For those that did not meet the normality assumption, non-parametric tests such as the Mann-Whitney U test were used.

The T-test compared the means of two groups to determine whether the observed difference was random or statistically significant. This test provided significant convenience for researchers, particularly when working with small sample sizes. On the other hand, the Mann-Whitney U test was employed when the data did not meet the assumptions of parametric tests and served as an alternative for testing the significance of differences between two means (Miran, 2002).

## Results and Discussion

***Phenological Observations of the Experimental Vineyard***

The phenological observations of the vineyard where the experiment was conducted were determined by taking into account the classification of the phenological stages of the grapevine made by Eichorn & Lorenz (1977) (Table 1).

On April 6, 2021, budburst (Stage 2) was observed, marking the initiation of bud development. Subsequently, the flowering stages were closely monitored, beginning of flowering (Stage 21) was recorded on May 14, 2021, when 25% of the caps had fallen. Full flowering (Stage 23) was recorded on May 17, 2021, when 50% of the caps had fallen.

Table 1. Phenological observations of the Sinceri grape cultivar

| Phenological Observations | Date* |
|---|---|
| Budburst | 06.04.2021 |
| Beginning of Flowering | 14.05.2021 |
| Full Flowering | 17.05.2021 |
| End of Flowering | 20.05.2021 |
| Berry Set | 26.05.2021 |
| Veraison | 28.07.2021 |
| Harvest | 30.08.2021 |
| Leaf Fall | 30.11.2021 |

*Phenological observations were determined on the same dates in all treatments. Especially since the shading materials were laid on the vines during the veraison state, the observations were the same until this state. Harvest was done on the same day to compare the effects of the treatments on the must parameters. All treatments did not have any effect on the leaf fall date.

Table 2. Determination of soil structure of the experimental vineyard

| Structure | Value |
|---|---|
| Clay (C) (%) | 44.5 |
| Silt (S) (%) | 42.5 |
| Sand (S) (%) | 13.0 |
| Texture Grade | SiC |
| Ph | 7.46 |
| Salt (µS) | 0.16 |
| Organic Matter (%) | 1.59 |
| Lime (%) | 41.15 |

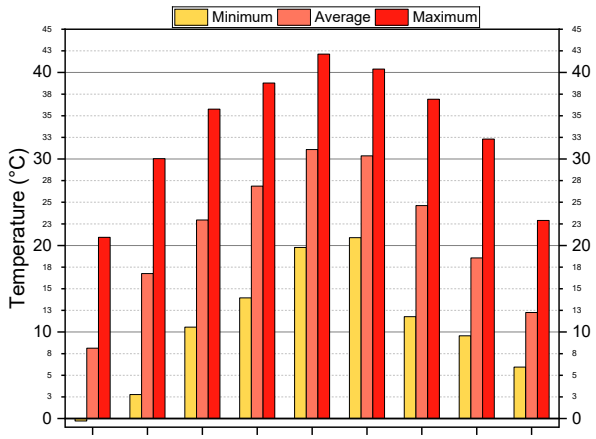


Figure 3. Monthly temperature data of the experimental area

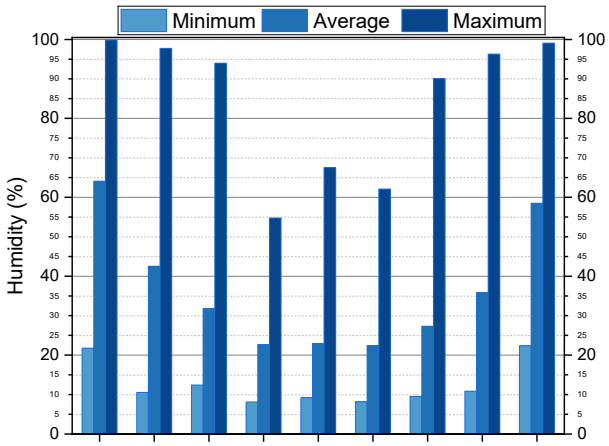


Figure 4. Monthly humidity data of experimental area

The end of flowering (Stage 25) on May 20, 2021, when 80% of the caps had fallen. Berry set (Stage 27) was observed on May 26, 2021, as berries reached a diameter of approximately 3–4 mm. The veraison stage (Stage 35), defined as the onset of berry softening, was documented on July 28, 2021. The harvest phase (Stage 38) was carried out on August 30, 2021, when the grapes reached maturity as determined by the maturity index. Finally, the leaf fall stage (Stage 47) was observed on November 30, 2021, with approximately when 70% of the leaves having fallen (Table 1).

***Climate Data of The Experimental Vineyard***

The climate data for the experimental vineyard were collected during the 2021 vegetation year. Monthly average temperature, minimum temperature, maximum temperature, average humidity, minimum humidity, and maximum humidity values were calculated for the period from budburst to leaf fall and are presented in Figure 3 and Figure 4.

As specified in Table 1, budburst for the Sinceri grape cultivar occurred on April 6, 2021, while leaf fall took place on November 30, 2021. Based on this period, monthly average temperature and humidity values were calculated.

The highest monthly average temperature was recorded in July at 31.08°C, while the lowest monthly average temperature was observed in March at 8.13°C. The minimum temperature occurred in March at -0.28°C, whereas the maximum temperature was recorded in July at 42.12°C (Figure 3).

The highest monthly average humidity was observed in March at 64.09%, while the lowest monthly average humidity was recorded in August at 22.42%. The minimum humidity was 8.13% in June, and the maximum humidity was 99.88% in March (Figure 4).

Lu et al. (2021) reported that the use of shading materials creates a microclimate area in the canopy of the grapevine to reduce the effects of environmental stress factors such as excessive sunlight and heat. Miccichè et al.

(2023) reported in their study that artificial shading, applied at full fruit set, interfered with the microclimate of the vines, causing partial effects on the grape ripening processes and delays leaf fall.

***Soil Analysis of the Vineyard Land***

The physical properties of the soils and their macro and micro nutrient element contents were evaluated according to Aksu (2008). The experimental vineyard soil is classified as silty clay (SiC) in texture, non-saline, highly calcareous, low in organic matter, and slightly alkaline (Table 2). When examining macro and micro nutrient concentrations, the levels of Cu and K were found to be adequate, while Mn and Fe concentrations were high. In contrast, Mg, Zn, and B were very low, and P and Ca were at low levels (Table 3).

Although viticulture can be practiced on a wide range of soil types, the ideal vineyard soils are loamy (L) or sandy-loamy (SL), slightly gravelly, well aerated, humus rich, and moderately calcareous. For vineyards, the most suitable soil pH is reported to be between 6 and 8 (Yetgin & Korkmaz, 1991; Çelik et al., 1998; Çelik, 2011).

Table 3. Determination of macro and micro nutrient elements of the experimental vineyard

| *Element* | *Value* |
|---|---|
| Cu (ppm) | 10.10 |
| Mn (ppm) | 91.74 |
| Fe (ppm) | 18.63 |
| Zn (ppm) | 0.18 |
| B (ppm) | 0.12 |
| $K_2O$ (kg/da) | 34.36 |
| $P_2O_5$ (kg/da) | 5.33 |
| Ca (kg/da) | 599.39 |
| Mg (kg/da) | 28.44 |

Table 4. Effects of shading treatments on yield parameters

| Treatment | Number of clusters (clusters/vine) | Average cluster weight (g) | Grape yield (g/vine) |
|---|---|---|---|
| Control (%0) | 15.59±0.05c | 181.75±5.27b | 2832.83±74.22c |
| %35 Shading | 17.72±0.15b | 194.67±4.06b | 3449.10±55.37bc |
| %55 Shading | 19.90±0.80a | 223.83±3.63a | 4459.95±213.28a |
| %75 Shading | 16.41±0.05bc | 237.25±2.43a | 3893.75±50.48ab |

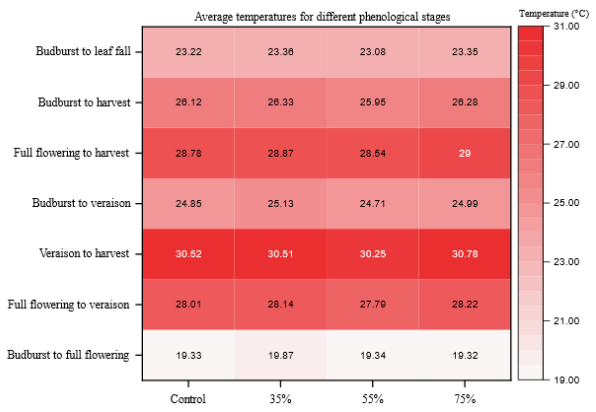


Figure 5. Heat map of average temperatures during different phenological stages

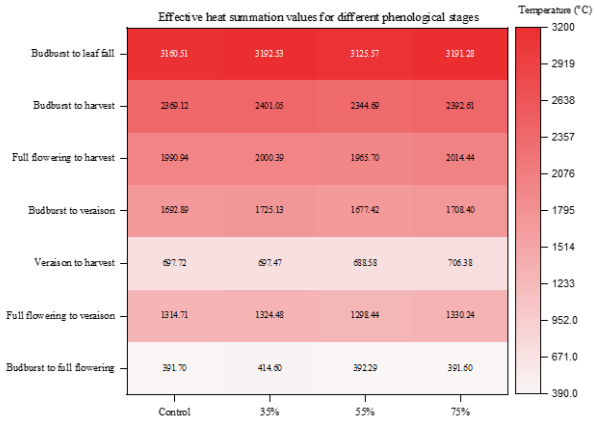


Figure 6. Heat map of Effective Heat Summation values for different phenological stages

***Average Temperatures and Effective Heat Summation (EHS) according to Phenological Stages***

Based on the data obtained from the HOBO devices installed in the experimental vineyard on March 1, 2021, Average temperatures and Effective Heat Summation (EHS) were calculated for the budburst-full flowering, full flowering-veraison, veraison-harvest, budburst-veraison, full flowering-harvest, budburst-harvest and budburst-leaf fall periods in each treatment (control and varying densities of shading materials). Average temperatures (Figure 5) and Effective Heat Summation (EHS) values (Figure 6) were calculated by considering the seven different phenological stages given above.

Effective Heat Summation (EHS) is one of the most critical parameters for determining the suitability of a region for viticulture and identifying which grape varieties can be cultivated in a specific ecology. This value is expressed as day-degrees (dd).

Except for the period between budburst and full flowering (since the shading materials were laid on the vines on June 19, 2021), the lowest result in terms of both average temperature and EST values in all other periods was determined in the 55% shading material treatment.

The lower limit of the Effective Heat Summation suitable for viticulture in an ecology is considered to be 900 dd (Eggeberger et al., 1975).

Their ecologies according to EHS values;
Cold=900-1400 dd
Cool=1401-1700 dd
Temperate=1701-1950 dd
Warm-temperate=1951-2250 dd
Hot= 2251 dd and above (Winkler et al., 1974).

According to the classification determined by Winker et al. (1974); it was determined that the ecology in which the study was conducted was in the hot climate class.

***Effects of Shading Applications on Yield Parameters***

Observations and calculations were conducted in the experimental vineyard, with the harvest taking place on August 30, 2021. The number of clusters (pcs), average cluster weight (g), and grape yield per vine (g) were calculated according to the specified methodology for each treatment, using five vines per replication. The effects of the shading treatments on yield parameters are summarized in Table 4.

The yield parameters in Table 4 shows the 55% shading treatment resulted in the highest values for both the number of clusters and grape yield per vine. According to Tukey's multiple comparison test, all differences were statistically significant at the 5% level.

In a previous study conducted on the Sinceri grape cultivar, Demirhan and Aslan (2022) reported a grape yield of 6.60 kg/vine and an average cluster weight of 323.05 g. Similarly, Koç (2018), in his research on local grape varieties in Muş province, determined the cluster weight of the Sinceri grape as 138.77 g and the grape yield as 5.16 kg/vine. Conversely, Miccichè et al. (2023) observed that shading significantly reduced berry size, resulting in lower cluster weight and vine yield. However, the findings of our study indicated that shading positively influenced yield parameters, particularly under the 55% shading treatment.

***The Effects of Shading Material Treatments on The Nutrient Element Levels in Grapevine Leaves***

As presented in Table 5, among the macro and micro elements analyzed, the highest levels of boron (B), magnesium (Mg), phosphorus (P), and nitrogen (N) were observed in the control treatment (0% shade), indicating that full exposure to sunlight supports higher concentrations of these elements. In contrast, the 35% shading treatment showed the highest levels of copper (Cu) and iron (Fe), suggesting that moderate shading may optimize the uptake or retention of these elements. For calcium (Ca), manganese (Mn), and zinc (Zn), the highest levels were detected under the 55% shading treatment, which may reflect a balance between light exposure and shading that favors the availability or mobility of these nutrients. Potassium (K), however, reached its maximum level under the 75% shading treatment, indicating that greater shading might enhance potassium accumulation in leaves, potentially due to reduced transpirational losses or specific physiological adaptations. These results highlight the differential impact of shading levels on the accumulation of macro and micro elements, suggesting that shading intensity can significantly influence nutrient dynamics in grapevine leaves.

***Economic Analysis***

As part of this study, a partial budget analysis was conducted using data obtained from the experimental results established in the producer vineyard. The most critical aspect of partial budgeting is the calculation of variable costs. Since all variable costs except those associated with shading materials were consistent across treatments, shading material costs were identified as the primary variable cost. In the control group, where no shading material was used, total variable costs were 0 TL/vine. In contrast, variable costs were calculated as 3.20 TL/vine for both the 35% and 55% shading material treatments, and 4.27 TL/vine for the 75% shading material treatment. The analysis was completed by determining the total gross profit following the partial budgeting process (Table 6).

Upon examining the data on gross production value, variable costs, and gross profit, it was found that the differences in gross profit across all three treatments were statistically significant at the 5% significance level according to the statistical analysis results. Based on the results, shading material was determined to have a statistically significant impact on yield, with the 55% shading treatment identified as the most economical option.

In a similar study, Cangi et al. (2011a) determined that "the second model with the highest total production costs (2144 TL/da) is the most profitable production model (due to the high amount of marketable grapes, the grapes being harvested at the latest and being sold at the highest prices)" in their study on the Sultani Cekirdeksiz grape variety.

Table 5. Macro and micro element levels in grapevine leaves

| Treatment | B | Ca | Cu | Fe | K | Mg | Mn | P | Zn | N |
|---|---|---|---|---|---|---|---|---|---|---|
| | (ppm) | (%) | (ppm) | (ppm) | (%) | (%) | (ppm) | (%) | (ppm) | (%) |
| Control (%) | 33.57 | 0.89 | 14.58 | 147.97 | 0.48 | 0.52 | 151.30 | 0.13 | 29.98 | 2.92 |
| %35 Shading | 14.16 | 0.81 | 14.99 | 149.57 | 0.58 | 0.37 | 104.59 | 0.12 | 33.21 | 2.12 |
| %55 Shading | 21.10 | 1.76 | 14.79 | 145.63 | 0.62 | 0.47 | 164.70 | 0.12 | 42.82 | 2.09 |
| %75 Shading | 21.19 | 1.36 | 14.87 | 137.77 | 0.74 | 0.51 | 127.20 | 0.11 | 31.98 | 2.05 |

Table 6. Economic analysis according to treatments

| Treatment | Gross production value (TL/vine) | Total variable cost (TL/vine) | Gross Profit (TL/vine) |
|---|---|---|---|
| Control (%) | 10.48 | 0.00 | 10.48 |
| %35 Shading | 12.76 | 3.20 | 9.56 |
| %55 Shading | 16.50 | 3.20 | 13.30 |
| %75 Shading | 14.41 | 4.27 | 10.14 |

The economic importance of grape production is steadily increasing in both local and international markets (Alston & Sambucci, 2019). Achieving sustainable and high-quality grape production requires attention to numerous factors influencing yield and quality (Reynolds, 2022). Consequently, implementing cultural practices that enhance grape yield, such as the use of shading materials, is essential for sustainable viticulture.

## Conclusion

Based on Effective Heat Summation (EHS) values across different phenological stages under various treatments, the 55% shade material application consistently recorded the lowest results for both average temperature and EHS values in all periods except the interval between budburst and full flowering. Regarding yield parameters, the 55% shade material application yielded the highest values for both the number of clusters and grape yield per vine.

In terms of macro- and micronutrient levels in the leaves, the shading treatments positively influenced the accumulation of most elements, except for boron (B), magnesium (Mg), phosphorus (P), and nitrogen (N), where no significant increases were observed.

In conclusion, economic analyses for the Sinceri grape cultivar indicated that shading material applications have a statistically significant impact on yield. Based on the data obtained, the most effective shading treatment was determined to be the use of nets with a 55% shading rate.

## Declarations

### *Data Availability*

The data to support the results and conclusions of this study is presented within the article. Detailed data is available upon request.

### *Conflicts of interest*

No potential conflict of interest was reported by the authors.

### *Funding statement*

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# Drone Use in Agricultural Spraying: An Examination in Terms of Occupational Health and Safety

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Technology

ABSTRACT

One of the cornerstones of a developed economy is undoubtedly the agricultural sector. Agriculture is at the center of both human nutrition and economic activities. The use of drones, especially in the process of spraying fields, has the potential to increase the efficiency of agricultural production. Drones can be programmed to scan the field and spray pesticides on these areas. This allows farmers to manage the processes of protecting their crops and removing pests more effectively. Spraying with drones minimizes the negative effects encountered in spraying with tractor-drawn machines. Drones can easily reach places that tractor-drawn machine cannot reach and can spray more precisely. In addition, thanks to drones, the need for labor is also reduced, so that one person can spray a large area in a short time. In this study, the process of spraying with drones was observed in detail by a company with an unmanned aerial vehicle-2 (UAV) license for agricultural spraying. This modern spraying method using drones was meticulously evaluated step by step. In traditional methods, farmers or workers may be directly exposed to pesticides while spraying with tractor-drawn machines, but thanks to drones, this exposure is minimized, which provides a great advantage in terms of occupational health and safety (OHS). In addition, the speed of the work process, less use of water and pesticides, and the need for labor are among the advantages. However, the problem of not being able to connect to GPS, accidents that may occur under the command of the drone, and limitations such as adverse weather conditions can be considered disadvantages of drone spraying. The findings reveal how drone spraying has transformed agriculture.

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## Introduction

The maintenance of food security and the increase in food production are increasingly becoming more problematic (Prosekov & Ivanova, 2018). The increase in the disruption of the fight against the spread of various harmful plants and plants also increases the environmental and worker parts (Shahrooz et al., 2020). Throughout history, management production methods have been developed and modernized, and agricultural technologies have been made more efficient and sustainable (Topal, 2010). With the widespread use of tractor-drawn machines, which are considered a revolution in annual agriculture, production capacities have increased, and large areas have become more cultivated in a shorter time and with less labor. However, today, the basis of this technological step has been passed. In recent years, the use of drone technologies in agriculture has become increasingly widespread. (Özgüven et al., 2022). Although the development of unmanned aerial vehicles (UAVs), which have an important place in the field of robotics, was initiated by military resources (Ergunşah & Koşunalp, 2022), it is present in many places today. UAVs are currently used in areas such as mapping (Avdan et al., 2014; Navia et al., 2016), agriculture (Mogili & Deepak, 2018; Ge et al., 2019), archaeology (Campbell, 2018), wind turbines (Wang & Zhang, 2017).

The use of drones in agriculture allows farmers to make the right decisions by providing real-time data (Emimi, et al., 2023), while the possibility of detailed imaging to monitor plant diseases and early detection of diseases allows intervention at certain times (Jain et al., 2023).

In their report, Ahirwar et al. (2019) argued that meeting the food needs of the growing world population can be achieved through the adoption of advanced technologies in agricultural production and stated that the use of advanced technologies such as drones offers the potential to face various challenges. Kalamkar et al., (2020) stated that today, farmers are turning to drone technology to obtain an effective solution to eliminate

problems such as weeds and insects that affect the success of farmers, and that unmanned aerial vehicles provide the opportunity to maximise yield in crop production.

Most of the economic loss in agriculture is caused by harmful insects and weeds (Mahajan et al., 2023), drone pesticide spraying provides faster, more efficient and targeted applications than manpower (Sahni et al., 2024).

Spraying drones used in agriculture can spray one hectare of land in about 10 minutes, depending on the model, and can speed up the normal spraying time (Shahrooz et al., 2020). There are many types of drones on the market according to their tank volume (10-50 liters) (Anonymous (a), 2024). Some of these have digital beam formation (DBF) radars that prevent diversity by sensing the environment (Puri et al., 2017) and normalized difference vegetation index (NDVI) sensors for creating maps (Meesaragandla et al., 2024).

The aim of this research is to determine the hazards and risks in this process by observing drone pesticide spraying on site. In addition, the precautions to be taken in terms of occupational health and safety in drone pesticide spraying were also mentioned.

## Materials and Methods

This research was designed as a qualitative field study. In order to examine the effects of drone use in agricultural spraying on occupational health and safety, the activities of an agricultural spraying company were observed. Data collection was carried out with the unstructured observation technique (Yıldırım & Şimşek, 2005). The flow of actions was monitored without intervention and the findings were recorded. In addition, visual materials (video and photographs) obtained during the observation were used to support the data. In the research, the location of the area to be sprayed, the preparation of the pesticide diluted with water, the activities of drone operators on the job, potential occupational health and safety risks encountered during spraying, the equipment used and protective measures were observed. The observations were carried out as an external observer without any influence and were supported by field notes and photographs. The collected field notes and visual materials were categorized within the framework of risks and measures taken in terms of OHS.

### *Technical Informations, Observation*

The drone in the study is the DJI brand T30 model and some of its technical specifications are as shown in Table 1.

The workflow is as follows:

- Mapping the area requested to be sprayed (at various points of the field depending on its geometry and whether it is hilly or flat) via GPS (Figure 1).
- Introducing obstacles that may collide in the mapping (such as electric poles, tall trees) one by one (Figure 2)
- First, mixing the pesticide and water to be used in the spraying process in buckets before filling the tank to obtain a homogeneous distribution (Figure 3)
- Filling the resulting mixture into the tank.
- Setting the distance and height at which the drone will spray by the drone operators (minimum 2.5 m for rice). (Uniform spraying is provided thanks to the altitude sensor) (Figure 4). The company is generally preferred for spraying rice fields, corn and canola fields.
- Ventilating and monitoring the drone from a safe distance (Figures 5)
- After the spraying process is completed, the device is closed and the tank is cleaned for next spraying (Figure 6).

Table 1. Some features of the drone used by the company (Anonymous (b),2024)

| | |
|---|---|
| Hourly operating efficiency | 40.000 $m^2$ |
| Total weight (excluding batteries) | 26,4kg |
| Maximum flight speed | 7m/sn |
| Nozzle quantity | 16 |
| Maximum effective spray width | 4-9 m (12 nozzles and 1.5 to 3 meters distance to the plant) |
| Battery | Two batteries that can be recharged 1000 times |
| Charger | Available |
| Obstacle avoidance radar | Available |
| Tank volume | 30 L |

Figure 1. Drone mapping

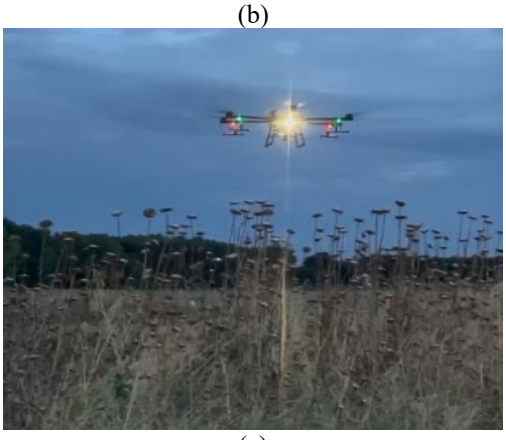
Figure 2. Drone flies through power lines

Figure 3. Preparation of the pesticides diluted with water

Figure 4. Drone spraying from a certain altitude (Still areas are sprayed areas)

(a)

(b)



(c)

Figure 5. (a)Taking off the drone from a safe distance, (b) Drone spraying monitoring, (c) Spraying of sunflower field in the evening hours

Figure 6. Cleaning the tank after spraying

**Findings**

The findings of the research based on the workflow and field observation are as follows:

- The company's spraying operations are carried out by two operators with UAV-1 and UAV-2 licenses serving the Thrace region. They have training and qualifications in this regard.
- The company uses the drone mostly for weed control, but also for insect control.
- In order for the crop area to be introduced to the drone, the drone operator must definitely establish a GPS connection and be at certain points in the crop area to determine which areas will be sprayed. Then, the drone autonomously adjusts its departure and arrival.
- During the windy, rainy and especially hot days in the summer months when the weather conditions are not suitable, spraying during the day is not preferred due to reasons such as battery heating and the spray drifting and blowing.
- Since there may be some dangerous situations (rough terrain, stones or pits) in the crop area, drone operators are at risk of falling and injury.
- The area visited may be a field where the spray has been applied before. In this case, the operators is likely to be exposed to the pesticide.
- There may be elements in crop fields that may pose a high safety risk for both the drone and the operators, such as power lines and high voltage.
- In the event of obstacles such as electric poles and large trees, these elements are introduced to the drone before the flight.
- The pesticide used in spraying the crop field is supplied by the field owner from the dealer selling agricultural pesticides.
- The dosage of the pesticide is mostly adjusted with the suggestion of the field owner, depending on the density of harmful weeds or insects. Sometimes, the dosage is adjusted based on the prescription given by the dealer selling the agricultural pesticide and in consultation with the company employees.
- After the amount of pesticide is determined, it is first mixed with water in buckets. At this stage, the company's drone operators may be exposed to high levels of pesticides. Drone operators use half-face masks with gas-vapor filters, nitrile gloves and daily long clothes to prevent contact with the body, especially against the risk of exposure to insecticides. When transferring the prepared pesticides diluted with water to the tank, the filling process is carried out again using personal protective equipment with the help of a dosing hopper, taking into account spillage and splashing situations.
- Failure to maintain a safe distance while the drone is taking off at the beginning of the spraying process may cause exposure to the sprayed pesticide. Drone operators start the drone at a certain distance from the drone and with protective clothing.
- Especially in the summer months when the air temperature is very hot, they also face possible risks such as exposure to sunlight due to being outdoors.
- The company sometimes sprays in the evening hours, taking into account conditions such as weather

conditions and explosions in the sun (as they negatively affect satellite and GPS systems). In spraying at night, both the battery heating is minimized and the pesticide sprayed from the nozzles is more effective due to excessive heat.

- One of the dangers during spraying of the drone is losing control of the drone. The software update of the device can lead to accidents. Drone operators have previously stated that the drone suddenly fell to the ground after an update.
- After the spraying process is over, the drone is turned off and loaded into the company's vehicle.
- White vinegar is generally preferred for tank cleaning. However, if oil-based pesticides are used in spraying, oil-dissolving chemicals on the market are also used to prevent accumulation or sedimentation at the bottom and thus reducing the effect of another pesticide.

## Conclusion and Recommendations

Drones are highly preferred technology in terms of reducing the duration of agricultural spraying and reducing human exposure to pesticides used to eliminate harmful weeds or insects. In traditional spraying methods (using tractor-drawn machine or backpack-borne sprayers), the risk of workers being exposed to pesticides is quite high. Since pesticides can enter the human body through inhalation or skin, drone spraying makes a serious contribution in this sense.

In addition, traditional methods are physically demanding and can cause musculoskeletal disorders in workers. Drone spraying significantly reduces the physical load because workers control the process only by directing the drone. Drone spraying offers a safe and effective method, especially in hazardous areas such as flooded paddy fields.

When performing agricultural spraying with drones, careful planning of the workflow is critical for the safe and efficient completion of the process. This workflow can be divided into three main stages: pre-flight, during flight, and post-flight.

### *Before Flight*

- Planning: The transportation route of the area to be sprayed must be determined and its geographical and topographic structure must be reviewed. In addition, the type of pesticide to be used, dosage, wind speed and air temperature must be taken into consideration to determine the time of spraying. It is important to check the mechanical and electronic parts of the drone, especially the nozzles, and to remove any blockages. Personal protective equipment (PPE) must also be ready at this stage.
- Pesticide Preparation: The pesticide must be prepared according to the instructions and mixed with water at the recommended dose. During this process, the drone operator must use appropriate PPE such as glasses, mask, gloves and protective clothing. In addition, precautions must be taken against leakage, spillage and splashing while filling the tank of the drone.

### *In the Flight Area*

- Field Observation: It must be ensured that there are no people or animals around the area to be sprayed.
- Technical Checks: It must be ensured that the drone's GPS connection and mapping system are working, the route is loaded correctly and the signal connections are healthy. The operator must be at a safe distance while the drone is taking off.

### *Post-Flight*

- Landing: After the drone completes its mission, it must land safely and wait for it to cool down.
- Equipment Cleaning: After the spraying process is completed, the drone's spray system, tank and propellers must be cleaned appropriately.

The following suggestions can be listed as improvements in agricultural spraying with drones:

Emergency action plans should be prepared for situations such as sudden health problems that employees may encounter, such as access to first aid and emergency health services, and for risks that may be encountered in transportation. Approaching power lines can be dangerous for both the drone and the operators. High voltage lines in particular pose a serious risk. On the other hand, insufficient knowledge and experience regarding the use of drones can endanger the safety of the device and the people and animals around it. In this context, operators need to have certain technical knowledge. Having an action plan in case of emergency ensures that the device is less affected by the possible negativities. If the periodic maintenance of the device is not done on time or is ignored due to its costs, the device may lose its reliability. This may have some negative consequences in terms of occupational health and safety and environmental safety.

## Declarations

### *Limitations of the Research*

The research only covers the flights and operations performed in the rice and sunflower fields within the scope of the specified drone brand. This situation expresses the limitation of the research. Since it cannot be generalized to other agricultural products, different regions and different device brands and models, it can be considered as an opportunity for future research.

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# Formulation of Multi-Source Edible Oils from Palm oil and African Walnut oil and Study of Their Effect on Hematological, Inflammatory and Oxidative Stress Markers in High Fat Diet Obese-Induced Wistar Rats

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ABSTRACT

The objective of this study was to evaluate the effects of palm oil, African walnut oil and their blends on hematological, inflammatory, and some oxidative stress markers in high fat diet (HFD) obese-induced Wistar rats. Obesity was induced for 60 days and treated for 28 days using edible oils [palm oil, African walnut oil, palm oil: African walnut oil (50:50) and palm oil : African walnut oil (60:40)] and orlistat (10 mg/Kg). Thereafter the animals were sacrificed, blood was collected for hematological studies and the preparation of the serum, while the organs harvested were used to prepare organ homogenates. Serum and organ homogenates were used for the evaluation of inflammation and oxidative stress markers. Results showed that the oils utilized were confirmed to be of high quality through their good stability indices (peroxide value: 2.52-3.87meq $O_2$/Kg; *p*-anisidine value: 8.24-12.33, TOTOX value: 13.37-19.46,). Looking at the haematological study, animals that received the HFD presented the lowest ($p<0.05$) hematocrit and Platelet. PO:WO (50:50 and 60:40) significantly ($p<0.05$) decreased the granulocytes concentration in the blood of rats. PO:WO (50:50) significantly ($p<0.05$) increased the lymphocyte concentration while 100% PO increased the mid-size white blood cells level in the animals. Serum levels of inflammation markers were higher ($p<0.05$) in the negative control group (354.44-385.82 pg/mL) compared to the other groups (147.22-271.55 pg/mL). The analysis of oxidative stress parameters revealed that the administered oils and orlistat generally exhibited good protections compared to the normal and negative control groups, which might be due to the presence of omega-3 fatty acids and bioactives such as β-carotene and vitamin E which have good antioxidant and anti-inflammatory properties. It can be concluded that these oils have a role in protecting against obesity through their effects on oxidative stress, hematology, and inflammatory cytokines.

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## Introduction

Over the last few years, the consumption of fast foods, high calorie foods, and processed foods has become a day-to-day activity resulting in medical conditions that show very clearly the implication of diet on health. Oils, being an essential and staple component of diet play a great role in an individual's overall health, as the type of fat consumed, method of preparation and frequency can ameliorate or deteriorate one's health. Obesity, a metabolic disorder characterized by fat accumulation in adipose tissues, has become increasingly prevalent and it is due to many factors amongst which to the consumption of high calorie foods, sedentary lifestyle and lack of exercise (Kelly & Uadia, 2020).

In recent years, the values representing statistics of obesity on a regional, national and international scale have been increasing. Statistics show that in 2022, one in eight people in the world were living with obesity. Obesity in adults has doubled since 1990 and has quadrupled in adolescent. Still in 2022, 2.5 billion adults (>18 year old) were overweight, from which 890 million were obese. 37 million children under age of 5 were reported to be overweight. Over 390 million children and adolescent aged 5-19 years were reported overweight in 2022, with 160 million of them living with obesity (WHO, 2024). The prevalence to obesity and overweight has risen worldwide in the pass decades also. According to the World Obesity

Atlas (2023), the estimated statistic of obese and overweight people in 2020 among the global population aged >5 was 38% which is expected to move to 51% by 2035 (Jeon et al., 2024).

According to a multicentric study, the prevalence of obesity in Africa ranges from 4.5 to 32.5% (Oladeji et al., 2021). Cameroon is not spared from this. The most recent data shows that the prevalence of obesity in Cameroon is approximately 9.4% among adults (WHO, 2015).

Obesity has been proven to be a major risk factor for developing several chronic diseases such as type 2 diabetes mellitus, cardiovascular diseases, cancer, kidney failure and so on because fat accumulation leads to complications affecting blood flow, insulin sensitivity, lipid metabolism, inflammatory responses and more (Shalom et al., 2020). According to WHO statistics from 2019, unhealthy fat intake and accumulation are the highest risk factors for developing heart diseases and stroke. In spite of the preventive measures put in place, the prevalence of obesity continues to increase and if this trend persists it is estimated that by 2025, 18% of men and 21% of women worldwide will be obese (Kinlen et al., 2018). Unhealthy dietary habits, particularly the consumption of high-fat diets is becoming rampant and plays a significant role in the development and progression of obesity.

The detrimental effects of excessive fat intake are well-documented (Schmidt et al., 2015). Consequently, there is a growing interest in exploring alternative dietary approaches that can mitigate the adverse effects of high-fat diets and their complications especially in developing countries like Cameroon, where consumption of pork, refined sugars and processed foods is on a rise. The Food Safety and Standards Authority of India (FSSAI) as of June 2021 recommended the consumption of balanced and diverse diets for maintaining optimal health (FSSAI, 2021). In line with these guidelines, the formulation of multi-source edible oils using a combination of two or more oils has gained attention due to its potential to provide a balanced fatty acid profile and a range of health benefits as oil blends are richer in nutrients especially in essential fatty acids (EFA), vitamins and are more stable than oils rich in polyunsaturated fatty acids alone (FSSAI, 2021; Dhyani et al., 2022).

Many oils are available in Cameroon and used individually with the most common being palm oil and its refined fraction-palm olein, which are consumed in all regions of Cameroon. Since the population is slowly becoming aware of the importance of consuming omega 3 and 6 rich oils, the oils available like soya bean oil and cotton seed oil are quite expensive and not accessible to everyone. Additionally, these oils are poor in omega 3 and omega 6 fatty acids. On the other hand, good plant sources of omega-6 and omega-3 fatty acids are available, that is the case of African walnut oil which contains about 14.41% of omega 6 fatty acids and 70.39% of omega 3 fatty acids. It will be important to evaluate the effect of the mixture of this oil with palm oil at different proportions according to FSSAI (2021) to see its effect on obesity.

Due to the rapid increase in the obese population, many drugs have been developed for their treatment and management purposes (for example Orlistat) but just like other synthetic drugs, these drugs are not always 100% effective, they are expensive, and may have side health effects depending on the individual (Yael et al., 2021). Orlistat was reported as very effective and well tolerated anti-obesity drugs which can be employed as adjunct to therapeutic lifestyle changes to achieve and maintain optimal weight (Jain et al., 2011). It acts by binding to serine residue found at the active site of gastric and pancreatic lipases. It acts by partially inhibiting the breakdown of triglycerides, thus reducing the absorption of free fatty acids and monoglycerides (Guerciolini, 1997). Diets rich in polyunsaturated fatty acids have proven to have very promising effects in combating non communicable diseases like obesity, and obesity related conditions (Duo, 2015). Since Cameroon has shown limited progress in using food ingredients and diets to address non-communicable disease (NCD), it is necessary to formulate and adopt diet changes using food staples that are readily available in Cameroon for obesity management (African Population and Health Research Center, 2018).

Palm oil is a widely consumed oil in all regions of Cameroon. It contains a balanced proportion of saturated fatty acids; particularly palmitic acid (44%), stearic acid (4.5%) and unsaturated fatty acids; oleic acid (39.2%), linoleic acid (10%), α-linolenic acid (0.4%). It also contains a high proportion of antioxidants, Vitamin E and Beta carotene (Annamaria et al., 2015). However, this oil was reported to inflame white adipose tissue and triggers metabolic disturbances in mice on a high-fat diet (Martins et al., 2024). Widjaja et al. (2018) reported that supplementation of coconut oil and palm oil in rats can increases body mass index, abdominal circumference, and fat mass. The effects recorded by these authors were related to the high content in saturated fatty acids of palm oil. On the other hand, omega-3 rich oils were reported to have improved body composition and counteract obesity-related metabolic changes (Albracht-Schulte et al., 2018). An example of omega-3 rich oil available in Cameroon and that can be exploited towards this direction is African walnut oil.

African walnut (*Tetracarpidium conophorum*) is a traditional oil source in Africa which has not been much exploited. It is highly available in Cameroon between July and September of each year and is often boiled, cracked and eaten like other traditionally boiled nuts. It is not exploited for its oil in Cameroon though it has been reported to contain 9.48% Oleic acid, 14.41% linoleic acid and 70.39% alpha-linoleic acid (Tchiegang et al., 2007). Similar composition was reported by Ghomdim et al. (2024) who showed that *Tetracarpidium conophorum* oil is composed of 14.35% oleic acid, 10.56% linoleic acid and 69.90% alpha-linolenic acid. African Walnuts was reported to attenuate ectopic fat accumulation and associated peroxidation and oxidative stress in monosodium glutamate-obese Wistar rats (Uti et al., 2020a). These same authors showed that African Walnuts modulate hepatic lipid accumulation in obesity via reciprocal actions on HMG-CoA reductase and paraoxonase (Uti et al., 2020b). It was also reported to have good hypolipidemic properties (Oriakhi and Uadia, 2020). Due to the fact that palm oil is the most consumed and available oil in Cameroon, blending it with omega-3 fatty acids-rich oil such as African walnut oil can help improve the health of obese patients. Multi-source edible oils or oil

blends from Palm and African Walnut oils can be a promising solution to reduce the prevalence of obesity in Cameroon if they are known and adopted by the population. Both oils have shown promising effects in various studies for their potential to modulate physiological processes and provide protective effects against certain diseases (Kaur et al., 2014). It can be hypothesized that multi-source edible oils from palm and walnut oils can significantly reduce the prevalence of obesity and some of its complications in high fat diet induced-obese Wistar rats. The objective of this study was to evaluate the effects of multi-source edible oils from palm and walnut oils in the management of obesity and some of its complications in high fat diet induced-obese Wistar rats.

## Materials and Methods

### *Materials*

Fresh African walnuts were purchased from a farmer in Muyuka, Fako Division, South-West Region of Cameroon in October 2023. 5 litres of pressed palm oil was purchased from Fontem, Lebialem Division, South-West Region of Cameroon. Forty-two albino Wistar rats aged 3-4 months and weighing 150-200 g were purchased from a farmer in Yaoundé, Centre Region of Cameroon. The reagents and other chemicals utilized were of good purity.

### *Methods*

*Extraction and partial purification of African walnut oil Extraction*

African walnuts were cracked to remove the shells. The obtained nuts were cut into small pieces and dried in an electric air-drying oven at 50°C for 24 hours. The dried nuts were cold pressed using the HD Manual Oil Press Machine Expeller Extractor Stainless Steel#304 to obtain the oil following the manufacturer's guide. About two kilograms of ground African walnuts were used. The extracted oil was collected in a beaker gradually and later stored in the freezer at -18°C for further analysis and the defatted cake discarded.

*Oils degumming*

The method reported by Moretto and Fett (1998) was used. In a beaker of 250 mL, 100 g of oil and 3 mL of warm water (70°C) were added. After stirring the mixture for 30 min, it was allowed to decant. The precipitate was removed and the oil filtered and dried using anhydrous sodium sulfate.

*Partial Purification of Oils*

Purification of oil samples was done following the method described by Ulfah et al. (2023) using palm kernel shell activated carbon and decolorized activated charcoal at 1.5%. Purification was done by adding 100 mL of oil into a 250 mL Erlenmeyer flask. After that, activated carbon was added (1.5%). The Erlenmeyer flask containing the oil and activated carbon was coated with aluminium foil, heated and stirred on a hot plate magnetic stirrer for 60 minutes at 70˚C. A vacuum filter was used to isolate the filtrate. The filtrate was transferred in bottles coated with aluminium foil and kept in the freezer at -18˚C for further analysis.

*Formulation of multisource edible oils*

Multisource edible oils from palm and African walnut were prepared in two different ratios, 50:50 and 60:40 as recommended by the Food Safety and Standards Authority of India (FSSAI, 2021). The uniform multisource oils were prepared by stirring the sample at 180 rpm (revolutions per minute) for 15 min as reported by Dhyani et al. (2022). It is important to note that saturated oils were warmed at their melting temperatures before use. The samples and their respective blends are presented in Table 1.

Table 1. Composition of different oil samples

| Samples | Composition |
|---|---|
| 1 | African walnut oil (100%) |
| 2 | Palm oil (100%) |
| 3 | Palm oil (50%) + African walnut oil (50%) |
| 4 | Palm oil (60%) + African walnut oil (40%) |

Upon formulation of the multisource oils, all the oils presented in table 1 were initially characterized for their physicochemical properties before being subjected to thermal treatment to study their stability. The parameters analyzed were the colour, peroxide, *p*-Anisidine, TOTOX, acid values and the Fourier-transformed infrared spectroscopy

*Oil quality analysis*

*Colour:* The change in colour of oil samples during storage was measured using a FRU WR10 Portable colorimeter. The device was calibrated using an empty petri dish lying on a white surface and the value of the blank recorded. After that, oil was introduced into the petri dish and scanned using the colorimeter. The value for the blank was deducted from the test value and recorded. Analyses were carried out in triplicate. The obtained results were presented as $L$, $a^*$ and $b^*$. L* is known as the lightness ($L$ = 0 (black), $L$ = 100 (white); $a^*$ ($-a$ = greenness, $+a$ = redness) and $b^*$ ($-b$ = blueness, $+b$ = yellowness).

*Quality indexes:* Standard methods were used to analyze oil quality. The peroxide value was determined using the IDF method (IDF, 1991), the p-anisidine and acid values using the AOCS standard method (AOCS, 2003), the thiobarbituric acid value following the method reported by Draper and Hadley (Draper and Hadley, 1990) and the TOTOX value calculated using the following equation as mentioned by Shahidi & Wanasundara (2008): TOTOX = 2PV + AV.

*Fourier transformed infrared spectroscopy:* The method of Liang et al. (2013) was used for the Fourier transformed infrared spectroscopy of oil samples. A Shimadzu IRPrestige-21 coupled with the DLATGS detector was used. Potassium bromide served as beam splitter and the IRsolution software was used as controller. About 20 µL of sample was dropped between two KBr disks, creating a thin film in the 4000-500 $cm^{-1}$ IR region. The measurements were carried out in duplicate against clean empty KBr disks that served as blank. After each reading, the disks were cleaned twice using hexane, dried with tissue before being washed again with acetone before drying again.

*Animal bioassay*

*Ethical clearance:* Animals were cared for and used in agreement with international standard guidelines for animal use. In order to carry out this study, an ethical clearance for animal handling and care was obtained from the University of Buea - Institutional Animal Care and Use Committee (IACUC) with permit UB-IACUC No 02/2024. Animals were under hygienic conditions, they were given water and food ad libitum and their sawdust was changed every two days. They did not feel any pain during the induction of obesity, since it was done by just modifying their diet into a high fat diet. Gavage was done carefully in order not to wound or stress the animals. During the sacrifice too, they were anesthetized so that they are spared from any pains. They were constantly monitored for safety.

*Preparation of animal feed:* The method described by Othman et al. (2019) with few modifications was used in the formulation of the normal and high fat diets. The normal diet included the following ingredients: 600 g of maize flour, 200 g of soyabeans, 290 g of fish powder, and 10 g of salt for each kilogram of food. The high fat diet (HFD) consisted of 600 g of normal diet, 110 g of boiled egg yolk as source of cholesterol and 290 g of lard, which is a saturated fat from pig. These proportions are for one kilogram of food. After a dough-like consistency was formed using water. The feed was shaped into small balls and dried in the oven at 40$^{o}$ C overnight and used to feed the rats the next morning. Feed was prepared everyday to avoid chemical and biological spoilage.

*Treatment of animals:* Rats were acclimatized under a 12-hour light-dark cycle at room temperature for 14 days in cages containing sawdust, with ad libitum access to food and water prior to the start of the experiment. A total of forty-two adult male rats was randomly distributed into seven groups of six rats each. Group 2, 3, 4, 5, 6 and 7 were fed with the high-fat diet for 60 days and the oils and Orlistat treatments was done on group 3,4,5,6, and 7 for 28 additional days. The characteristic of each group is presented in Table 2.

After 28 days of treatment, the rats were fasted overnight and anesthetized using Ketamine 60mg/Kg and Diazepam 10mg/kg body weight and the blood collected by cardiac puncture using a 5 mL syringe. The blood was divided into two portions; the first one was introduced in tubes with EDTA and was used for hematological evaluation. The second was introduced in a tube without EDTA and the serum obtained by centrifugation (3000 rpm for 15 min). The serum was used for the evaluation of oxidative stress and inflammatory cytokines. Organs of interest (heart, kidney, pancreas, brain, spleen and liver) were collected and weighed. They were used to prepare organs homogenates (22 g of organ/100 mL of distilled water) which were centrifuged at 3000 rpm for 10 min and used for the determination of oxidative stress markers.

*Determination of hematological parameters:* Hematological analyses were carried-out on blood samples introduced in tubes containing EDTA. They were done using an automatic hematological analyzer (SFRI H18 LIGHT auto Hematology Analyzer). The parameters analysed were: White blood cells (WBC), Red blood cells (RBC), Hemoglobin (HGC), MID (Mid-size white blood cells), Haematocrit (HCT), Mean copuscular volume (MCV), Granulocytes (GRAN), Platelet (PLT), Mean Platelet Volume (MPV), Platelet Large Cell Ratio (PLCR), Platelet Distribution Width (PDW), Mean Corpuscular Haemoglobin (MCH) and MCHC.

*Analysis of some inflammatory cytokines:* Serum was used for the analysis of inflammatory cytokines. TNF-α, INF-γ, IL-1β, and IL-6 were quantified using an ELISA kit (Quantikine Colorimetric ELISA Kits (Quantikine®), following instructions provided by the manufacturer. The ELISA kits were obtained from R&D Systems Biotechne, USA.

*Determination of oxidative stress parameters:* Serum and organ homogenates were used for the measurement of oxidative stress parameters.

*Malondialdehyde (MDA)*

Malondialdehyde (MDA) level was measured following the method described by Yagi (1976). About 100 µl of serum/ organ homogenate was introduced into test tubes followed by 500 µL of 1% thiobarbituric acid (prepared in 1% trichloroacetic acid) and 500 µL of 1% phosphoric acid. The mixture incubated at 100°C for 15 min and then allowed to cool for 30 min. The tubes were later centrifuged at 3000 rpm for 10 min and the supernatant collected. The absorbance of the supernatant was recorded at 532 nm. The blank was prepared under similar conditions but the sample was replaced by distilled water.

The MDA concentration was calculated using the extinction coefficient 1.56 × 105 $M^{-1}cm^{-1}$ and expressed as µmol of MDA per g of tissue following the formula:

$$[\text{MDA}]\ (\mu\text{mol/g tissue}) = \frac{A}{E \times L}$$

Where; A= Absorbance, Vt=the total volume of the medium (ml), V1=volume of sample (in cuvette); m = weight of tissue used (g); L = light path = 1cm; E= Extinction coefficient =1.56 × 105 $M^{-1}cm^{-1}$

Table 2. Animal group distribution and characteristics

| Groups | Characteristics |
|---|---|
| Group 1 (normal) | Healthy rats fed with the normal rodent chow |
| Group 2 (negative control) | Obese rats + distilled water (250 mg/kg bodyweight (BW) |
| Group 3 (positive control) | Obese rats + oral administration of Orlistat (10 mg/ kg) for 28 days |
| Group 4 (test group 1) | Obese rats + oral administration of 1000 mg/ kg of African walnut oil for 28 days by gavaging |
| Group 5 (test group 2) | Obese rats + oral administration of 1000 mg/ kg of palm oil oil for 28 days by gavaging |
| Group 6 (test group 3) | Obese rats + oral administration of 1000 mg/ kg of a 50:50 (PO:WO) multisource edible oil from palm and African walnut oils for 28 days by gavaging |
| Group 7 (test group 4) | Obese rats + oral administration of 1000 mg/ kg of a 60:40 (PO:WO) multisource edible oil from palm and African walnut oils for 28 days by gavaging |

*Reduced glutathione (GSH)*

Reduced glutathione (GSH) level in organ homogenates and serum was assayed as described by Ellman (1959). About 100 µL of organ homogenates or serum was added to test tubes followed by 900 µL of Ellman reagent (0.4 mg/mL) (prepared in tris-HCl buffer (0.1M, pH 6.5). After stirring the tubes using a vortex, the mixture was incubated at room temperature for 30 min. The absorbance was recorded at 412 nm against a blank which contained 900 µL of the reactive solution and 100 µL of NaCl 0.9% and incubated under the same conditions. The concentration of Thiol groups (SH) was measured by the extinction coefficient (Ɛ) of 13600 $M^{-1}cm^{-1}$ and expressed as µmol/g of total protein. The concentration of GSH was determined as follow:

$$A=Ɛ\,L$$

Where; A = absorbance, L = path length in centimetres, C = concentration in Moles/litter (M), Ɛ the extinction coefficient

*Catalase activity (CAT)*

The method used by Sinha (1972) was applied for the determination of the catalase activity. About 750 µL of phosphate buffer (pH 7.4, 0.1M) was introduced into a test tube, followed by the addition of 50 µL of sample (organ homogenate or serum). The reaction was initiated by the addition of 100 µL of $H_2O_2$ (50mM) and a minute after, 1 ml of potassium dichromate (5%) which was prepared in 1% acetic acid. The tubes were then incubated at 100 ˚C for 10 min and the absorbance recorded at 570 nm against a blank (50 µ NaCl 0.9%) prepared under similar conditions. The Catalase activity, expressed as µmol $H_2O_2$ used/min/mg of protein was determined using the formula:

$$\text{Catalase activity} = Ab \times 1000 \times Vt \times Ɛ \times V$$

Where: Ab: mean of absorbances, **Ɛ**: molar absorbtion coefficient. ( 40 M $cm^{-1}$); V: volume of organ homogenate or serum; Vt: Total volume of reaction medium

*Nitric oxide (NO) level*

The nitric oxide (NO) level determined following the method reported by Montgomery and Doymock (1961). About 100 µL of organ homogenate/serum was mixed with 100 µL of sulfanilamide 1% (prepared in phosphoric acid 5%) followed by incubation at room temperature for 5 min. After that, 100 µL of 0.1% Naphtylethelene diamine (NED) was added and the mixture was incubated once more at room temperature for 5 min. Formation of a purple magenta was indicative of the presence of nitrite formed. The absorbance was recorded at 540 nm. The concentration of NO was determined from the standard curve using different concentrations of $NaNO^2$. NO concentration was determined using its molecular coefficient of **Ɛ**=39500$M^{-1}.cm^{-1}$and calculated as thus:

$$\text{NO Concentration (µM/L)} = \frac{\text{Absorbance}}{Ɛ \times L}$$

***Statistical Analysis***

The obtained data (Mean ± Standard deviation) were subjected to one-way analysis of variance (ANOVA) using Statgraphics Centurion version XVI in order to evaluate the statistical significance of the data. A probability value at $p<0.05$ was used for statistical significance.

## Results

***Oil Quality***

The initial physicochemical properties of oil samples used in this study are presented in Table 3. The colour of PO was found to be more black, more red and more yellow (*L*, *a**, *b** respectively); WO was more black, normal and more yellow; 50:50 (PO:WO) more black, more red, more yellow and 60:40 (PO:WO) more black, more red and more yellow. The peroxide, p-anisidine, TOTOX, acid and iodine values were respectively between 2.52-3.87meq $O_2$/Kg, 8.24-12.33, 13.37-19.46, 0.32-20.34 mg KOH/g and 55.21-166.41 g $I_2$/100g. The highest iodine value was recorded with 100%WO followed by 50:50 (PO: WO), 60:40 (PO: WO) and 100%PO respectively.

The Fourier Transform Infrared Spectroscopy of oil samples are presented in Figure 1 (A-D). The results revealed no significant ($p > 0.05$) peak between 3250 and 3750 $cm^{-1}$. On all the 4 figures, a peak was recorded at 3000 $cm^{-1}$, and another peak at 2900 $cm^{-1}$, an abundant peak was recorded at 1750 $cm^{-1}$. No peak was found at 2750 $cm^{-1}$ and 1600 $cm^{-1}$. Looking at the fingerprint region, a peak was recorded around 900 $cm^{-1}$ with the highest intensity found in100%PO. Another peak was found at 750 $cm^{-1}$.

Table 3. Characterization of the oils physicochemical properties

| Parameter | | Palm oil (PO) (100%) | Walnut oil (WO)(100%) | 50:50 (PO:WO) | 60:40 (PO:WO) | Standard CSX 329-2017 (WHO/FAO) |
|---|---|---|---|---|---|---|
| Colour | *L** | -30.13±0.00$^a$ (More black) | -27.15±0.94$^a$ (More black) | -28.52±0.04$^a$ (More black) | -29.41±0.26$^a$ (More black) | / |
| | *a** | *4.075±0.00$^a$ (More red) | *-0.58±0.00$^b$ (Normal) | *4.55±0.31$^a$ (More red) | *2.40±0.04$^c$ (More red) | / |
| | *b** | *7.27±0.00$^a$ (More yellow) | *4.52±0.82$^b$ (More yellow) | *9.01±0.31$^a$ (More yellow) | *8.72±0.33$^a$ (More yellow) | / |
| Peroxide value (meq $O_2$/Kg) | | 3.16±0.21$^a$ | 2.52±0.00$^a$ | 3.87±0.00$^a$ | 2.56±0.00$^a$ | ≤15 meq $O_2$/Kg |
| *p*-Anisidine value | | 9.39±0.07$^a$ | 12.33±0.00$^b$ | 11.72±0.00$^b$ | 8.24±0.00$^a$ | ≤20 in fish oil |
| TOTOX value | | 15.72±2.51$^{ac}$ | 16.83±0.00$^a$ | 19.46±0.00$^b$ | 13.37±0.00$^c$ | ≤26 in fish oil |
| Acid value (mg KOH/g) | | 0.32±0.00$^a$ | 20.34±0.22$^b$ | 12.17±1.35$^c$ | 10.25±0.45$^c$ | 4 mg KOH/g |
| Iodine value (g$I_2$/100 g) | | 55.21±1.24$^a$ | 166.41±3.54$^b$ | 97.45±1.32$^c$ | 85.41±3.42$^d$ | / |

n=3 values are presented as mean±SD. $^{a-d}$Values with different superscript letters in the same row are significantly different at $p<0.05$ indicating significance. WO= African walnut oil; PO= Palm oil; 50:50 (PO:WO)= 50:50 (Palm oil : African walnut oil); 60:40 (PO:WO)= 60:40 (Palm oil : African walnut oil).

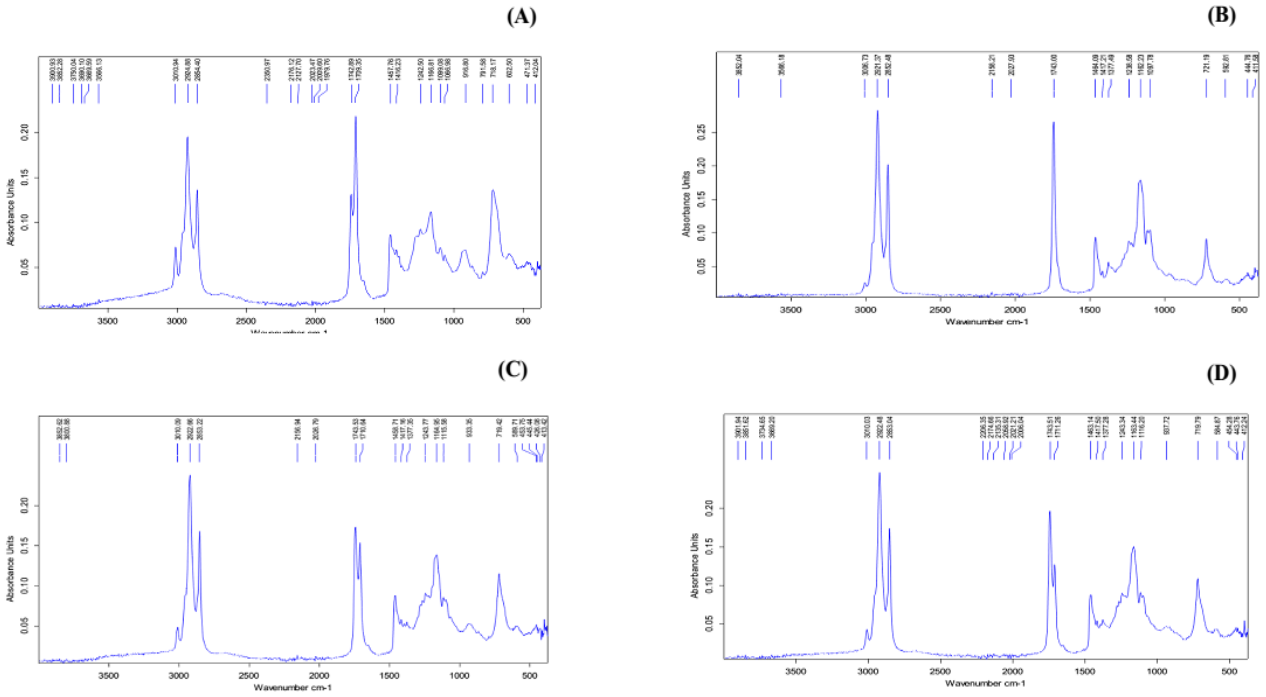


Figure 1 (A-D): Fourier Transfer Infrared Spectroscopy of A: African walnut oil; B: Palm oil; C: 50:50 (Palm oil: African walnut oil); D: 60:40 (Palm oil: African walnut oil)

Table 4. Effect of African walnut oil, palm oil and their blends on Hematological parameters in HFD obesity-induced Wistar rats

| | WBC ($\times10^9$/L) | LYM (%) | MID (%) | GRAN (%) | RBC ($\times10^{12}$/L) | HGB (g/dL) | HCT (%) | MCV (fL) |
|---|---|---|---|---|---|---|---|---|
| Normal | 2.90±0.72$^{a}$ | 66.00±3.45$^{a}$ | 16.87±1.25$^{a}$ | 18.46±2.67$^{a}$ | 7.29±0.82$^{a}$ | 14.36±0.98$^{a}$ | 54.86±2.08$^{a}$ | 54.86±1.36$^{ab}$ |
| Negative control | 3.00±0.14$^{ab}$ | 61.46±7.01$^{a}$ | 19.53±1.24$^{a}$ | 22.10±3.25$^{a}$ | 7.51±1.16$^{a}$ | 14.07±1.60$^{a}$ | 43.86±4.09$^{b}$ | 56.35±2.16$^{a}$ |
| Orlistat (10 mg/kg) | 5.46±1.49$^{b}$ | 66.95±7.24$^{a}$ | 18.72±2.29$^{a}$ | 21.36±0.55$^{a}$ | 7.65±0.44$^{a}$ | 13.16±0.98$^{a}$ | 39.50±2.48$^{c}$ | 51.70±1.52$^{b}$ |
| 100% WO[1] | 5.32±1.10$^{b}$ | 61.90±4.71$^{a}$ | 23.67±3.21$^{ab}$ | 20.86±3.20$^{a}$ | 7.82±0.77$^{a}$ | 13.65±1.61$^{a}$ | 41.22±3.96$^{b}$ | 52.60±1.56$^{ab}$ |
| 100% PO[1] | 4.56±0.55$^{b}$ | 51.8±10.01$^{a}$ | 26.23±4.78$^{b}$ | 25.96±3.00$^{a}$ | 8.05±0.53$^{a}$ | 13.30±2.10$^{a}$ | 42.02±4.00$^{b}$ | 52.71±1.70$^{ab}$ |
| 50:50 (PO: WO)[1] | 4.95±0.77$^{b}$ | 81.65±3.04$^{a}$ | 11.70±1.27$^{c}$ | 6.65±1.76$^{b}$ | 7.46±0.45$^{a}$ | 12.50±0.95$^{a}$ | 38.40±2.16$^{c}$ | 51.56±0.95$^{ab}$ |
| 60:40 (PO: WO)[1] | 10.75±3.32$^{c}$ | 65.06±5.32$^{a}$ | 18.63±2.40$^{a}$ | 16.30±3.76$^{c}$ | 8.31±0.43$^{a}$ | 14.07±0.35$^{a}$ | 42.30±0.88$^{b}$ | 51.15±1.81$^{ab}$ |
| | MCH (pg) | MCHC (g/dL) | PLT ($10^9$/L ) | MPV (fL ) | PCT (%) | P-LCR (%) | PDW (fL) | |
| Normal | 17.86±1.22$^{a}$ | 32.64±1.72$^{a}$ | 829.66±104.88$^{a}$ | 6.48±0.23$^{a}$ | 0.546±0.08$^{a}$ | 8.27±1.03$^{a}$ | 8.42±0.26$^{a}$ | |
| Negative control | 17.52±1.06$^{a}$ | 31.84±3.35$^{a}$ | 734.50±52.84$^{ab}$ | 6.04±0.30$^{a}$ | 0.42±0.18$^{a}$ | 5.66±1.12$^{b}$ | 7.84±0.99$^{a}$ | |
| Orlistat (10 mg/kg) | 17.15±0.70$^{a}$ | 33.25±0.68$^{a}$ | 774.00±78.31$^{ac}$ | 6.31±0.24$^{a}$ | 0.48±0.05$^{a}$ | 8.15±1.96$^{a}$ | 8.46±0.58$^{a}$ | |
| 100% WO[1] | 16.63±1.34$^{a}$ | 31.73±2.13$^{a}$ | 679.00±0.00$^{d}$ | 6.30±0.04$^{a}$ | 0.04±0.00$^{a}$ | 7.52±0.70$^{a}$ | 8.21±0.93$^{a}$ | |
| 100% PO[1] | 17.03±1.07$^{a}$ | 32.41±1.40$^{a}$ | 715.33±27.20$^{ac}$ | 6.40±0.24$^{a}$ | 0.48±0.07$^{a}$ | 8.03±2.19$^{a}$ | 8.30±0.18$^{a}$ | |
| 50:50 (PO: WO)[1] | 16.70±0.52$^{a}$ | 32.46±0.68$^{a}$ | 807.00±16.82$^{a}$ | 6.33±0.20$^{a}$ | 0.50±0.02$^{a}$ | 7.33±0.20$^{a}$ | 8.30±0.30$^{a}$ | |
| 60:40 (PO: WO)[1] | 16.90±0.48$^{a}$ | 33.12±0.47$^{a}$ | 599.00±70.01$^{c}$ | 6.40±0.31$^{a}$ | 0.36±0.04$^{a}$ | 7.82±2.08$^{a}$ | 8.15±0.51$^{a}$ | |

1: (1000 mg/kg), n=6 values are presented as mean±SD. $^{a-d}$Values with different superscript letters in the same column are significantly different at $p<0.05$ indicating significance. White blood cells (WBC), Lymphocytes (LYM), granulocytes (GRAN), red blood cells (RBC), Haemoglobin (HGB), Haematocrit (HCT), (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelets (PLT), Mean platelet volume (MPV), platelet count (PCT), Patelet larger cell ratio (PLCR), Platelet distribution width(PDW). Palm oil (PO), African walnut oil (WO) and PO:WO (50:50) and PO:WO(60:40).

***Hematological Parameters***

The effect of oils on the hematological parameters of rats is presented in Table 4. No significant ($p > 0.05$) difference was observed between the WBC of the normal and negative control. However, the WBC levels were significantly higher ($p<0.05$) in rats treated with Orlistat and oils. For the lymphocytes, no significant difference was recorded between the LYM% of all groups except for the rats treated with 50:50 (PO:WO) which was significantly higher ($p < 0.05$). The rats fed with 100%PO presented the highest MID compared to the other groups, while the lowest value was recorded in rats fed with 50:50 (PO:WO). 50:50 (PO:WO) and 60:40(PO:W0) significantly ($p < 0.05$) decreased the GRAN concentration in the blood of rats compared to the other groups. No significant difference ($p > 0.05$) was recorded in the values for RBC, HGB, MCV, MCH, MCHC, MPV, PLT, PCT, P-LCR, PDW across the groups. The concentration of HCT significantly reduced in the rats fed with the HFD compared to the normal group. Similar observations were made with PLT number.

***Inflammatory Cytokines***

Results for inflammatory cytokine are presented on Figure 2 (A-D). Serum levels of TNF-α, INF- γ, IL-1β and IL-6 were significantly ($p<0.05$) higher in negative control group as compared to the normal group, Orlistat, 100% WO, 100%PO, 50:50 (PO:WO), and 60:40 (PO:WO).

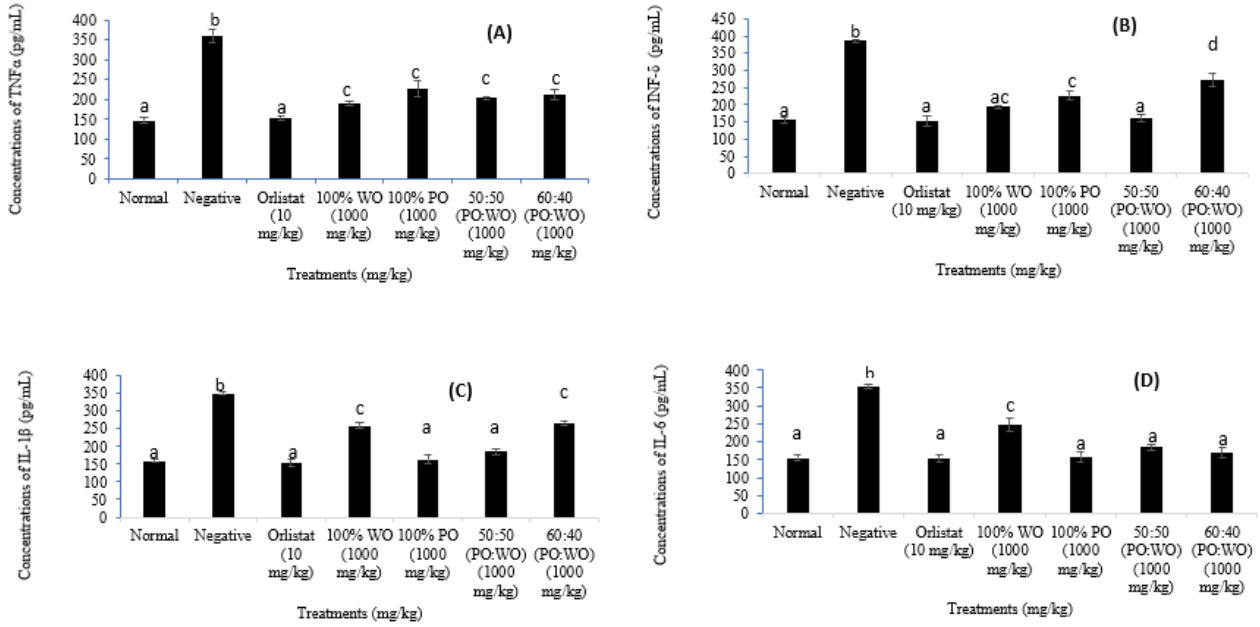


Figure 2. (A-D): Inflammatory cytokine: TNF-α (A), INF-δ (B), IL-1β (C) and IL-6 (D) levels in experimental groups
n=6 values are presented as mean ± SEM. Values with different superscripts are significantly difference at ($p<0.05$). PO; Palm oil, WO; African Walnut oil, 50:50 (PO:WO); 50:50 (Palm oil: African walnut oil), 60:40 (PO:WO); 60:40 (Palm oil: African walnut oil), TNF-α; Tumor necrotic factor-α, INF- γ; Interferon-δ, IL-1β; Interleukin- 1β and IL-6; Interleukin-6

Table 5. Effect of African walnut oil, palm oil and their corresponding blends on CAT levels in organ homogenate and serum (µmol $H_2O_2$/min/mg

| | Spleen | Kidney | Heart | Brain | Pancreas | Liver | Serum |
|---|---|---|---|---|---|---|---|
| Normal | 10.92±1.10$^{a}$ | 11.49±0.46$^{a}$ | 15.12±1.01$^{a}$ | 18.48±0.00$^{a}$ | 14.10±0.00$^{a}$ | 17.16±0.00$^{a}$ | 20.37±2.50$^{a}$ |
| Negative control | 10.80±0.00$^{a}$ | 9.90±0.42$^{a}$ | 14.70±1.78$^{a}$ | 16.56±4.32$^{ab}$ | 12.69±1.90$^{ab}$ | 20.01±0.04$^{b}$ | 18.92±1.41$^{ab}$ |
| Orlistat (10 mg/kg) | 9.82±0.24$^{a}$ | 11.08±2.06$^{a}$ | 15.86±1.17$^{a}$ | 17.62±1.86$^{a}$ | 10.20±0.33$^{b}$ | 17.19±3.86$^{ab}$ | 20.72±1.78$^{a}$ |
| 100% WO[1] | 12.62±1.02$^{ab}$ | 11.67±1.14$^{a}$ | 13.00±0.29$^{a}$ | 21.66±1.27$^{b}$ | 11.32±1.05$^{b}$ | 15.09±1.82$^{ac}$ | 14.32±2.43$^{b}$ |
| 100% PO[1] | 12.63±0.63$^{ab}$ | 10.36±0.78$^{a}$ | 13.74±1.56$^{a}$ | 17.72±1.88$^{a}$ | 10.56±2.12$^{ab}$ | 13.11±1.14$^{c}$ | 22.90±2.82$^{a}$ |
| 50:50 (PO: WO)[1] | 10.44±0.00$^{a}$ | 11.04±0.00$^{a}$ | 15.06±0.00$^{a}$ | 20.94±0.00$^{ab}$ | 12.06±0.00$^{ab}$ | 17.88±0.00$^{ab}$ | 22.26±5.15$^{a}$ |
| 60:40 (PO: WO)[1] | 13.68±0.00$^{b}$ | 9.00±0.00$^{a}$ | 14.79±1.23$^{a}$ | 22.29±1.48$^{ab}$ | 13.44±1.78$^{ab}$ | 16.86±0.00$^{abc}$ | 22.44±3.49$^{a}$ |

1: (1000 mg/kg), n=6 values are presented as mean±SD. a-cValues with different superscript letters in the same column are significantly different at $p<0.05$ indicating significance. Palm oil (PO), African walnut oil (WO) and PO:WO (50:50) and PO:WO(60:40).

Table 6. Effect of African walnut oil, palm oil and their corresponding blends on GSH levels in organ homogenate and serum (µmol)

| | Spleen | Kidney | Heart | Brain | Pancreas | Liver | Serum |
|---|---|---|---|---|---|---|---|
| Normal | 40.07±0.00$^{a}$ | 117.50±25.37$^{a}$ | 142.27±7.27$^{ab}$ | 64.33±0.00$^{a}$ | 60.07±0.00$^{a}$ | 129.08±0.67$^{ac}$ | 45.58±0.51$^{a}$ |
| Negative control | 126.02±0.00$^{b}$ | 112.31±18.04$^{a}$ | 134.55±12.16$^{ab}$ | 104.55±0.00$^{bc}$ | 27.94±14.14$^{bc}$ | 132.79±0.00$^{a}$ | 67.94±10.08$^{b}$ |
| Orlistat (10 mg/kg) | 24.41±0.00$^{c}$ | 110.77±2.85$^{a}$ | 141.37±3.63$^{ab}$ | 75.18±23.24$^{ab}$ | 37.23±18.54$^{b}$ | 139.70±1.08$^{b}$ | 97.53±10.76$^{c}$ |
| 100% WO[1] | 62.38±10.24$^{d}$ | 112.67±20.67$^{a}$ | 124.60±20.87$^{ab}$ | 115.51±6.23$^{c}$ | 21.94±8.57$^{bc}$ | 127.89±17.46$^{abc}$ | 59.74±1.81$^{b}$ |
| 100% PO[1] | 141.43±7.64$^{e}$ | 101.91±12.45$^{a}$ | 116.88±20.64$^{a}$ | 73.757.27$^{ab}$ | 13.63±1.2$^{c}$ | 133.65±7.19$^{ab}$ | 52.27±14.97$^{b}$ |
| 50:50 (PO: WO)[1] | 97.05±0.00$^{f}$ | 102.20±0.00$^{a}$ | 134.26±0.00$^{b}$ | 105.73±0.00$^{b}$ | 68.45±0.00$^{a}$ | 133.75±0.00$^{a}$ | 45.33±1.50$^{a}$ |
| 60:40 (PO: WO)[1] | 57.64±0.006$^{d}$ | 127.42±6.13$^{a}$ | 133.52±0.18$^{b}$ | 122.09±4.10$^{b}$ | 39.26±0.00$^{b}$ | 117.86±5.40$^{c}$ | 90.34±8.51$^{c}$ |

1: (1000 mg/kg), n=6 values are presented as mean±SD. $^{a-d}$Values with different superscript letters in the same column are significantly different at $p<0.05$ indicating significance. Palm oil (PO), African walnut oil (WO) and PO:WO (50:50) and PO:WO (60:40).

***Effect on Oxidative Stress Parameters***

*Effect of Oils on CAT Activity in Organ Homogenate and Serum*

The catalase activity of organ homogenates and serum of normal and HFD rats is presented in Table 5. Generally, no significant ($p > 0.05$) difference in this parameter was observed in the spleen, of the normal and HFD groups except for the rats that received 60:40 (PO:WO) who presented a significantly higher ($p < 0.05$) catalase activity. Similarly, no significant ($p > 0.05$) change in CAT activity was recorded in the kidney and heart of the animals across all groups. At the level of the brain, rats treated with 100% PO, 50:50 (PO:WO) and 60:40 (PO:WO) presented the highest CAT activities compared to the normal group and negative control. At the level of the pancreas, rats that received Orlistat and 100% PO presented significantly ($p < 0.05$) lower CAT activities compared to the rats of the other groups.

*Effect of oils on GSH activity in organ homogenate and serum*

Table 6 presents the effect of oils and Orlistat on the GSH activity of HFD rats. A significant increase ($p < 0.05$) in this parameter was recorded in the spleen, of the negative control and the rats treated with 100%PO compared to the other groups where GSH activity was significantly ($p < 0.05$) lower. No significant ($p > 0.05$)

difference was recorded in the GSH activity of the kidney homogenates in all groups. At the level of the heart, the GSH activity was higher in the normal group but lower in the rats that received the HFD even though it was only significant in the rats treated with PO:WO (60:40) and PO;WO (50:50). At the level of the brain, a significant increase ($p < 0.05$) in GSH activity was observed in all the groups that received the HFD compared to the normal group. The group treated with Orlistat and 100% PO exhibited the lowest GSH activities compared to the negative control. At the level of the pancreas, the rats of the normal group and those that received PO:WO (50:50) presented the highest GSH activities compared to the other groups. At the level of the liver, the highest GSH activity was obtained with the group treated with Orlistat and the lowest in the one treated with PO:WO (60:40). No significant difference ($p < 0.05$) was observed in this parameter among other groups. Looking at the GSH of the serum, it generally significantly increased ($p > 0.05$) in groups that received the HFD compared to the normal group. The group treated with Orlistat and 60:40 (PO:WO) exhibited the highest GSH levels.

*Effect of oils on MDA levels in organ homogenate and serum*

The level of malondialdehyde in organ homogenates and serum of rats is presented in Table 7. At the level of the spleen, almost all the rats fed with the HFD presented significantly ($p < 0.05$) higher MDA concentrations compared to the normal group, and the highest value recorded with the negative control group. No significant ($p > 0.05$) difference was observed between the MDA level of the normal group and the rats treated with 100% WO, PO:WO (60:40). At the level of the kidney, the negative control group exhibited significantly ($p < 0.05$) higher MDA levels compared to all the other groups. A significant ($p < 0.05$) increase was seen in the MDA levels in the heart of all the rats that received the HFD compared to the normal group. This parameter was higher in the heart of the rats that received Orlistat or oil, compared to the negative control though this was not significant. At the level of the brain, the rats treated with Orlistat and PO:WO (50:50) presented significantly ($p < 0.05$) lower MDA levels compared to the other groups in which the MDA levels remained similar. For the pancreas, the rats fed with Orlistat, 100%WO and 100%PO plus those of the normal group showed significantly ($p < 0.05$) lower MDA levels compared to the other groups. In the liver, the negative control and the group treated with 100%PO presented the highest MDA concentration compared to the other groups. A similar observation was made at the level of the serum.

*Effect of oils on Nitric Oxide (NO) levels in organ homogenate and serum*

Table 8 shows the NO levels of HFD rats treated and untreated compared to the normal group. Generally, this parameter increased in the groups treated with oils as well as the normal group compared to the negative control and the group treated with Orlistat. At the level of the spleen, no significant ($p > 0.05$) difference was observed between the NO levels of all rats treated with PO:WO (50:50) in which the NO levels were significantly ($p < 0.05$) higher. At the level of the kidney, the homogenates from rats treated with Orlistat, 100% PO, PO:WO (50:50) showed significantly higher NO levels compared to the other groups. The NO level of the heart was significantly ($p < 0.05$) higher with the normal group, compared to the groups that received the HFD. No significant ($p > 0.05$) difference was recorded between the negative control and the treated groups. The NO levels in the brain of rats that received the HFD and treated were generally significantly ($p < 0.05$) higher compared to that of the negative control. However, the NO level in the group treated with 100% PO was similar to that of the negative control. At the level of the pancreas, the NO level in the normal group was significantly ($p < 0.05$) higher than that of the other groups, the negative control presenting the lowest value. At the level of the liver, the NO level of the negative control was significantly ($p < 0.05$) lower than that of the rat treated with Orlistat and oil but was similar to that of the normal group. At the level of the serum, the normal group presented a significantly ($p < 0.05$) lower NO value, compared to the groups that received the HFD and were treated. However, its value was not different from that of the negative control.

Table 7. Effect of African walnut oil, palm oil and their corresponding blends on MDA levels in organ homogenate and serum (µmol/L)

| | Spleen | Kidney | Heart | Brain | Pancreas | Liver | Serum |
|---|---|---|---|---|---|---|---|
| Normal | 1.88±0.69 $^{a}$ | 1.04±0.26 $^{a}$ | 1.83±0.02 $^{a}$ | 2.56±0.60 $^{ab}$ | 1.56±0.00 $^{a}$ | 1.03±0.07 $^{a}$ | 1.86±0.44 $^{a}$ |
| Negative control | 4.22±0.00 $^{b}$ | 3.27±0.00 $^{b}$ | 2.59±0.47 $^{b}$ | 2.51±0.00 $^{a}$ | 4.59±0.00 $^{b}$ | 2.74±0.30 $^{b}$ | 3.01±0.39 $^{b}$ |
| Orlistat (10 mg/kg) | 3.07±0.79 $^{b}$ | 1.40±0.00 $^{a}$ | 3.91±0.79 $^{bc}$ | 1.98±0.02 $^{b}$ | 3.20±0.04 $^{c}$ | 1.16±0.00 $^{a}$ | 2.35±0.52 $^{ab}$ |
| 100% WO[1] | 2.08±0.97 $^{a}$ | 2.22±0.07 $^{c}$ | 4.00±0.42 $^{c}$ | 2.25±0.21 $^{a}$ | 1.74±0.11 $^{a}$ | 1.21±0.00 $^{a}$ | 1.85±0.61 $^{c}$ |
| 100% PO[1] | 3.70±0.83 $^{b}$ | 1.00±0.23 $^{a}$ | 3.18±0.00 $^{bc}$ | 2.77±0.14 $^{a}$ | 3.40±0.65 $^{bc}$ | 2.60±0.36 $^{b}$ | 2.42±0.37 $^{ab}$ |
| 50:50 (PO: WO)[1] | 2.97±0.00 $^{b}$ | 2.46±0.00 $^{a}$ | 3.41±0.00 $^{bc}$ | 1.97±0.00 $^{b}$ | 3.85±0.00 $^{bc}$ | 1.58±0.00 $^{a}$ | 2.20±0.33 $^{ab}$ |
| 60:40 (PO: WO)[1] | 2.03±0.00 $^{a}$ | 1.72±0.00 $^{a}$ | 3.58±0.83 $^{bc}$ | 2.30±0.70 $^{a}$ | 4.49±0.10 $^{b}$ | 1.66±0.00 $^{a}$ | 2.71±0.70 $^{ab}$ |

1: (1000 mg/kg), n=6 values are presented as mean±SD. $^{a-c}$Values with different superscript letters in the same column are significantly different at $p<0.05$ indicating significance. Palm oil (PO), African walnut oil (WO) and PO: WO (50:50) and PO:WO(60:40).

Table 8. Effect of African walnut oil, palm oil and their corresponding blends on NO levels in organ homogenate and serum (µM/L)

| | Spleen | Kidney | Heart | Brain | Pancreas | Liver | Serum |
|---|---|---|---|---|---|---|---|
| Normal | 16.84±5.3$^{ab}$ | 11.11±0.00$^{a}$ | 13.72±3.07$^{a}$ | 10.81±0.00$^{a}$ | 37.73±3.16$^{a}$ | 28.34±0.69$^{a}$ | 1.31±0.00$^{a}$ |
| Negative control | 8.46±1.41$^{a}$ | 12.27±0.00$^{a}$ | 8.94±3.63$^{ab}$ | 7.40±0.41$^{b}$ | 13.46±0.57$^{b}$ | 22.45±3.22$^{a}$ | 3.15±1.55$^{a}$ |
| Orlistat (10 mg/kg) | 10.27±3.29$^{ab}$ | 34.31±2.41$^{b}$ | 9.59±2.75$^{ab}$ | 12.58±1.57$^{a}$ | 22.05±1.67$^{c}$ | 36.15±0.75$^{b}$ | 6.97±0.91$^{b}$ |
| 100% WO[1] | 15.98±2.51$^{ab}$ | 9.65±1.05$^{a}$ | 6.60±2.62$^{b}$ | 18.84±1.19$^{c}$ | 29.03±0.00$^{d}$ | 35.53±2.63$^{b}$ | 11.24±4.18$^{b}$ |
| 100% PO[1] | 14.07±3.16$^{ab}$ | 33.65±2.16$^{b}$ | 6.66±2.80$^{b}$ | 7.56±0.75$^{b}$ | 24.40±5.33$^{cd}$ | 36.35±3.49$^{b}$ | 8.37±0.00$^{b}$ |
| 50:50 (PO: WO)[1] | 23.59±0.00$^{c}$ | 21.51±0.00$^{c}$ | 6.70±1.05$^{b}$ | 20.37±0.00$^{c}$ | 16.73±0.00$^{b}$ | 35.39±0.00$^{b}$ | 5.54±0.00$^{a}$ |
| 60:40 (PO: WO)[1] | 14.44±6.03$^{ab}$ | 14.65±0.00$^{a}$ | 6.77±1.73$^{b}$ | 12.73±2.79$^{a}$ | 25.64±0.00$^{cd}$ | 35.06±1.93$^{b}$ | 11.44±0.00$^{b}$ |

1: (1000 mg/kg), n=6 values are presented as mean±SD. $^{a-d}$Values with different superscript letters in the same column are significantly different at $p<0.05$ indicating significance. Palm oil (PO), African walnut oil (WO) and PO:WO (50:50) and PO:WO(60:40).

## Discussion

### *Initial Oil Quality*

The colour characteristics of oils can be attributed to various pigments and compounds present in the oil, such as carotenoids and chlorophylls. The L*, a*, b* scale has been adopted for determining the colour of edible oils and on this scale, L* is light/dark with L values from 0-50 representing lightness and L* values from 51-100 representing darkness, a* is red/green with positive a values representing redness and negative a values representing greenness. b* is yellow/blue with positive b values representing yellowness and negative b values representing blueness (Klisic et al., 2023). Results showed that palm oil (PO) exhibited higher values of blackness, redness, and yellowness compared to African walnut oil (WO). This could be attributed to its high content in carotenoids compared to WO. WO had higher blackness and yellowness values but showed a normal level of redness. In the case of palm oil, its characteristic deep red colour is predominantly due to the presence of carotenoids, specifically β-carotene (Tan et al., 2021). The darker colour observed in palm oil compared to walnut oil could be attributed to a higher concentration of these pigments. Walnut oil, on the other hand, contains a lower number of carotenoids (Oloko, 2019) and so it exhibited a normal a* value. The variations in colour observed in the oil -50:50 (PO:WO) and 60:40 (PO:WO) blends, can be attributed to the combined pigments from both palm oil and walnut oil. The addition of palm oil to African walnut oil may contribute to the increased redness and yellowness, resulting in a more intense color profile than with African walnut oil alone.

Peroxide value indicates the extent of primary oxidation in an oil particularly the presence of hydrogen peroxide (Ali et al., 2020). The peroxide values for all oil samples analysed ranged between 2.52 and 3.87 meq $O_2$/kg which was within the safety range established by *Codex Alimentarius* (≤15 meq $O_2$/Kg) for crude and virgin edible oils (FAO/WHO, 2009).

*P*-anisidine value is a measure of secondary oxidation products in oils, marked by the formation of aldehydes, especially 2-alkenals and 2,4-dienals (He and Liu, 2019). The P-anisidine value of all the oil samples analysed ranged from 8.24-12.33 and were within the standard (≤20 in fish oil) established by *Codex Alimentarius*, indicating moderate levels of secondary oxidation in the oils and blends (FAO/WHO, 2009).

TOTOX value measures the overall oxidation state of an oil, combining peroxide and p-anisidine values (Bannenberg et al., 2017). This value better describes the total oxidative state to which a fat or oil has been exposed. Results showed that the TOTOX values obtained in this study ranged from 13.37 to 19.46 which are within the accepted range established by *Codex Alimentarius* (≤26 in fish oil) (FAO/WHO, 2009). The moderate TOTOX values suggest a moderate level of oxidative deterioration in the oils

Acid value is an indicator of oil degradation and it informs on the breakdown of triglycerides into free fatty acids (Sharma and Jain, 2015). The acid value of all the tested oil samples (from 0.32 to 20.34%), was generally higher than 0-4 mg KOH/g which is the recommended range for crude and virgin oils. However, it was lower than 45 mg KOH/g which is the recommended range in fish oils with a high phospholipid concentration of 30 percent or more such as krill oil (FAO/WHO, 2009). The high in acid value found with African walnut oil (20.34 mg KOH/g) suggests its high potential for degradation among the samples.

Iodine value is a measure of the degree of unsaturation of fats and oils. Higher iodine values indicate the presence of multiple double bonds and hence higher levels of unsaturated fatty acids (Geng et al., 2023). The results in this study showed that the iodine values ranged from 55.21 to 166.41 g $I_2$/100g with the highest iodine value observed in 100% WO, followed by 50:50 (PO: WO), 60:40 (PO:WO), and 100% PO. The higher iodine value of African walnut oil compared to palm oil indicates a higher content of unsaturated fatty acids in walnut oil. This result goes to support the findings of Tchiegang et al. (2007) who revealed that African walnut oil contains a higher proportion of unsaturated fatty acids; 9.48% Oleic acid, 14.41% linoleic acid and 70.39% alpha-linolenic acid. Blending it with palm oil reduced its iodine value indicating the blend has a lesser degree of unsaturation and hence is more stable to oxidation.

Fourier Transform-Infrared Spectroscopy (FT-IR) is a technology for acquiring emission spectra or infrared absorption from solid, liquid or gas samples, as molecules absorb radiation at very specific wavelengths (Kassem et al., 2023). The FTIR spectrum employed for the four samples in this study was measured within the wave numbers between 400 $cm^{-1}$ to 4000 $cm^{-1}$. Almost all edible oils consist of triacylglycerol (92%), low concentrations of di- and monoacylglycerols (5%), and low levels of other components (Jamwal et al., 2019). Thus, the spectra of these oil samples exhibit numerous similarities among absorbance bands in this study. This region is similar to the region chosen by other researchers (Fridah, 2015). The spectral analysis revealed several important peaks that provide insights into the chemical composition of the oils. The results for all oil samples showed there was an absence of peaks between 1600 $cm^{-1}$ - 2750 $cm^{-1}$ and 3250 $cm^{-1}$ - 3750 $cm^{-1}$, indicating the absence of Aldehydes (C=O), Aldehydes (C-H), and Hydrogen peroxide vibrations respectively, in this spectral region (Poiana et al., 2015). This showed the oils were free of primary and secondary oxidation products. In all oils, a peak was consistently recorded at 3000 $cm^{-1}$, which corresponds to the stretching vibrations of C-H bonds in the oil samples (Poiana et al., 2015). This peak suggests the presence of hydrocarbon chains in both palm oil (PO) and African walnut oil (WO). This result is not uncommon as it a common functional group found in oils. Another prominent peak observed in the spectra was located at 1750 $cm^{-1}$, which represents the stretching vibration of the carbonyl (C=O) group, indicating the presence of fatty acid esters or other carbonyl-containing compounds in the oil samples analyzed (Wang et al., 2024). This proved oil quality, indicating that the fatty acids are still linked to glycerol. Furthermore, peaks were found at the wave number 2900 $cm^{-1}$; indicating the presence of methylene/ methyl groups

which are mostly found in hydrocarbons, and at 3000 $cm^{-1}$; indicating the presence of a cis bond (C-H) (Poiana et al., 2015). These results revealed that all the samples analysed had some degree of saturation and unsaturation respectively. In the fingerprint region, a peak was recorded around 900 $cm^{-1}$, with the highest intensity found in 100% palm oil (PO). This peak indicates the presence of isolated trans bonds (C=C-H bend) (Poiana et al., 2015) and hence indicates the presence of unsaturated compounds in this oils. Additionally, another peak at 750 $cm^{-1}$ was observed in the FTIR spectra. This peak corresponds to $CH_2$ rocking vibrations present in the oil samples (Poiana et al., 2015), supporting the previous vibrations in the functional group zone that suggested the presence of unsaturated compounds in the oils.

***Hematology***

The blood count is the primary biological test used to screen for hemopathies (Lounguaing et al., 2022). White blood cells (WBC) source out, attack and destroy disease causing microorganisms on a daily basis and therefore play a crucial role in the body's immune response. Results showed no significant ($p>0.05$) difference in the WBC and its sub types in the normal and negative control groups but an increase in the groups treated with orlistat, and the oils. There was an important increase ($p<0.05$) in the LYM % of the rats treated with 50:50 (PO:WO), and in the MID of rats treated with 100% PO compared to the other groups. This increase in white blood cell count could indicate that consumption of orlistat or the various oils in their corresponding groups did not have a negative impact on the defensive function of WBC (Babalola et al., 2016). These results are contrary to the findings of Gu et al. (2018) who concluded that the presence of elevated WBC in obesity is a predisposing factor to developing type 2 diabetes. Granulocytes (GRAN) on the other hand are a type of white blood cells which comprise of neutrophils, eosinophils, and basophils and play an important part in innate immune response and fighting against infections (Gigon et al., 2021). Results found that, treatment with 50:50 (PO:WO) and 60:40 (PO:WO) significantly decreased the GRAN concentration in the blood of rats in this group compared to the other groups. This suggests a reduced need for protection against infection or inflammation in these groups and hence efficacy of the corresponding oil blends in combating the latter.

The absence of variations in RBC, HGB, MCV, MCH, MCHC, MPV, PLT, PCT, P-LCR, PDW suggests that none of the feeding methods, positive control drug (orlistat) or lipid sources used induced anaemia in the experimental rats since RBC, HGB and PCV concentrations are basic values indicating the degree of anaemia (Tirado, 2023). These results were not different from the findings of Babalola et al. (2016) who made similar conclusions from their findings.

Results revealed that the HFD groups exhibited significantly ($p<0.05$) lower values for HCT and PLT compared to the normal group. Similar results were obtained by Adullah et al. (2018). The decrease in platelet number in the high fat diet groups could be due to free radical damage to the stem cells which are the site of production of platelets (Loungaing et al., 2022). However, these findings contradict those of Saputri et al. (2023) who attributed HFD to enhanced PLT activation.

***Inflammatory Cytokines***

Recent research has shown the link between High fat diet consumption and low-grade inflammation (Moghbeli et al., 2020). Results showed that the negative control group which received the high fat diet alone with no treatment exhibited the highest levels of inflammatory cytokines. This can be explained by the fact that, persistent ingestion of a high fat diet causes changes in the gut microbiota. This leads to increased production of lipopolysaccharides (LPS) which increases the production of inflammatory cytokines (Thomas et al., 2022). This finding can help agree that the rats of the negative control group were obese since obesity elevates the number of blood cytokines such as TNF-α, IL-1β, IL-8 and IL-6 in the circulation (Ashraf et al., 2018). This work was in accordance with a similar study carried out by Schmidt et al. (2015) where there were significant elevations of INF-γ and TNF-α in case of obesity. The results obtained in this study showed that using the oils as an intervention for inflammation in high fat diet induced obesity improved the inflammatory conditions by lowering pro-inflammatory cytokine levels. Interestingly, African walnut oil exerted better improvement effects than palm oil, as evident from the significantly reduced TNF-α and INF-γ levels. This can be attributed to the anti-inflammatory nature of omega-3 rich oils (Guo et al., 2023). African walnut oil is rich in α-linolenic acid (about 69.90%) (Ghomdim et al., 2024). These fatty acids might be responsible for the observed anti-inflammatory activity. Giacobbe et al. (2020) reported that omega-3 fatty acids have the ability to reduce symptoms and have anti-inflammatory property by producing omega-3 fatty acids metabolites such as maresins, revolvins and protectins, which are promediators that act as strong anti-inflammatory agents.

Palm oil on the other hand had better anti-inflammatory effects in reducing the levels of IL-1β and IL-6. This can be attributed to its high content of tocotrienols (a type of vitamin E), which act as a potential antioxidant, and which equally exhibit anti-inflammatory activities (Dauqan et al., 2012; Tan et al., 2020). Wu et al. (2008) demonstrated that tocotrienol-rich fraction of palm oil exerts good anti-inflammatory property by suppressing the expression of inflammatory mediators in human monocytic cells. These results are similar to the findings of Ajuwon et al. (2022) where red palm oil reduced the levels of TNF-α, IL-1β and IL-6. Contrary to these results were the findings of Martins et al. (2024) where consumption of palm oil led to higher plasma levels of IL-6. This could be due to the fact that the palm oil used in this study was inter-esterified palm oil.

As to what concerns the oil blends, interestingly PO:WO (50:50) had better effects against inflammation as it significantly reduced TNF- alpha, INF-γ and IL-1β levels in serum. This can be due to the fact that a diet containing a low ratio of saturated to unsaturated fatty acids is more effective in reducing inflammation due to a higher proportion of anti-inflammatory omega-3 PUFA's, as revealed in the findings of Thomas et al. (2022) who obtained similar results.

***Oxidative Stress***

Reactive oxygen species have been found to be involved in the control of body weight by exerting different effects on hypothalamic neurons, which control satiety and hunger behaviour (Drougard et al., 2015). HFD induced obesity is associated with mitochondrial dysfunction and increased oxidative stress. It has been reported that mitochondria are the main sources of reactive oxygen species in mammalian cells. Their production leads to mitochondrial damage in a range of pathologies amongst which obesity (Andreyev et al., 2005; Balaban et al., 2005). Research has shown that in excessive adiposity, reactive oxygen species levels increase and exceed the capacity of the antioxidant system (Balan et al., 2024). A protective effect against oxidative stress is seen from an oil's ability to reduce lipid peroxidation and increase anti-oxidant enzyme activity (Dauqan et al., 2012). For example, Omega-3 fatty acids were reported to have antioxidant and anti-inflammatory properties. They are efficient in reducing oxidative stress and inflammation in macrophages from patients with small abdominal aortic aneurysms (Rocha et al., 2022). Heshmati et al. (2019) reported that omega-3 fatty acid supplementation improves antioxidant, glutathione peroxidase and malondialdehyde activities. Thus, omega-3 fatty acids and omega-3 fatty acids-rich oils can be mentioned as enhancer elements in antioxidant activity against Reactive oxygen species. This can help improve the pathological status of many diseases.

In this study, lipid peroxidation examined by organ/serum levels of MDA was shown to be significantly ($p<0.05$) higher in the negative control group than the normal group. Results showed that, the greatest extent of lipid peroxidation was observed in the spleen, kidney, pancreas, liver, and serum of the negative control. This could be attributed to the fact that this group was not treated and receive just the high fat diet throughout the experimental period. Multiple studies have shown that obese patients have higher MDA levels than non-obese patients (Yesilbursa et al., 2005; Huang et al., 2023). This result was also consistent with available information in the literature which noted that increase lipid peroxidation occurs in response to overwhelming rise in ROS (Ichipi-Ifuor et al., 2022). Results revealed that the liver exhibited the highest CAT activity and MDA level in the negative control group. This can be explained by the fact that obesity is characterised by a fatty liver resulting in a higher-than-normal liver weight in a condition referred to as non-alcoholic fatty liver disease (NAFLD). NAFLD is characterized by fat accumulation in the liver, which can lead to increased lipid peroxidation. It is a metabolic disease that leads to fat accumulation in the liver, a condition known as hepatic steatosis. This can appear if the liver cannot properly process fats or if a patient has other conditions like obesity, diabetes, or high triglycerides (Martín-Fernández et al., 2022). The increase CAT activity as shown in this study is an effort to protect the liver from the harmful effects of the reactive oxygen species resulting from this lipid peroxidation (Nandi et al., 2019). Results of this study also found that the heart and brain showed significantly lower MDA levels, with a significantly higher GSH activity in the negative control group. This reflects an effort to protect the cells of the heart against oxidative damage and can be explained by the fact that the glutathione (GSH) system, has been proven to be one of the most powerful endogenous antioxidant systems in the cardiovascular system due to its key contribution to detoxifying xenobiotics (Tan et al., 2023). Similar results were obtained by Meral (2022) who revealed that the low level of MDA in the brain of obese rats indicates the presence of a protective mechanism against hypoxia since the enlargement of adipocytes leads to local hypoxia (Dos Santos Cardoso, 2013).

Looking at the group of rats that received 100%WO, results showed that lipid peroxidation products (MDA) are decreased upon treatment with African walnut oil with a corresponding decrease in GSH and catalase activities in most tissues/ serum. This can be explained by the fact that omega-3 fatty acid rich nature of African walnut oil is able to counteract oxidative stress and lipid peroxidation due to its antioxidant capacity (Rocha et al., 2022; Heshmati et al., 2019). These results are similar to those obtained by Uti et al. (2019) and Abam et al. (2013) who showed that lipid peroxidation in the liver, kidney and brain tissues of rats treated with cadmium was successfully attenuated upon administration of African walnut oil at 2.0 g/kg body weight. In the group treated with PO (100), results showed a decrease in lipid peroxidation in most organs and serum. This effect of palm oil can be related to the β-carotene present that has the ability to quench free radicals and prevent tissue damage, and also due to the fact that vitamin E present in palm oil inhibits the production of lipid hydroperoxides (Dauqan et al., 2012). At the level of the brain the group treated with 100% PO exhibited the lowest GSH activity, and highest CAT activity respectively. Also, the MDA levels were high in the liver and serum of rats in 100% PO. This work is in agreement to the findings of Ichi-Ifukor et al. (2022) who showed that crude palm oil reduced GSH levels in the brain, has no significant difference in CAT activity of the brain but reduces serum and liver levels of MDA in rats exposed to oxidative stress by acute cadmium poisoning.

As to what concerns the blends, the results obtained showed that the multisource oil blends PO: WO (60:40) exhibited high GSH activity in the serum, and high CAT activity in the spleen brain and serum. Also, PO: WO (50:50) showed low levels of MDA at the level of the brain, with high CAT activity in the brain and high GSH activity at the level of the pancreas. Oxidative stress is characterized by depletion in antioxidants/antioxidant enzymes and a high level of lipid peroxidation (Oyem et al., 2021). The results obtained from the administration of this multisource oil are indicative of the fact that the oils had better effects in fighting the adverse effect of the high fat diet by improving the animals' oxidative state and lipid peroxidation, owing to the combined potentials from the omega-3 rich PUFAs African walnut oil (omega-3 fatty acids) (Rocha et al., 2022; Heshmati et al., 2019) and the antioxidant packed palm oil (Vitamin E, β-caroten) (Dauqan et al., 2012).

Nitric Oxide (NO) serves as an important marker of oxidative stress. This study showed that Obesity induction caused significant decrease in NO levels in rats of the negative control group. This can be due to the fact that High fat diet impairs nitric oxide bioavailability (Razni et al., 2011). Eccleston et al. (2011) reported that chronic exposure to a HFD leads to hepatic steatosis, impairs nitric

oxide bioavailability, and modifies the mitochondrial proteome in mice. It can further be explained from the quenching of NO by its action with resulting free radicals, loss of endothelial NO synthase (eNOS) activity or downregulation of eNOS (Sansbuty & Hill, 2014). Similar results were obtained by Niwanthi et al. (2016) who explained that reduced NO availability as seen in high fat diet consumption is caused by impaired transport of its substrate, L-arginine. Results showed that treatment with 100%WO improved and upregulated the NO levels of rats in this group at a similar level to that of normal and orlistat groups, all higher than NO levels in the negative control group. These treatments raised the NO levels in the spleen, brain, pancreas, and liver, with a marked increase in the serum. Although the mechanism of action was not exploited in this study, the results obtained by Niwanthi et al. (2016) permits to suggest that African walnut oil may be involved in the augmentation of endothelial L-arginine transport which results in the production of more NO. Concerning the NO concentration in 100% PO group, all organs and serum of rats in this group had high NO levels when compared to the negative control group except for the heart. There was a significant increase in NO at the level of the kidney and the liver. The significantly higher NO levels seen in the kidney can be due to the fact that fresh palm oil supports glomerular flow rate, renal plasma flow (Beshel et al., 2014). It can also be due to the fact that NO is involved in modulation of renal autoregulation as well as tubular fluid and electrolyte transport (Carlstrom, 2021). The high NO levels revealed in all groups might be attributed to the fact that NO maintains liver homeostasis and inhibits the pathological conditions of the liver. The increase is contrary to the findings of Albrahim et al. (2022) who showed low levels of MDA results from the administration of red palm oil to rats fed a hypercholesterolemic diet.

## Conclusion

The objective of this work was to evaluate the effects of palm oil, African walnut oil and their blends (50:50 and 60:40) on hematological, inflammatory and some oxidative stress markers in high fat diet obese-induced Wistar rats. Results showed that oil quality indices were all within the norm and of good quality. The analysis of oxidative stress parameters revealed that the catalase activity was not significantly different amongst all test groups in kidney and heart. GSH activity amongst test groups of kidney homogenates was not significantly different. NO and MDA levels showed a significant difference for organ homogenates amongst all test groups. Hematological parameters of LYM, RBC, HGB, MCH, MCHC, MPV, PCT, and PDW had no significant difference amongst the test groups. Serum levels of TNF-α, INF- γ, IL-1β and IL-6 were significantly higher in the negative control group. These markers improved in the test groups treated with the oils. Therefore, it can be concluded that multisource edible oils from palm oil and African walnut oil have a role in the protection from obesity and its effects on oxidative stress, hematology, and inflammatory cytokines. It will be important in future studies to look into the effect of such formulation on the lipid profile and serum enzymes of rats.

## Declarations

### *Ethical Statement*

This is to inform you that in this study, animals were involved. Animals were cared for and used in agreement with international standard guidelines for animal use. In order to carry out this study, an ethical clearance for animal handling and care was obtained from the University of Buea - Institutional Animal Care and Use Committee (IACUC) with permit UB-IACUC No 02/2024.

### *Author Contribution Statement*

Fabrice Tonfack Djikeng: Conceptualization, Data collection, investigation, Methodology, formal analysis, writing the original draft.

Mundi Eunice-Laura Lemnyuy: Data collection, investigation, Methodology, formal analysis, writing the original draft.

Veshe-Teh Zemoh Sylvia Ninying: Conceptualization, Data collection, investigation, Methodology, formal analysis, writing the original draft.

Tiencheu Bernard: Project administration, Conceptualization, Supervision, review and editing.

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### Conflict of interest

The authors declare no conflict of interest

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# Sustainability and Challenges of Water Occupancy Rates of İmranlı Dam Lake in the Rural Region of Sivas Province, Türkiye

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ABSTRACT

Studies on water occupancy rates have an important share in the sustainability of dam lakes, which are an important component of aquatic ecosystems. İmranlı Dam Lake was built between 1994-2002 for energy and agricultural irrigation purposes. This study is about the water occupancy rates of the İmranlı Dam Lake, located in Sivas province of Türkiye, between the years 2010-2021. The highest water occupancy rate of İmranlı Dam Lake was determined as 76.20 percent in 2018, while the lowest water occupancy rate was 27.70% in 2012. The average annual water occupancy rate of İmranlı Dam Lake between 2010-2021 was calculated as 56.86 percent. According to these values, it was determined that the water occupancy rates of the İmranlı Dam Lake did not face a significant decrease between the years 2010-2021. As a result, there is no short-term problem in terms of water occupancy rates in İmranlı Dam Lake. However, this does not mean that it will not be a problem in the long run. For this reason, taking into account the possible effects of climate change (CC), the water of İmranlı Dam Lake should be used sparingly and consciously. As a result of all these measures that can be taken, sustainability will be ensured in the İmranlı Dam Lake, and at the same time, water scarcity will be prevented, the continuity of aquatic vitality will be ensured and losses will be prevented.

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## Introduction

Global climate variability is directly related to hydrology and the availability of water resources in the world and has a great importance. As a natural consequence of the increase in greenhouse gases in the atmosphere, changes in temperature, evaporation, air and soil moisture and precipitation regimes affect the hydrological process, the condition of water resources, agriculture, mining, hydroenergy activities, aquatic and land life. CC change accelerates the global hydrological cycle with the increase in surface temperatures, changes in precipitation regime and evaporation rate. Changes in the amount, intensity and frequency of precipitation will affect the amount and frequency of rivers, and flood and drought events that cause significant effects on water resources on a local and regional scale will be experienced. With the decrease in the amount of precipitation and the increase in evaporation, the decreasing amount of runoff threatens the water resources needed for human and industrial activities (Adiller, 2014). Lakes are critical natural resources sensitive to CC. The water cycle is dynamic and naturally variable. Societies and ecosystems continue their lives within this variability. However, CC poses risks on a global scale, especially for lakes that are freshwater sources, and threatens the sustainability of lakes, by changing the water cycle in various ways on different time scales and geographical areas. Risk management is very important for estimating when and how these risks will materialize, and the estimation of the losses that will occur in case of realization. In this context, by developing resilience strategies against anthropogenic climatic events, increasing the lake ecosystem's ability to cope with this threat and adapt to new situations; It is very important for the sustainability of lakes (Işıldar and Ercoşkun, 2021).

With the world population increasing day by day, the importance of water storage is increasing within the framework of more effective and efficient use of water resources, which are decreasing in parallel with industrial and technological developments. Dam lakes are aquatic ecosystem areas built by humans that allow natural environments to be operated by humans. These ecosystem environments offer many natural and human opportunities and they continue to exist with these opportunities

(drinking and irrigation water, energy production, aquaculture, flood management, tourism and leisure activities). Dams are built to control water in many river basins (Atayeter et al. 2022). Dam lakes provide socio-economic and environmental benefits in rural areas. Bringing irrigation and hydroelectric energy projects, which are the most important pillars of sustainable development, to the country's economy is possible with the rational planning and management of the owned water resources.

Türkiye is located in one of the most problematic regions of the world in terms of water resources. Apart from the existing water resources being under threat, it is possible that the existing water resources will not be able to meet the needs with the increasing population. It is obvious that the years 2030-2080 will be years of serious and important water problems due to global warming and CC, which are among the most important problems of the world agenda. In addition to the rapidly increasing need for water throughout Türkiye, the fact that in Türkiye rainfall tends to decrease can be considered a sign in terms of water problems that will arise in the near future. The most distinctive feature of the drought is the scarcity of precipitation and the inability to use the available water consciously and to operate it healthily (Gerek et al. 2008). For this reason, the water occupancy rates of the dam lakes, which are the water collection areas in the future of global CC, are important in terms of the sustainability of water resources, alternative planning and operation. This study was carried out in line with this idea. In this study, it was aimed to investigate the water occupancy rates of the İmranlı Dam Lake, one of the important artificial water resources in Sivas province of Türkiye, between the years 2010-2021.

## Materials and Methods

İmranlı Dam Lake is located within the borders of İmranlı district of Sivas Province in Türkiye. It is approximately 110 kilometers from Sivas city center. İmranlı Dam Lake is approximately 2 kilometers from İmranlı town center. Some technical information of İmranlı Dam Lake is summarized in Table 1. İmranlı Dam Lake was built on the Kızılırmak River in Sivas between 1994-2002 for irrigation and energy generation. The height of the dam, which is an earth body fill type, from the river bed is 49.00 meters and the lake area at normal water level is 6.50 square kilometers (Table 1). İmranlı Dam Lake provides irrigation service to an agricultural area of 11220 hectares (URL-1, 2022).

The distrcit of İmranlı, which bears the same name as the İmranlı Dam Lake, is in the east of Sivas province and has a height of 1650 meters from the sea. İmranlı has a mountainous structure compared to Sivas province in general. The land is generally steppe and consists of hills. The area suitable for agriculture is 12700 hectares and the total forest area is 4635 hectares. The mountains descend in an inclined manner towards the Kızılırmak Valley from the north and south. Kızıldağ Mountain, located in the northeast of İmranlı, is the highest mountain of Sivas province with its height of 3025 meters and is located at the intersection of Central Anatolia, Eastern Anatolia and Black Sea Regions. Kızıldağ Mountain is also the birth place of Kızılırmak River. The river is the longest river of our country, which originates within the borders of Türkiye and empties into the sea again within the territory of Türkiye, with a length of 1151 kilometers. It arises from the southern slopes of Kızıldağ Mountain in the east of İmranlı in three branches. These three branches converge near Çukuryurt village, 6 kilometers away from İmranlı district, and merge into İmranlı Dam Lake, taking the name Kızılırmak River. Since the waters of İmranlı Dam Lake are close to the source, they are cold, clean and clear (Dirican, 2022a). İmranlı is a mountainous district dominated by a continental climate with hot, dry and windy summers and cold winters. The number of summer days is 78 days. The total annual precipitation in İmranlı is 412 mm (Yıldırım, 2018). The coldest place in Sivas is İmranlı. The average temperature of İmranlı in winter is below zero degrees Celsius. Therefore, the surface of İmranlı Dam Lake freezes in winter.

In this study, water occupancy rate data between 2010 and 2021 obtained from the General Directorate of State Hydraulic Works in Türkiye for İmranlı Dam Lake were used. The occupancy rate includes the data of the monitored dams (with an active water volume of 3 million cubic meters and above). The changes in the water occupancy rates and sustainability of the İmranlı Dam Lake between the years 2010-2021 were examined with these data. The changes in the water occupancy rates of İmranlı Dam Lake were analyzed and synthesized in accordance with the purpose of the study. Occupancy rate of İmranlı Dam Lake was calculated as the ratio of active dam volume to total active dam volume. The occupancy rate of İmranlı Dam Lake is expressed in percent (%).

Table 1. Some technical information about İmranlı Dam Lake.

| N | Features | Value |
|---|---|---|
| 1 | Country | Türkiye |
| 2 | Province | Sivas |
| 3 | Build Start Year | 1994 |
| 4 | Build Completion Year | 2002 |
| 5 | Goal of the Dam | Energy, Irrigation |
| 6 | River | Kzılırmak River |
| 7 | Body Fill Type | Soil |
| 8 | Height | 49.00 m |
| 9 | Lake Volume | 62.50 $hm^3$ |
| 10 | Lake Area | 6.50 $km^2$ |
| 11 | Irrigation Area Gross | 11.220 ha |

## Results and Discussion

The water occupancy rates of İmranlı Dam Lake show a tendency to fluctuate in a narrow area between 2010-2021 (Figure 1). The water occupancy rate of the İmranlı Dam Lake in 2010 was determined as 52.94 percent. In 2011 and 2012, the water occupancy rate decreased to 43.62 percent and 27.70 percent, respectively. In 2013 and 2014, the water occupancy rate increased to 29.50 percent and 73.10 percent, respectively. In 2015, the water occupancy rate decreased to 58.60 percent. In 2016, the water occupancy rate increased to 73.00 percent. In 2017, the water occupancy rate decreased to 64.70 percent.

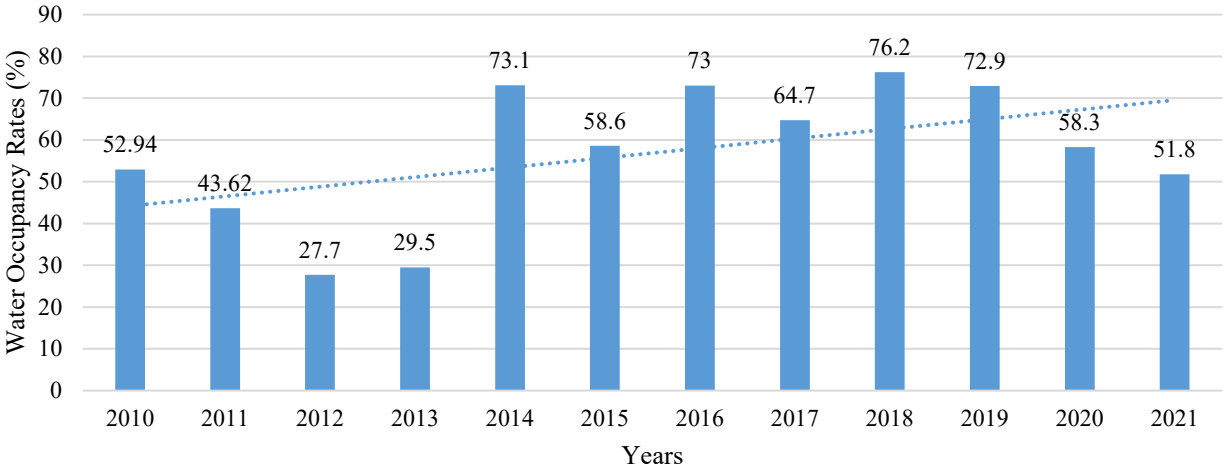


Figure 1. Changes in the water occupancy rates of İmranlı Dam Lake between 2010-2021

In 2018, the water occupancy rate increased to 76.20 percent. In 2019, 2020 and 2021, the water occupancy rate decreased to 72.90 percent, 27.70 percent and 51.80 percent, respectively (Figure 1). While the lowest water occupancy rate of İmranlı Dam Lake was observed in 2012 with 27.70%, the highest water occupancy rate was observed in 2018 with 76.20%. The average water occupancy rate of the İmranlı Dam Lake between 2010-2021 was calculated as 56.86±16.62 percent. Accordingly, it can be said that the water occupancy rates of İmranlı Dam Lake are in good condition.

While the water occupancy rate of the İmranlı Dam Lake was below the 50 percent band in 2010-2013, the water occupancy rate was above the 50 percent band in 2014-2021 (Figure 1). Water resources are of great importance for both society and ecosystem. Human beings and all other living things on Earth need clean water resources in order to sustain their lives in a healthy way. In addition, water is needed for agriculture, energy production, recreation and various production activities. This situation puts pressure on water resources and causes concern about the gradual decrease of available resources due to CC. In many areas, it is possible that CC will double its negative effects by increasing the need for water on the one hand and reducing water resources on the other (Adiller, 2014). İmranlı Dam Lake has first class water quality and the annual average nitrate value is 1.5 mg/L (SEP, 2021). According to TWPCR (2008), the water of the İmranlı Dam Lake, which has first class water quality, can be disinfected and used not only for drinking purposes, but also for recreational purposes, rainbow trout farming, animal production and other purposes. Therefore, İmranlı Dam Lake is an important water source for the region and Türkiye. İmranlı Dam Lake was opened for fish farming in 2010, and there is one farm in this area that produces rainbow trout in 42 net cages designed by the Sivas Agriculture and Forestry Provincial Directorate. This farm, which produces rainbow trout in İmranlı Dam Lake, has an annual production capacity of 950 tons (Dirican, 2022a). Fish species such as barbel, bleak, carp, chub, freshwater perch, khramulya and rainbow trout live in İmranlı Dam Lake. For this reason, angling is also carried out in the İmranlı Dam Lake (Dirican, 2022a). The fishing rights of the hunting area of İmranlı Dam Lake were leased from 17.08.2021 to 16.08.2026. However, one boat was licensed for fishing in the İmranlı Dam Lake (Dirican, 2022b). İmranlı Dam Lake is an important water source where economic activities such as rainbow trout farming in cages, angling and commercial fishing are carried out as well as energy and agricultural irrigation.

With this study, it has been determined that approximately 50 percent of the İmranlı Dam Lake is full, according to the water occupancy rates between the years 2010-2021. According to the water occupancy rates of the last twelve years in İmranlı Dam Lake, it has been determined that the slope line is also upwards (Figure 1). In this case, it indicates that the water occupancy rates of the İmranlı Dam Lake are at a good level. Therefore, it can be said that the water occupancy rates for energy, agricultural irrigation, rainbow trout farming in cages, angling and commercial fishing are close to normal levels in İmranlı Dam Lake. For now, it can be said that there is no risk for the İmranlı Dam Lake in the short term in terms of energy, agricultural irrigation, rainbow trout farming in cages, angling and commercial fishing.

In recent years, one of the most important issues on the world agenda is global CC. It is estimated that there will be increases in temperatures in latitudes, including Türkiye, as well as decreases in precipitation and soil water content. According to studies conducted with global climate models, a large part of Türkiye will be under the influence of a very dry and hot climate in 2030. Temperatures in Türkiye will increase by 2 ºC in winter and 2-3 ºC in summer. While precipitation will decrease by 5-15% in summer, there will be a slight increase in winter (Gerek et al. 2008). A large part of Türkiye is under the influence of arid and semi-arid climate, and there is a serious water shortage, especially in summer. In countries where these problems are experienced, it is an inevitable fact that dam constructions are needed for the efficient use of water, taking into account the sustainability of resources and the economic development of the country (Engindeniz et al. 2014). According to these, the effects of drought will be felt more in the future in Türkiye, which is arid and semi-arid, and the importance of water will increase for Türkiye in the future. For these reasons, it is very important to investigate the water occupancy rates of the İmranlı Dam Lake in terms of the planning, operation and management

of water resources. Considering the possible effects of CC, it is necessary for the country's economy to develop, revise, and use water resources sparingly and consciously. As long as we continue to use the İmranlı Dam Lake correctly and sparingly, take precautions and comply with the necessary legal regulations, it will continue to maintain its continuity in the living things in its waters and the vitality connected to this food chain. The most important step of CC is to protect our waters and the creatures in it and to ensure the continuity of life. As a result of drought, the greatest vital problem that we will face will arise with the danger of being without water and food. The world, water and living things should be protected and precautions should be taken. Laws should be revised and even new ones should be enacted, training should be given due importance and climate crisis awareness should be created. After that, all precautions should be taken in this direction. With the aim of meeting the needs of the present without preventing future generations from meeting their needs, the necessary adaptation, mitigation and combating methods against CC should be jointly developed in accordance with local locations and practices that can sustain sustainable development should be given priority. Freshwater ecosystems and dam lakes enable many people living in rural areas to continue their lives, making it possible for every society to reach very valuable goods and services. If water resources are not managed effectively and competently, environmental problems may arise due to their depletion.

## Conclusion

In this study, it was observed that the water occupancy rate of the İmranlı Dam Lake between the years 2010-2021 was good. However, it was determined that the water occupancy rate, which was 76.20 percent in 2018, decreased to 72.90 percent in 2019, 58.30 percent in 2020 and 51.80 percent in 2021 for İmranlı Dam Lake. It has been determined that the water occupancy rates of the İmranlı Dam Lake are generally in good condition in the short term between 2010-2021 with this study. With the emergence of the effects of CC on human activities depending on location and time, social, economic, industry, agriculture, forest, water resources and the like in countries are affected, and it is inevitable that if greenhouse gas emissions are not prevented in the coming years, there will be increasing effects. The multidimensional and comprehensive potential impact power of CC makes the solution of this problem dependent on the development of environmental awareness, solidarity and cooperation around the world. Therefore, one of the prerequisites for coping with CC is to take measures to develop positive attitudes and behaviors to increase awareness levels.

## Declarations

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# Detection of Structural Damage After an Earthquake Using GIS and Remote Sensing Methods

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ABSTRACT

Developments in Geographic Information Systems and Remote Sensing (RS) technologies and innovative approaches emerging in deep learning (DL) supported analysis methods have an important place in disaster research as in every field. Convolutional neural networks such as Mask RCNN, U-NET, one of the deep learning methods for disaster damage impact assessment and classification, have started to show successful results. However, high-resolution geospatial imagery and drones provide faster and more accurate detection of structural damage. In this study, damaged building detection was performed using Göktürk-1 satellite images from 6 February 2023 using Mask-RCNN architecture. In this study, deep learning methods were used to detect the collapsed buildings in the city of Malatya during the 6 February 2023 earthquakes. The study aims to emphasize the significance of GIS and remote sensing for the timely and efficient evaluation of building damage after a disaster. Considering this, high quality images of Malatya city before and after the earthquake were analyzed and data sets were created by masking using Mask RCNN deep learning method through ArcGIS Pro 3.4.0 software. According to the results of the research, it quickly detected damaged buildings with an accuracy rate of 70% according to satellite images after the earthquake. As a result, GIS and deep learning models were used to detect and map the initial damage after the earthquake.

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## Introduction

Cities are settlements formed in a socio-economic context where thousands of people live together. Besides, the overuse of resource consumption caused by the increasing rate of urbanization, insufficient infrastructure, disorganized planning, and poor services contribute to pose risks in urban areas (Büyüközkan et al., 2022). This situation negatively affects the resilience of cities after disasters such as earthquakes and brings along social, economic, and environmental problems.

Rapid assessment of infrastructure damage after a major disaster plays a crucial part in disaster response coordination and recovery efforts (Moradi & Shah-Hosseini). In this context, the construction of earthquake-resistant structures is of great importance. However, in cities where rapid urbanization and uncontrolled construction are common, disasters such as earthquakes can result in massive destruction, leading to significant loss of life and property. Therefore, pre- and post-disaster damage assessments have become a major focus of interest among researchers and practitioners working in the field of disaster management (Yamazaki & Matsuoka, 2007).

The first hours after the earthquake are very important. It is one of the first tasks to detect the first effects of the earthquake in these hours and to establish emergency response systems. (Dell'Acqua & Gamba, 2012; Eguchi et al., 2009; Nex et al., 2019). GIS and remote sensing technologies and the increase in satellite image quality have made it possible to use rapid assessment after disasters. (Yamazaki & Matsuoka, 2007). Considering this, the analysis of post-earthquake images derived from satellite datasets in highly urbanized areas is an effective method for visualizing the extent of initial damage. Change detection approaches involve post-classification comparison methods and image enhancement techniques, which are used to identify differences in building conditions across different time periods (Dong & Shan, 2013).

The first stage of post-earthquake emergency planning begins with assessing the current situation. Remote sensing data collected before and after a disaster offer a rapid evaluation of the built environment, making them highly valuable. Specifically, the comparison of old images and post-earthquake satellite images accelerates damage assessment in terms of time and cost. The advanced spatial analysis capabilities of GIS facilitate rapid and spatially informed decision-making for authorities. In this context, analyses based on satellite imagery play a vital role in emergency response since numerical data and satellite image analyses of pre-disaster settlements reveal post-disaster changes and provide estimations about affected populations.

After disasters, especially those affecting large areas such as earthquakes, floods, and wildfires, GIS and RS-based analyses have become widely used. High-resolution satellite imagery and aerial photographs taken by UAVs help detect the impacts of disasters within a few hours. In this context, high-resolution SAR satellite images, GÖKTÜRK satellites, etc., can quickly identify areas affected by disasters. Following analyses based on these images, the affected areas can be mapped, providing initial findings that serve as a basis for assessing the impacts of the disaster (Brunner et al., 2010).

Matsuoka and Yamazaki (2005) identified and mapped collapsed buildings using satellite imagery following the 2003 earthquake in Iran. Similarly, Balz and Liao (2010) employed SAR satellite imagery after the Sichuan earthquake to map the areas affected by the disaster. More recently, following the February 6, 2023, Kahramanmaraş (Turkey) earthquakes, numerous researchers utilized high-resolution satellite images, aerial photographs, and orthophotos to detect and map structural damage in the affected areas. In this context, Wang et al. (2023) used Sentinel-1 data to be detecting the structural damage caused by the earthquakes in Nurdağı, Kahramanmaraş, Hatay, Türkoğlu, and İslahiye. In a similar study, Du et al. (2024), Vitale and Milillo (2024), Wu et al. (2024), and Yu et al. (2024) employed SAR satellite imagery with new methods and analyses to detect building damage caused by the earthquake.

This study aims to identify, and map collapsed buildings in the Malatya caused by the February 6, 2023, earthquakes using GIS and remote sensing analysis methods based on pre- and post-earthquake orthophotos and GÖKTÜRK satellite images. ArcGIS Pro software's deep learning tools were utilized for detecting the damaged buildings in the study. The Mask R-CNN architecture, one of the deep learning methods, was employed in the analysis.

## Materials and Methods

### *Study Area and Data Management*

On February 6, 2023, two major earthquakes with magnitudes of 7.8 Mw and 7.5 Mw struck approximately nine hours apart, centered in the Elbistan and Pazarcık districts of Kahramanmaraş. These earthquakes caused widespread building collapses and severe structural damage in the eleven provinces (Kirici & Soyluk, 2023).

The designated study area, Malatya, is a mid-sized Anatolian city located near the East Anatolian Fault Zone. Geographically, Malatya lies between the coordinates 38° 21' 19.3032'' N and 38° 20' 0.6972'' E. The total population of the province is 742,725, with the Battalgazi and Yeşilyurt districts, which form the core of the study area, having a combined population of 556,068. According to post-earthquake damage assessments conducted throughout Malatya, out of 155,658 residential units in the province, 5,610 were completely destroyed, 1,840 were marked for urgent demolition, 35,620 sustained severe damage, and 2,480 were moderately damaged (Şıkoğlu, 2024).

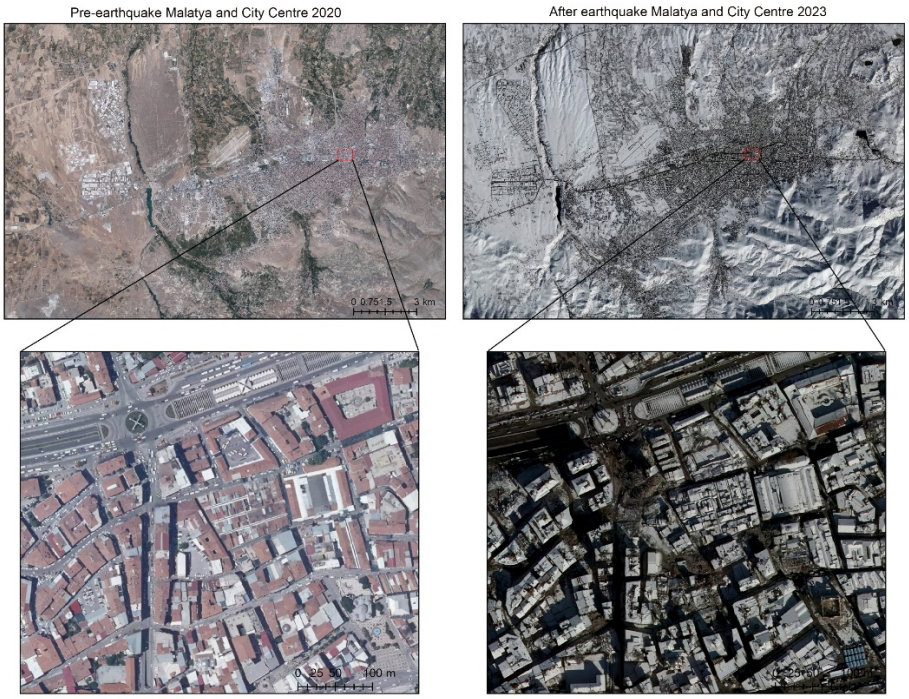


Figure 1. Study Area Before and After Earthquake

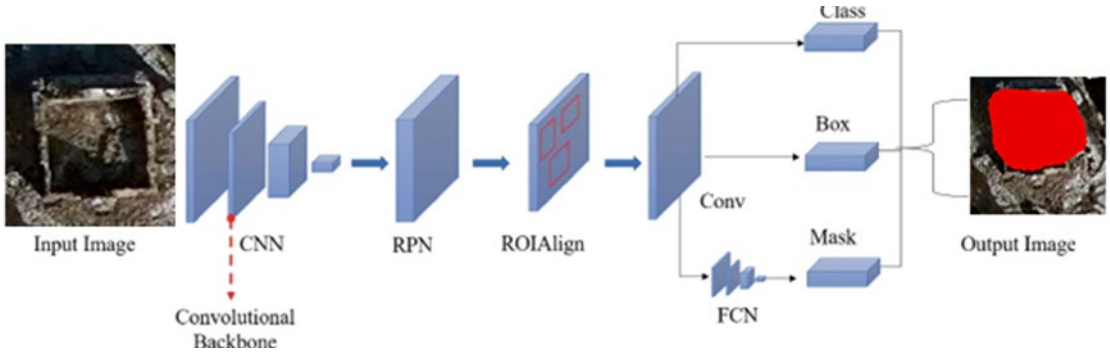


Figure 2. General architecture of the mask R-CNN (modified from Al Deen Taher & Dang, 2023)

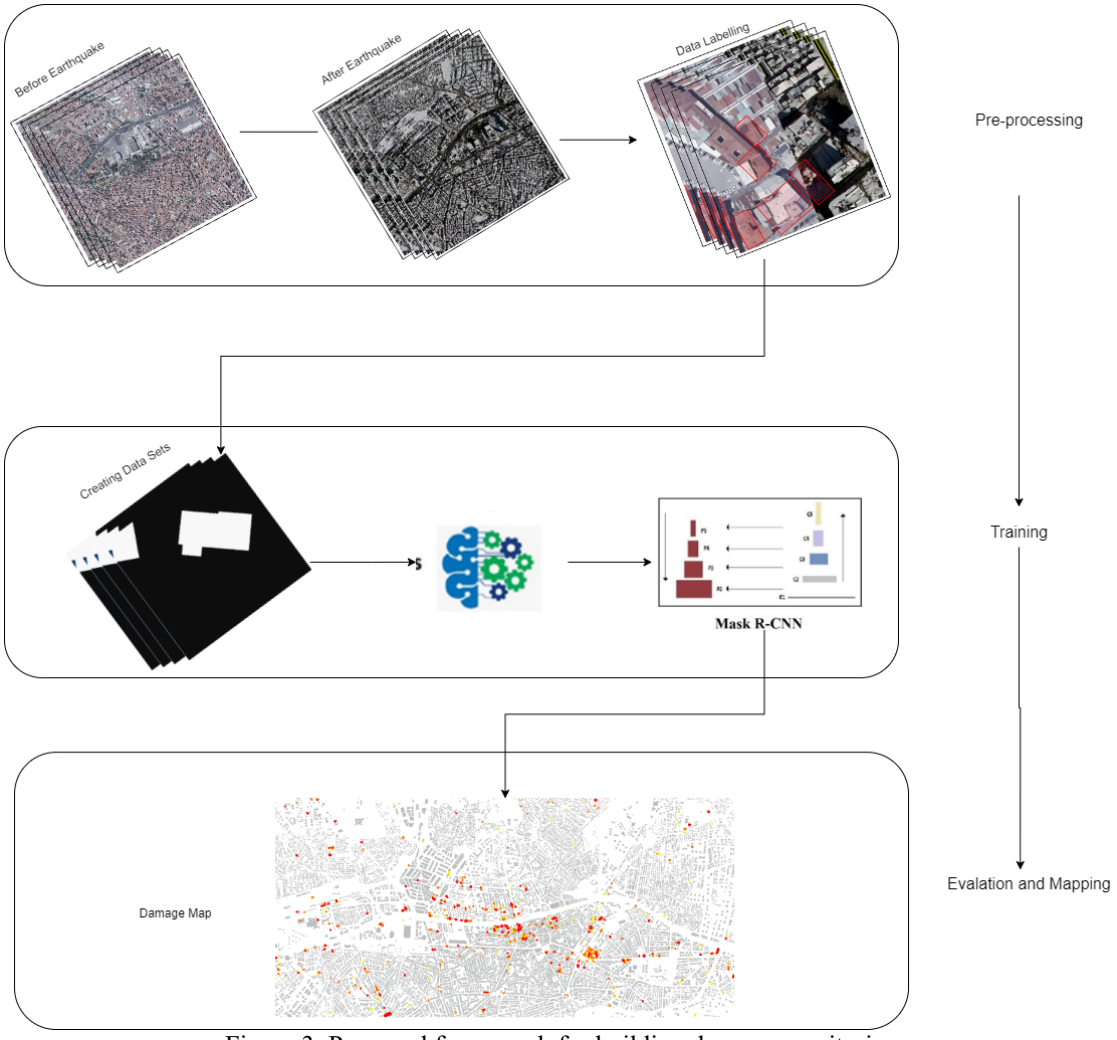


Figure 3. Proposed framework for building damage monitoring

The study, pre-earthquake 2020 orthophoto imagery provided by the General Directorate of Mapping and the post-earthquake Göktürk I-satellite imagery captured on February 9, 2023, were used. The pre-earthquake orthophoto image has a pixel size of 0.330 meters and consists of three bands. The post-earthquake satellite image has a pixel size of 0.217 meters and also consists of three bands. Both images possess high-resolution values, enabling detailed analyses for detecting structural damage following the earthquake. As shown in Figure 1, satellite images show the Malatya center, which was among the most affected areas. The test and training datasets used in the study were selected from the city center.

***Methods***

GIS and DL algorithms based on artificial neural networks were used to detect collapsed buildings following the February 6, 2023, earthquakes. ArcGIS Pro 3.4.0 software was utilized within the scope of the research. Deep learning algorithms are widely applied through ArcGIS Pro tools to solve spatial problems, detect objects, and perform pixel classification (Esri, 2024a).

A two-stage deep learning network was designed to address the building damage assessment problem, which involved building detection and damage grading. In the first stage, the semantic segmentation method was employed to segment buildings, drawing on methods used in previous studies. In the second stage, the damage levels

of the identified buildings were classified. The ArcGIS Pro software utilized the Mask R-CNN deep learning method for the analysis. Mask R-CNN is a model capable of performing both object detection and object segmentation simultaneously, making it particularly effective for damage assessment scenarios where understanding the extent of damage is crucial (Esri, 2024b). The deep learning workflow used in the study is illustrated in Figure 2. Mask R-CNN consists of two stages. The first stage is similar to Faster R-CNN, where the Region Proposal Network (RPN) suggests a series of Regions of Interest (ROIs) along with probability scores indicating whether the regions contain objects. The key difference is that Mask R-CNN uses ROIAlign, an enhanced version of the ROIPool operation. In the second stage, the Faster R-CNN classifier is combined with an additional mask prediction head to predict both the class of the object within the ROIs and the corresponding mask (Al Deen Taher & Dang, 2023). The proposed analytical framework is based on the ArcGIS Pro 3.4.0 Mask R-CNN implementation and can be used with the licensed ArcGIS Pro software.

The Mask R-CNN algorithm was used because of its fast and high learning capability based on test data, which facilitates the processing of large data. Figure 3 shows the general workflow diagram of the method proposed in the research.

***Pre-processing***

Pre- and post-earthquake satellite images obtained from the February 6, 2023 earthquake were aligned using ArcGIS Pro software (Figure 4). Both satellite images were enhanced using the pan-sharpening method to improve spatial resolution. At this stage, buildings were classified based on their damage status. In the classification, buildings were labeled as '0' for damaged and '1' for undamaged. Additionally, roads and parks were also labeled. During the data labeling process, collapsed buildings were marked in red, undamaged buildings in yellow, roads in blue, and green areas in green. To increase accuracy, data processing was conducted in the UTM/WGS84 coordinate system. Next, the Label Objects for Deep Learning tool was used to prepare the training datasets from the post-earthquake satellite image for model training. The prepared dataset was then exported using the Export Training Data for Deep Learning tool. Finally, the data was used to train the Mask R-CNN model using the ArcGIS Pro deep learning library.

***Network Architecture***

Mask R-CNN is a deep learning model used for object-based boundary detection. The model was developed based on the Faster R-CNN model. Faster R-CNN consists of region-based convolutional neural networks (Region-based CNNs) that return a bounding box, a class label, and a confidence score for each object (Esri, 2024).

Table 1. Technical specifications of Göktürk-1 satellite image

| | |
|---|---|
| Launch date | 9 February 2023 |
| Orbit | ~ 681 km |
| Time of crossing the equator | 10:30 (Local time of ascending node) |
| Resolution | 2 m |
| Radiometric Resolution | 11 Bit |
| Spectral band | 0.45–0.52(Blue),<br>0.52–0.60 (Green),<br>0.63–0.69 (Red),<br>0.76–0.90 (Infrared) |

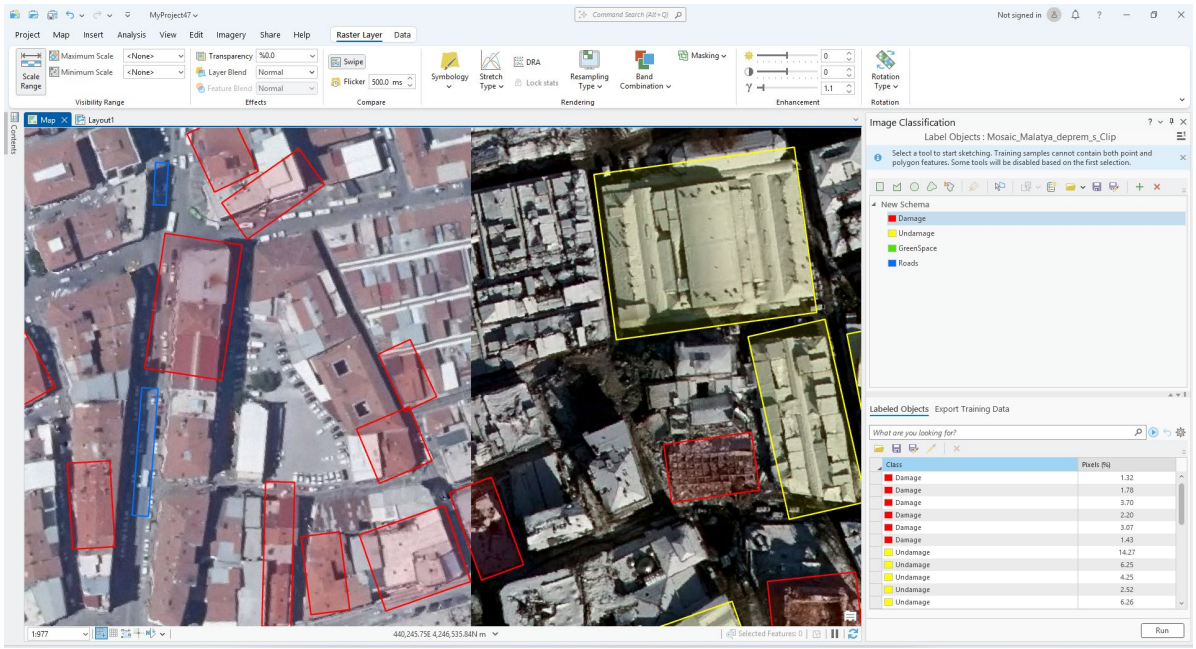


Figure 4. Creating a data set on ArcGIS Pro.

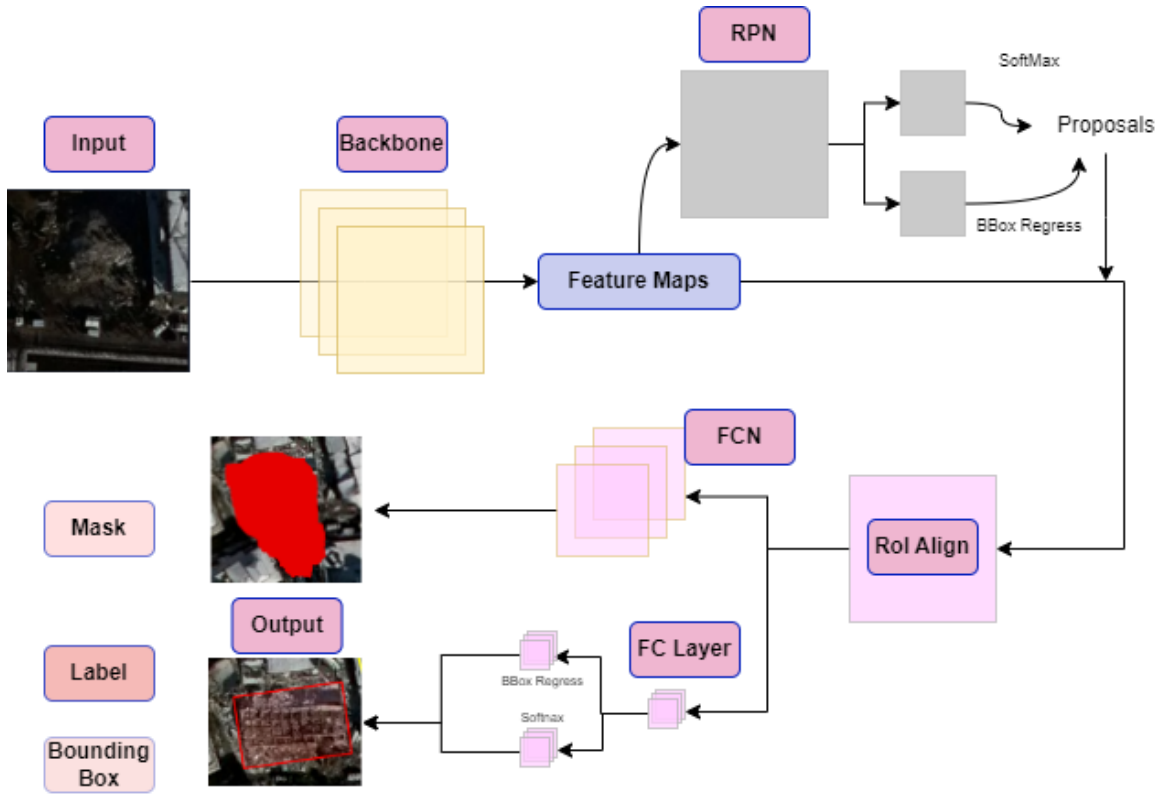


Figure 5. Mask R-CNN algorithm structure (Modified from Zhan et al., 2022)

Table 2. Summary statistics of training data

| Feature | Value | CN | CV | NI | NF | MA | AA | MA |
|---|---|---|---|---|---|---|---|---|
| Total Number of Images | 414 × 3 × 256 × 256 pixels | Damaged | 0 | 208 | 257 | 0 | 440.5 | 3000.69 |
| Total Number of Features | 532 | Undamaged | 1 | 206 | 275 | 0.02 | 302.83 | 2762.93 |
| Features per Image | Min: 1, Average: 1.29, Max: 4 | | | | | | | |

CN: Class Name; CV: Class Value; NI: Number of Images; NF: Number of Features; MA: Min Area (m²); AA: Average Area (m²); MA: Max Area (m²)

The workflow of Mask R-CNN is as follows:

- 'Mask R-CNN processes the image through a residual network to extract features and create multi-scale feature maps.
- Side-joining is performed, and the feature maps at each stage are doubled by performing a tensor summation with adjacent lower layers.
- The feature maps are fed into the Region Proposal Network (RPN) to generate candidate regions of varying sizes. These candidate regions are transferred along with the feature maps to the Region of Interest Align (RoI Align) layer, producing bounding boxes.
- The bounding boxes are classified, and their positions are refined. A high-quality instance segmentation mask is generated for the detected object.' (Zhan et al., 2022).

### *Model Training*

The model training was conducted on a system equipped with a 13th generation Intel(R) Core(TM) i7-13700H 2.40 GHz processor, an NVIDIA GeForce RTX 4060Ti graphics card, and 32 GB of RAM. For the study, the Train Deep Learning Model tool in ArcGIS Pro, which runs on the Keras deep learning framework, was used for the Mask R-CNN model. This tool utilizes the dataset created by the Export Training Data for Deep Learning tool and generates definitions based on the observed features. It iteratively processes the provided data to ensure the generated definitions align with the dataset (Arnold, 2023). While preparing the training data in the Export Training Data for Deep Learning tool, a coverage limitation was applied, and a damage classification layer was created. A total of 414 training samples were used, consisting of 208 samples representing collapsed buildings after the earthquake and 206 representing other structures. The Train Deep Learning Model tool was configured with ResNet50 as the backbone model.

## Results

In this study, post-earthquake damage assessment was conducted using deep learning algorithms applied to satellite imagery within ArcGIS Pro 3.4.0 software. The Mask R-CNN model was leveraged, trained with the ResNet50 backbone architecture. For training the Mask R-CNN approach for building damage detection, 20 epochs were defined. Although ArcGIS Pro offers the maximum epochs value as an optional setting, it was set to 20 by default in this study to ensure balanced training of the data. Increasing this value could lead to overfitting. Additionally, raising the number of epochs prolongs the data analysis process. Considering the critical importance of the first 72 hours following a disaster such as an earthquake, keeping the number of epochs reasonable and expediting the analysis process is crucial.

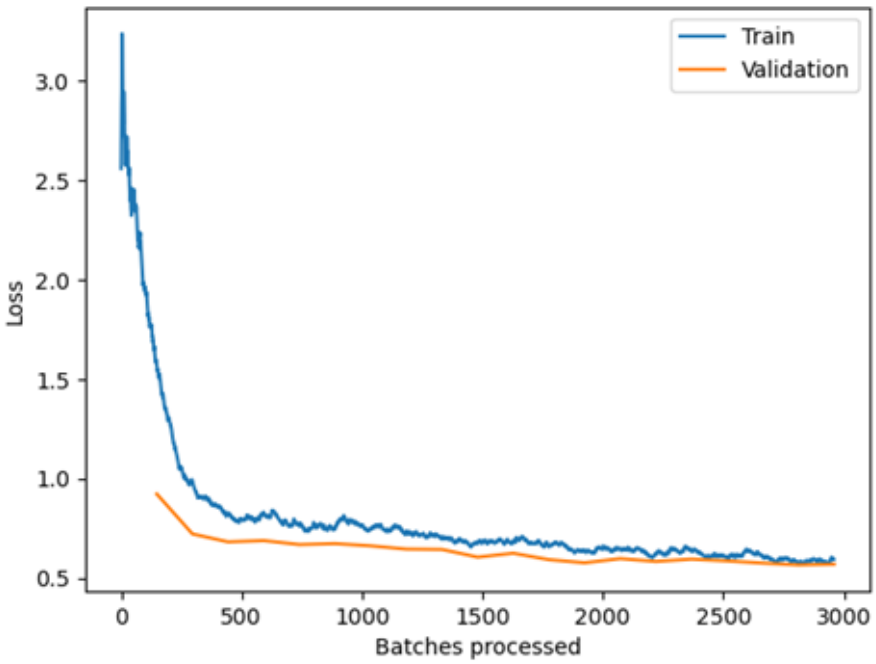


Figure 6. The relationship between the loss of training data and epochs

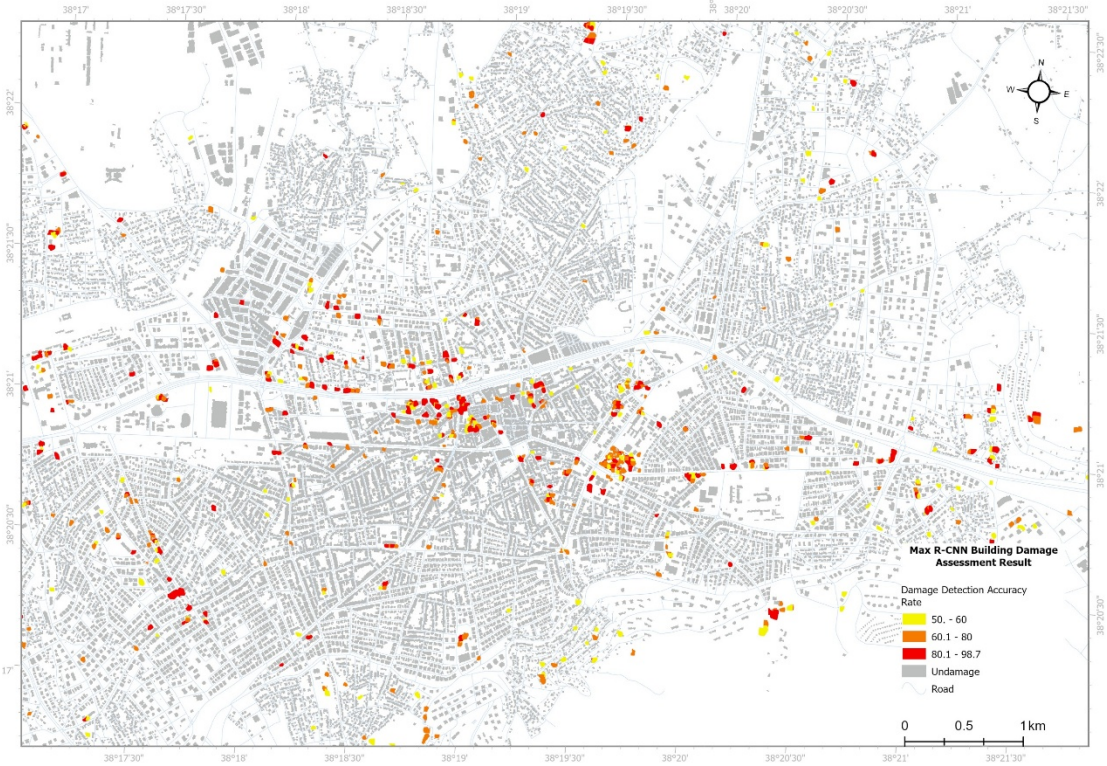


Figure 7. Deep learning result map generated using Mask R-CNN algorithm

The accuracy rate of the analyses conducted in the context of the study was calculated as 71%. This indicates that the proposed method in the article is sufficiently accurate. However, in analyses performed using methods such as deep learning after a disaster like an earthquake, a higher confidence interval is generally expected. Since this study focuses on identifying collapsed buildings after an earthquake, each vector data point during the assessment was scored with an accuracy rate ranging from 0 to 100. The closer the accuracy rate is to 100, the greater the damage in that area caused by the earthquake. When Figure 7 is examined, it is evident that the city center and its surroundings were significantly affected by the earthquake. Based on the analyses, areas with an accuracy rate of 80 or higher were determined to be completely destroyed. The undamaged data from the map basemaps represent the rasterized version of the existing building stock.

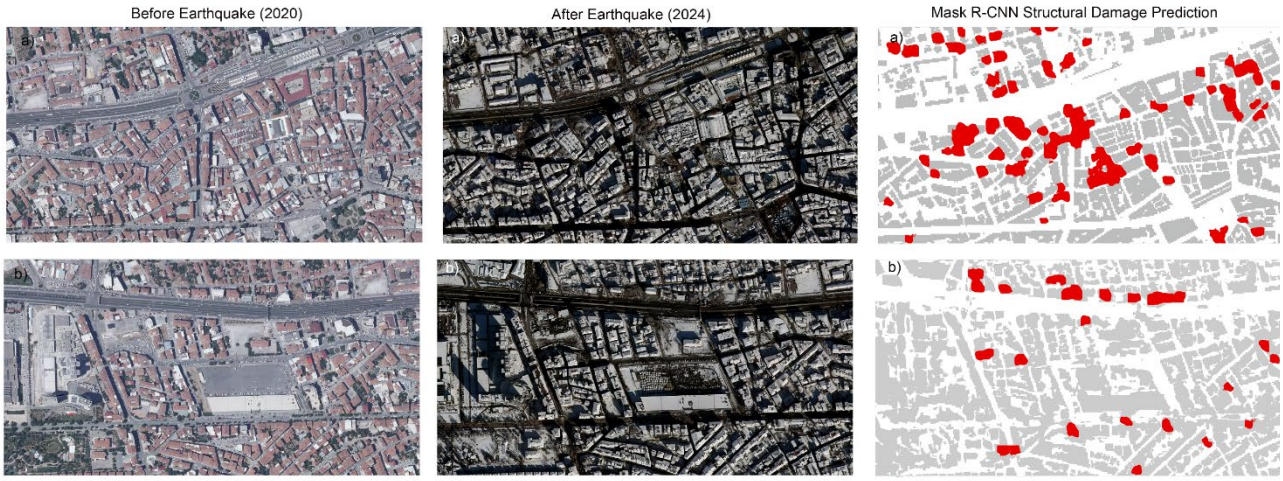


Figure 8. Visualization problems

In the satellite image-based analyses conducted using the Mask R-CNN algorithm, it was observed that some classifications were incorrectly labeled by the algorithm. The primary reason for this issue is that the analysis was performed solely based on the rooftops of the buildings. Additionally, the presence of snow cover in the satellite imagery used in the analysis further complicated the model's learning process (Figure 8).

## Discussion

The analyses conducted on pre- and post-earthquake images demonstrate that the identified collapsed buildings closely match the actual damage results. However, since the images used do not contain information on the structural components of buildings (e.g., columns, beams), the detected damage is limited to the roof. Therefore, significant differences may exist between the damage identified using satellite imagery and the actual structural damage. Nevertheless, the purpose of this study is to rapidly identify collapsed buildings after an earthquake, estimate the number of structures requiring inspection, and assess the number of affected individuals to initiate an effective search and rescue process.

In Türkiye, post-earthquake damage assessments are primarily conducted based on external and, if necessary, internal evaluations according to ATC-20 assessment criteria (ATCouncil, 2005; Özerol Özman et al., 2024). While this method is effective for long-term structural damage assessment, its impact during the immediate response phase is limited (Lozano et al., 2023). Traditional post-disaster assessments rely heavily on visual inspections by trained field teams, leading to inefficiencies, prolonged assessment timelines, and risks to personnel due to hazardous materials and debris exposure (Braik & Koliou, 2024; Korkmaz, 2009). However, advanced technologies such as remote sensing, artificial intelligence (AI), and data analytics hold the potential to revolutionize damage assessment processes (Braik & Koliou, 2024). In this context, Kaplan and Kaplan (2021) demonstrated that structural damage estimation supported by remote sensing data could reduce the number of buildings requiring expert inspection by approximately 50% after the 2020 Samos earthquake. Detecting and mapping the adverse effects of a disaster using traditional methods is quite challenging (Mahabir et al., 2018). Compared to conventional methods, GIS and remote sensing significantly reduce the time required for such assessments (Arikan İspir & Yildiz, 2025). In their study, Braik and Koliou (2024) utilized deep learning methods for the detection and analysis of damaged buildings, producing highly accurate maps. In many studies, an accuracy rate of 70% is considered successful (Arikan İspir & Yildiz, 2025; Atik, 2023; Pan et al., 2020). Similar results were obtained in this study, where post-earthquake satellite imagery identified collapsed buildings with 71% accuracy.

While the research provides promising results for large-scale damage mapping, there is still room for improvement in terms of image quality and analysis duration. Despite the high resolution of the Göktürk satellite image used in the study, the analysis was limited to identifying only collapsed buildings, restricting the scope of general damage assessment.

## Conclusion

The earthquakes on February 6, 2023, which caused massive destruction in densely populated 11 urban areas, have highlighted the need for GIS-based analysis. GIS plays key role not only in numerous fields but also in identifying areas suitable for settlement and regions with high damage risk by considering the influence and interaction of geographical factors (Sönmez, 2011). With advancements in GIS and remote sensing technologies and the emergence of innovative approaches in AI-supported analytical methods, disaster research has also significantly benefited. For disasters such as earthquakes, industrial accidents, and fires, determining the affected areas immediately after the event is critical. For this reason, GIS, remote sensing and deep learning methods that enable holistic evaluation of the disaster area after disasters that cause great destruction such as earthquakes can offer effective solutions.

After disasters, the primary and most crucial data sources are existing geographic datasets and remote sensing imagery. These datasets can be used to identify affected areas and perform earthquake impact analyses based on the images. UAVs and drones play a significant role in this process (Maraş & Sarıyıldız, 2023). In this study, similar images were used to detect and map

destroyed buildings after the earthquake. ArcGIS Pro 3.4.0 software was utilized for the detection of collapsed buildings using post-disaster remotely sensed imagery with deep learning-based Mask R-CNN architecture. To validate the method used, a building damage detection dataset was created using GÖKTÜRK satellite images obtained three days after the February 6, 2023, Kahramanmaraş earthquake, pre-earthquake orthophotos from 2020, and geographic vector data. Subsequently, the dataset of collapsed and intact buildings was trained and validated using the Mask R-CNN architecture in the ArcGIS Pro deep learning tool "Train Deep Learning Model." The analysis results demonstrated that the Mask R-CNN model, combined with the ResNet-50 backbone and trained with two different batch sizes for 20 epochs, produced results close to accuracy.

The key contributions of this study are (i) the rapid damage assessment based on GIS and RS datasets after a disaster and (ii) providing information that can help identify priority areas for search and rescue efforts and accelerate early intervention processes. As a result, the study has proven that GIS and deep learning models can facilitate the initial detection and mapping of earthquake-induced damage, supporting decision-makers effectively.

## Declarations

### *Author Contribution Statement*

All data collection, analysis and text writing of the study were carried out by the author.

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# A Graphical Approach as Multiple Comparison Method for the Balanced and Partially Balanced Lattice Designs

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ABSTRACT

This study proposes a reliable and easy understandable statistical solution for the selection of varieties in the balanced and partially balanced lattice experiments, which are widely used in plant breeding studies. For this purpose, the Analysis of Means (ANOM) was adapted to the balanced, simple and triple lattice designs and an R function is developed for it. The adapted ANOM approach was compared with the Tukey, Duncan and Fisher's LSD tests with respect to the actual type I error rate in all of the balanced, simple and triple lattice designs. In addition to this, the ANOM approach and Tukey test were examined comparatively using a hypothetical example. According to the simulation results, LSD and Duncan could not maintain the actual type I error rate at 5.00% under any conditions. This situation became more dramatic with the increase in the number of groups. While the actual type I error rate for LSD and Duncan tests varied between 54.36%-100.00% and 37.49%-99.96%, respectively, for ANOM and Tukey tests it varied between 4.64%-6.08% and 4.62%-6.45%, respectively. ANOM and Tukey tests were quite successful in terms of maintaining the actual type I error rate. However, since the number of groups in lattice designs was quite high, the given hypothetical example showed that it would be more understandable to use the ANOM method.

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## Introduction

Due to the soil structure, the experimental designs containing blocks are widely used in agricultural studies. The completely randomized block design is the leading of these experimental designs. In the completely randomized block design, each treatment or group takes place once in each block at equal intervals. The purpose of blocking is to separate the heterogeneous material of the experiment into homogeneous parts within itself. However, as the number of groups compared increases, it is almost not possible to find homogeneous blocks that can contain all groups. As in areas such as the stock market, medical research, and automotive industry, also in some cases in agriculture, the number of groups (varieties or treatments) compared especially in breeding studies is quite large (Wu & Liao, 2004). Therefore, it is not appropriate to use completely randomized block design. As a solution to this situation, Yates (1936a) proposed Balanced Incomplete Block (BIB) design, where not every group takes part in each block, but the number of pairs of each group taking part together is equal. Although balanced incomplete block designs are effective, they are not suitable. Because the minimum number of blocks required is impractical when the number of groups is large. Hence, Yates (1936b) developed a new experimental design called Lattice or Quasi-factorial for cases where the number of groups is large. As with all experimental designs, also in Lattice, the analysis of variance only shows whether the factor (such as variety, treatment or application) is statistically effective (Glass & Hakstian, 1969). However, it does not show the difference stems from which group or groups (levels of the factor) (Duncan, 1955). Therefore, if the effect of the factor as a result of the analysis of variance is statistically significant, multiple comparison tests are used. Numerous multiple comparison tests have been developed to compare group averages. At this point, it is very important to determine which multiple comparison tests should be used. Multiple comparison tests are divided into categories such as all-contrast comparisons (ACC), all-pairwise multiple comparisons (MCP) (Duncan, 1955; Fisher, 1937; Tukey, 1949) multiple comparisons with the best (MCB) (Hsu,

1996), multiple comparisons with the control (MCC) (Dunnett, 1955), and multiple comparisons with the overall average (MCA), depending on the purpose of use (Hsu, 1996). MCP tests can be used easily when the number of groups compared is not large. However, as the number of groups (k) increases, the number of pairwise comparisons (kC2) also increases rapidly. For example, when k = 3, 5, 10, and 50, the number of pairwise comparisons are 3, 6, 10, 45, and 1225, respectively. Absorbing the results becomes increasingly difficult when the number of groups being compared is large. Therefore, in such cases, it is much more understandable to compare the groups with the best group, the control group, or the overall average rather than comparing them with each other. Especially in experimental designs such as lattice where breeding or selection studies are carried out, the differences of quite a large number of varieties from the population average are examined. Therefore, using MCA may be much more useful than MCP, MCB, and MCC. The multiple comparisons with the overall average is also called the Analysis of Means (ANOM). ANOM is a graphical method used to compare groups in terms of averages, variances, rates or proportions, correlation coefficients, and regression coefficients (Ott, 1983; Nelson, 1983, 1985, 1989, 1993; Wludyka & Nelson, 1997; Nelson & Dudewicz, 2002; Pran Kumar & Rao, 1998; Rao, 2005; Nelson et al., 2005). Since it is a graphical method, the results are very easy to understand. The use of ANOM is detailed by Nelson et al.(2005). In this study, ANOM test was adapted to the balanced and partially balanced lattice designs. Also, an user friendly function was developed with the R programming language to use the improved ANOM approach for the balanced and partially balanced lattice designs. The adapted ANOM was compared with Tukey (Tukey, 1949), Duncan (Duncan, 1955) and LSD (Fisher, 1937) tests which are common multiple comparison tests in practice (Genç & Soysal, 2018), in terms of the actual type I error rate via monte carlo simulation technique. Finally, the use and interpretation of the ANOM approach were explained by comparing it with the Tukey test through a hypothetical example. The aim of this study is to provide a reliable solution based on statistical approaches for the selection of varieties in the balanced and partially balanced lattice experiments, which are widely used in plant breeding studies.

## Materials and Methods

### *The Balanced Lattice Designs*

Every replication must be an absolute square in balanced and partially balanced lattice desings. The number of blocks (k) in each replication must be equal to the square root of the number of groups (k2). Also, in balanced lattice designs, the number of replications (r = k + 1) must be one more than the number of blocks. Calculation steps for balanced lattice designs are as follows.

1. Compute the block totals, the replication totals, the group totals (t) and grand total (G).
2. For each group, compute totals ($B_t$) for all blocks in which the groups are located.
3. $W = kt - (k+1)B_t + G$
4. Sum of squares (s.s.) for the total, replications and groups are found classically.
5. Compute the adjusted sum of squares for the blocks, $SSB_{adj}=(\sum W^2)/(k^3+k^4)$
6. Compute the adjustment factor, $\mu = (E_b - E_e)/(k^2 E_b)$
7. $E_b = SSB_{adj}/(k^2-1)$ and $E_e = SSE/(k-1)(k^2-1)$, SSE is found by subtracting replications s.s., groups s.s. and adjusted blocks s.s. from total s.s.
8. The adjusted group total is $t + \mu W$.
9. Compute sum of squares for the groups based on the adjusted group total.
10. Effective error ($E_e'$) is $E_e(1 + k\mu)$
11. Compute the F-value by dividing the adjusted groups s.s. by the effective error (Cochran & Cox, 1960).

### *The Partially Balanced Lattice Designs*

In the partially balanced lattice, the number of replications does not have to be more than the number of blocks. A partially balanced lattice designs are named according to the number of replications. When the number of replications (r) are 2, 3, 4, etc., it is called as simple lattice, triple lattice, quadruple lattice, etc., respectively. In this study also, simple and triple lattice are emphasized. Calculation steps for partially balanced lattice designs are as follows.

1. Compute the block totals (B), the replication totals, the group totals (t) and grand total (G).
2. For each block compute,
   $C = \text{Sum (OR) of all groups in the block} - rB$
   OR: overall replicates
3. All sum of squares are computed classically.
4. Compute the adjusted sum of squares for the blocks,
   $SSB_{adj} = (\sum C^2)/[kr(r-1)] - (\sum R_c^2)/[k^2 r(r-1)]$,
   $R_c$ *is* is the sum of C values in each replication.
5. The adjustment factor (μ) is $(E_b - E_e)/[k(r-1)E_b]$
6. $E_b = SSB_{adj}/r(k-1)$ and $E_e$ is found like in the balanced lattice designs.
7. The adjusted group total is $t + \mu c$
8. Compute sum of squares for the groups based on the adjusted group total.
9. Effective error ($E_e'$) is $E_e[1 + (rk\mu)/(k+1)]$
10. Compute the F-value like in the balanced lattice designs (Cochran & Cox, 1960).

### *The Basic Anom Procedure*

The basic ANOM procedure is introduced for balanced one-way analysis of variance. In the ANOM chart, there are two decision lines, namely lower (LDL) and upper (UDL).

$$LDL = \bar{X}_{..} - h(\alpha, k, v)\sqrt{MSE}\sqrt{(k-1)/(kn)} \quad (1)$$

$$UDL = \bar{X}_{..} + h(\alpha, k, v)\sqrt{MSE}\sqrt{(k-1)/(kn)} \quad (2)$$

If the average of which group is outside of these decision lines, it is understood that the average of that group is statistically significantly different from the overall average. This is corresponding to comparing with h(α, k, v).

$$|T_i| = |\bar{X}_{i.} - \bar{X}_{..}| / \left[\sqrt{MSE}\sqrt{(k-1)/(kn)}\right] \quad (3)$$

The assumption that $X_{ij} \sim N(\mu_i, \sigma^2)$ are independent are met and if $H_0: \mu_1 = \mu_2 = \cdots = \mu_k$, $(T_1, T_2, \ldots, T_k)$ has a multivariate t distribution with equicorrelations $\rho = -1/(k-1)$ and $v = k(n-1)$ df. Under $H_0$, the critical values $h(\alpha, k, v)$ meet,

$$P[|T_1| \leq h(\alpha, k, v), |T_2| \leq h(\alpha, k, v), \ldots, |T_k| \leq h(\alpha, k, v)] = 1 - \alpha$$

Where, $\bar{X}_{i.}$ is average of i*th* group, $\bar{X}_{..}$ is the overall average, MSE is the mean square error, α is significance level, k is the number of groups (treatments or varieties), n is the number of replications (Nelson, 1993).

***Adaptation of ANOM for the Balanced and Partially Balanced Lattice Designs***

Lattice designs adjust $E_e$ and group totals so as to take account of sampling errors in the block correction. Therefore, the ANOM method should be applied to adjusted means. The Adjusted overall average is

$$\tilde{X}_{...} = \left(\sum \tilde{X}_{i..}\right)/k^2$$

The Adjusted average of i*th* group is

$\tilde{X}_{i..} = (t_i + \mu W_i)/(k+1)$ for the balanced lattice,

$\tilde{X}_{i..} = (t_i + \mu c)/r$ for the partially balanced lattice.

Therefore, the variance for difference of the adjusted averages of the groups from the adjusted overall average is

$Var(\tilde{X}_{i..} - \tilde{X}_{...}) = E'_e\,(k^2-1)/(k^3+k^2)$ for the balanced lattice,

$Var(\tilde{X}_{i..} - \tilde{X}_{...}) = E'_e\,(k^2-1)/(rk^2)$ for the partially balanced lattice.

Thus,

$|T_i| = \left|\tilde{X}_{i..} - \tilde{X}_{...}\right| / \sqrt{E'_e(k^2-1)/(k^3+k^2)}$ for the balanced lattice,

$|T_i| = \left|\tilde{X}_{i..} - \tilde{X}_{...}\right| / \sqrt{E'_e(k^2-1)/(rk^2)}$ for the partially balanced lattice.

The assumption that $X_{ijk} \sim N(\mu_i, \sigma^2)$ are independent is met and if $H_0: \mu_1 = \mu_2 = \cdots = \mu_k$, $(T_1, T_2, \ldots, T_k)$ has a multivariate t distribution with equicorrelations $\rho = -1/(k^2-1)$ and degree of freedom $v = (k-1)(k^2-1)$ for the balanced lattice and $v = (k-1)(rk-k-1)$ for the partially balanced lattice. Under $H_0$, the critical values $h(\alpha, k^2, v)$ are

$$P[|T_1| \leq h(\alpha, k^2, v), |T_2| \leq h(\alpha, k^2, v), \ldots, |T_{k^2}| \leq h(\alpha, k^2, v)] = 1 - \alpha$$

Accordingly, decision lines are

$$\left.\begin{aligned} LDL &= \tilde{X}_{...} - h(\alpha, k^2, v)\sqrt{E'_e}\sqrt{(k^2-1)/(k^3+k^2)} \\ UDL &= \tilde{X}_{...} + h(\alpha, k^2, v)\sqrt{E'_e}\sqrt{(k^2-1)/(k^3+k^2)} \end{aligned}\right\}$$

for the balanced lattice,

$$\left.\begin{aligned} LDL &= \tilde{X}_{...} - h(\alpha, k^2, v)\sqrt{E'_e}\sqrt{(k^2-1)/(rk^2)} \\ UDL &= \tilde{X}_{...} + h(\alpha, k^2, v)\sqrt{E'_e}\sqrt{(k^2-1)/(rk^2)} \end{aligned}\right\}$$

for the partially balanced lattice.

If an adjusted average of group falls outside these decision lines, it is statistically significantly different from the adjusted overall average.

Also, the required $h(\alpha, k^2, v)$ values according to different significance levels (α = 0.25, 0.10, 0.05, 0.01, 0.001) for all available the balanced, simple and triple lattice experimental designs were given in the appendix B (Table 8, 9 and 10).

***The R Function for ANOM Approach***

The function (ANOMLattice) developed using the R programming language (R Core Team, 2021) for the ANOM approach were given in appendix C. While writing the "ANOMLattice" function, "agricolae" (de Mendiburu, 2021), "MASS" (Venables & Ripley, 2002), "ggplot2" (Wickham, 2016) and "mvtnorm" (Genz et al., 2021) packages were used. Therefore, the aforementioned packages must be installed in order to use the "ANOMLattice" function. Then the following steps should be applied.

Firstly, installed packages must be loaded.

```
library(agricolae)
library(MASS)
library(ggplot2)
library(mvtnorm)
```

Secondly, it should be loaded by running the "ANOMLattice" function given in the appendix C.

Thirdly, the block, group, replication and response vectors required for "ANOMLattice" function should be created and the alpha value should be determined according to the desired significance level.

The "ANOMLattice" function were explained through two different examples for the balanced and partially balanced lattice designs.

***An Example for The Balanced Lattice Designs***

An application of the balanced lattice design were examined in a study investigating the effect of 9 feeding treatments on the growth rates of pigs by Comstock et al. (1948). The use of the "ANOMLattice" function for the balanced lattice were also explained through the same study. The R function is

"ANOMLattice(block,treat,rep,response,alpha)".

Vectors must be created for all inputs required by the function.

```
library(agricolae)
library(MASS)
library(ggplot2)
library(mvtnorm)
blk   =rep(1:12,each=3)
trt  = c(1,2,3,4,5,6,7,8,9,
         3,4,8,2,6,7,1,5,9,
         1,4,7,2,5,8,3,6,9,
         3,5,7,2,4,9,1,6,8)
rep = rep(1:4, each=9)
growth = c(2.20,1.84,2.18,2.05,0.85,1.86,0.73,1.60,1.76,
           1.71,1.57,1.13,1.76,2.16,1.80,1.81,1.16,1.11,
           1.19,1.20,1.15,2.26,1.07,1.45,2.12,2.03,1.63,
           2.04,0.93,1.78,1.50,1.60,1.42,1.77,1.57,1.43)
ANOMLattice(blk, trt, rep, growth, alpha = 0.05)
```

When the codes written above are run, figure 1 was generated directly.

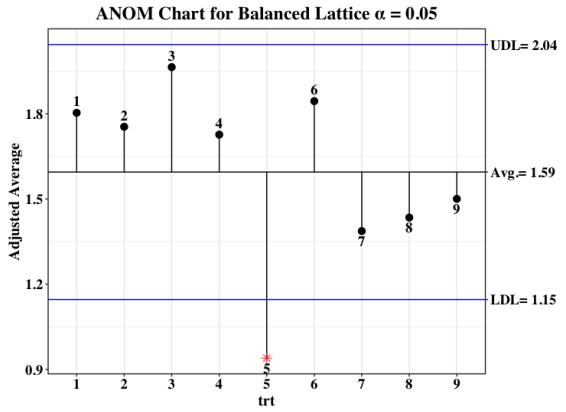


Figure 1. ANOM Chart for Balanced Lattice Design

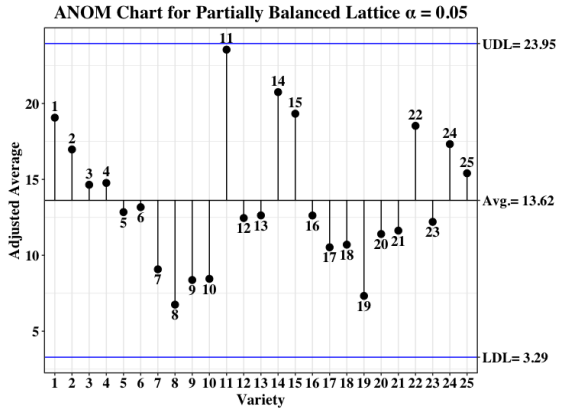


Figure 2. ANOM Chart for Partially Balanced Lattice Design

Figure 1 just showed that the average of the treatment 5 was statistically significantly smaller than the overall average, while the other treatment averages were not statistically significantly different from the overall average.

***An Example for The Partially Balanced Lattice Designs***

The use of the "ANOMLattice" function for the partially balanced lattice designs were explained through an example given by Cochran and Cox (1960). In this example, 25 soybean varieties are compared with respect to yield in a simple lattice design.

```
library(agricolae)
library(MASS)
library(ggplot2)
library(mvtnorm)
block = rep(1:10,each=5)
Variety =
c(1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,
21,22,23,24,25,1,6,11,16,21,2,7,12,17,22,3,8,13,18,23,
4,9,14,19,24,5,10,15,20,25)
rep = rep(1:2,each=25)
yield =
c(6,7,5,8,6,16,12,12,13,8,17,7,7,9,14,18,16,13,13,14,14,15,
11,14,14,24,13,24,11,8,21,11,14,11,23,16,4,12,12,12,17,10,
30,9,23,15,15,22,16,19)
ANOMLattice(block, Variety, rep, yield, alpha = 0.05)
```

When the codes written above are run, figure 2 was generated directly.

Figure 2 showed that the average of any variety was not different from the overall average in terms of yield. However, variety 11 was fairly close to UDL. This situation can be practically important (Nelson et al., 2005).

***Design of the Simulation Study***

ANOM approach together with Tukey (Tukey, 1949), Duncan (Duncan, 1955) and Fisher's LSD (Fisher, 1937) tests, which are widely used in practice, were examined in terms of the actual type I error rates in the balanced and partially balanced lattice designs under the assumptions of normality and homogeneity of variances. Random numbers which were generated using "rnorm" function of R project (R Core Team, 2021) were used in order to examine the all tests in terms of the actual type I error rates. Six different designs (k=3, 4, 5, 7, 8 and 9) for the balanced lattice and nine different designs (k=3, 4, 5, 6, 7, 8, 9, 10 and 12) for partially balanced lattice were considered in the study. Each experimental condition was repeated 10,000 times. The actual type I error rates for all tests were estimated as

$$\frac{\text{The number of falsely rejected } H_0 \text{ hypothesis}}{10{,}000}$$

Bradley (1978) reported that the actual type I error rate of a robust test should be between 4.50% and 5.50% when testing at the 5.00% level. In this work, Bradley's conservative criterion was taken into account as a measure of robustness.

**Results and Discussion**

***Results of the Simulation Study***

The actual Type I error rates were obtained as a result of simulation trials were given in Tables 1, 2 and 3. The actual type I error rates within Bradley's limits were written in bold.

In the balanced lattice designs, the actual type I error rates for the ANOM approach, Tukey, Duncan and LSD tests were in the range of 4.64-5.71%, 4.62-5.74%, 37.49-98.31% and 54.36 100.00%, respectively. Bradley's criterion was met for the ANOM approach and Tukey test in the all balanced lattice designs except 3x3 design. In the 3x3 design also, the actual type I error rates for ANOM and Tukey which were 5.76% and 5.74%, respectively deviated negligibly from Bradley's criterion. However, performances of the Duncan and LSD tests were dramatically badness under the same experimental conditions (Table 1).

In simple lattice designs, the actual type I error rates varying between 4.76-6.08% for ANOM, 4.75-6.45% for Tukey, 44.59-99.96% for Duncan and 44.31-100.00% for LSD were estimated. The ANOM and Tukey could not meet Bradley's criteria only in 3x3 and 4x4 designs. However, this situation was negligibly compared to the Duncan and LSD (Table 2). The actual type I error rates for the ANOM (6.04%) and Tukey (6.33%) fallen outside Bradley's limits only in 3x3 triple lattice design. However, the actual type I error rates for the Duncan and LSD tests could not met Bradley's criterion in any triple lattice designs (Table 3).

Table 1. The actual type I error rates for the balanced lattices.

| k×k | ANOM | Tukey | Duncan | LSD |
|---|---|---|---|---|
| 3×3 | 5.71 | 5.74 | 37.49 | 54.36 |
| 4×4 | **4.82** | **4.96** | 55.01 | 81.63 |
| 5×5 | **4.94** | **4.91** | 71.45 | 95.31 |
| 7×7 | **4.82** | **4.99** | 90.86 | 99.95 |
| 8×8 | **4.64** | **4.62** | 95.94 | 100.00 |
| 9×9 | **5.03** | **5.05** | 98.31 | 100.00 |

Table 2. The actual type I error rates for the simple balanced lattices.

| k×k | ANOM | Tukey | Duncan | LSD |
|---|---|---|---|---|
| 3×3 | 6.08 | 6.45 | 44.59 | 44.31 |
| 4×4 | 5.98 | 5.97 | 63.06 | 74.10 |
| 5×5 | **5.49** | **5.50** | 77.22 | 91.19 |
| 6×6 | **5.16** | **5.35** | 86.74 | 97.80 |
| 7×7 | **5.07** | **5.19** | 92.90 | 99.66 |
| 8×8 | **5.19** | **5.23** | 97.01 | 99.96 |
| 9×9 | **4.97** | **4.94** | 98.70 | 99.99 |
| 10×10 | **4.98** | **4.75** | 99.36 | 100.00 |
| 12×12 | **4.76** | **4.75** | 99.96 | 100.00 |

Table 3. The actual type I error rates for the triple balanced lattices.

| k×k | ANOM | Tukey | Duncan | LSD |
|---|---|---|---|---|
| 3×3 | 6.04 | 6.33 | 40.74 | 53.34 |
| 4×4 | **5.11** | **5.25** | 58.29 | 79.45 |
| 5×5 | **5.07** | **5.14** | 73.31 | 93.97 |
| 6×6 | **5.10** | **5.07** | 84.92 | 98.64 |
| 7×7 | **5.00** | **5.14** | 92.59 | 99.76 |
| 8×8 | **4.95** | **4.95** | 96.72 | 99.98 |
| 9×9 | **4.73** | **4.74** | 98.46 | 100.00 |
| 10×10 | **5.24** | **4.93** | 99.55 | 100.00 |
| 12×12 | **5.10** | **4.98** | 99.91 | 100.00 |

As the number of groups increases, the total number of observations increases, so the effect of the number of replications on the actual type I error rate decreases. Since the number of groups is less in 3×3 and 4×4 designs compared to the other designs, the actual type I error rates for the ANOM and Tukey deviated slightly. On the other hand, the actual type I error rates for the Duncan and LSD tests were seriously high under all experimental conditions.

***A Numerical Example***

A hypothetical example was given in which averages of 49 varieties (groups) from any plant species (for example wheat, barley, oat, etc.) were compared in terms of any traits (for example yield, length, etc.) in the balanced lattice design (Table 7 in appendix A). Calculations can be made easily by applying the formulas given in section 3 for the partially balanced lattices. While creating this example, the balanced lattice plan given by Cochran and Cox was used (1960). Since the number of varieties compared ($k^2$) was 49, the number of blocks must be $k = \sqrt{49} = 7$ and the number of replications must be r=7+1 = 8.

$B_t$ and W for Treatment 3,

First, the blocks in which variety 3 was located were determined. These were blocks 1, 10, 17, 24, 31, 38, 45 and 52.

The sum of these blocks given the Bt for Variety 3.

$B_t = 76 + 68 + 65 + 75 + 75 + 73 + 81 + 66 = 579$

$W = kt - (k+1)B_t + G$

$W = (7)93 - (7+1)579 + 3921 = -60$

Similarly, $B_t$ and W were calculated for all treatments (Table 4). After the Bt andWvalues were obtained, the analysis of variance table (Table 5) were easily created by using the formulas given in section 2.

The adjusted mean square for treatment (23.29) was found by dividing the adjusted sum of squares for treatment (1117.95) by degrees of freedom (d.f.) for treatment ($k^2 - 1 = 48$). The effective error was found by $E'_e = E_e(1 + k\mu) = (2.07)[1 + (7)(0.0029)] = 2.11.$ An approximately F value, 23.29/2.11 or 11.04, had 48 and 288 d.f. . Because the F value (11.04) was greater than $F_{0.05,48,288}$ critical table value (1.40), at least the average of one variety was statistically significantly different from the others. Therefore, after this point, it was necessary to determine which variety or varieties caused the difference. For this purpose, the ANOM approach and the Tukey test, which was quite common in practice, were used in this study. Variety averages were compared with both Tukey and ANOM approach at 0.05 significance level. The results of the Tukey test and ANOM approach were given in the table 6 and figure 3, respectively.

Table 6 showed that Tukey test results were highly complex. Since the number of varieties with common letters was considerably large, it was very difficult to select varieties based on Tukey test results. For example, the varieties with the highest average (31, 45 and 22) shared a letter with the 26 varieties that follows itself, and the variety with the lowest average (24) shared a letter with the 22 varieties whose average was higher. On the other hand, variety 20 shared letters with all the other varieties. When this was the case, it became very complicated to distinguish between varieties.

Therefore, it was more understandable and useful to compare with the general average using the ANOM method instead of pair-wise comparison. After obtaining the results of the analysis of variance, it was quite simple to apply the ANOM method. What needs to be done was to calculate the UDL and LDL.

Table 4. t, $B_t$, W and adjusted total values

| Treat. | t | $B_t$ | W | Adj. Tot. | Treat. | t | $B_t$ | W | Adj. Tot. | Treat. | t | $B_t$ | W | Adj. Tot. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 88 | 558 | 73 | 88.21 | 18 | 87 | 567 | -6 | 86.98 | 34 | 67 | 561 | -98 | 66.72 |
| 2 | 95 | 571 | 18 | 95.05 | 19 | 94 | 568 | 35 | 94.10 | 35 | 61 | 538 | 44 | 61.13 |
| 3 | 93 | 579 | -60 | 92.83 | 20 | 79 | 559 | 2 | 79.01 | 36 | 62 | 562 | -141 | 61.59 |
| 4 | 82 | 559 | 23 | 82.07 | 21 | 59 | 544 | -18 | 58.95 | 37 | 62 | 545 | -5 | 61.99 |
| 5 | 74 | 564 | -73 | 73.79 | 22 | 100 | 569 | 69 | 100.20 | 38 | 72 | 549 | 33 | 72.10 |
| 6 | 83 | 568 | -42 | 82.88 | 23 | 92 | 587 | -131 | 91.62 | 39 | 80 | 562 | -15 | 79.96 |
| 7 | 65 | 550 | -24 | 64.93 | 24 | 56 | 542 | -23 | 55.93 | 40 | 74 | 538 | 135 | 74.39 |
| 8 | 69 | 556 | -44 | 68.87 | 25 | 81 | 590 | -232 | 80.33 | 41 | 88 | 560 | 57 | 88.17 |
| 9 | 83 | 583 | -162 | 82.53 | 26 | 73 | 547 | 56 | 73.16 | 42 | 94 | 570 | 19 | 94.06 |
| 10 | 76 | 551 | 45 | 76.13 | 27 | 62 | 531 | 107 | 62.31 | 43 | 91 | 552 | 142 | 91.41 |
| 11 | 70 | 543 | 67 | 70.19 | 28 | 75 | 562 | -50 | 74.85 | 44 | 96 | 563 | 89 | 96.26 |
| 12 | 96 | 577 | -23 | 95.93 | 29 | 94 | 567 | 43 | 94.12 | 45 | 100 | 557 | 165 | 100.48 |
| 13 | 65 | 551 | -32 | 64.91 | 30 | 69 | 551 | -4 | 68.99 | 46 | 65 | 542 | 40 | 65.12 |
| 14 | 92 | 574 | -27 | 91.92 | 31 | 102 | 573 | 51 | 102.15 | 47 | 62 | 526 | 147 | 62.43 |
| 15 | 58 | 543 | -17 | 57.95 | 32 | 80 | 565 | -39 | 79.89 | 48 | 98 | 591 | -121 | 97.65 |
| 16 | 95 | 570 | 26 | 95.08 | 33 | 96 | 573 | 9 | 96.03 | 49 | 91 | 569 | 6 | 91.02 |
| 17 | 75 | 570 | -114 | 74.67 | | | | | | | | | | |

$G = 3921, \sum B_t = 27447, \sum(t + \mu W) = 3921, \bar{X}_{...} = 10.00$

Table 5. Results of the analysis of variance.

| Source | d.f. | s.s. | m.s. | F | P |
|---|---|---|---|---|---|
| Replications | $k = 7$ | 18.92 | | | |
| Treatments (adj.) | $k^2 - 1 = 48$ | 1117.95 | 23.29 | 23.29/2.11=11.04 | 0.000 |
| Blocks (adj.) | $k^2 - 1 = 48$ | 115.60 | 2.41 | | |
| Intra-block error | $(k - 1)(k^2 - 1) = 288$ | 594.86 | 2.07 | | |
| Total | $k^3 + k^2 - 1 = 391$ | 1843.00 | | | |

Table 6. Results of the Tukey test.

| Variety | $\bar{X}$ | Grouping | Variety | $\bar{X}$ | Grouping | Variety | $\bar{X}$ | Grouping |
|---|---|---|---|---|---|---|---|---|
| 31 | 12.77 | a | 1 | 11.03 | a b c d e f g | 38 | 9.01 | d e f g h i j k |
| 45 | 12.56 | a | 41 | 11.02 | a b c d e f g | 11 | 8.77 | e f g h i j k |
| 22 | 12.53 | a | 18 | 10.87 | a b c d e f g | 30 | 8.62 | f g h i j k |
| 48 | 12.21 | a b | 6 | 10.36 | a b c d e f g h | 8 | 8.61 | f g h i j k |
| 44 | 12.03 | a b c | 9 | 10.32 | a b c d e f g h | 34 | 8.34 | g h i j k |
| 33 | 12.00 | a b c | 4 | 10.26 | a b c d e f g h i | 46 | 8.14 | g h i j k |
| 12 | 11.99 | a b c | 25 | 10.04 | a b c d e f g h i j | 7 | 8.12 | g h i j k |
| 16 | 11.88 | a b c d | 39 | 9.99 | a b c d e f g h i j | 13 | 8.11 | g h i j k |
| 2 | 11.88 | a b c d | 32 | 9.99 | a b c d e f g h i j | 47 | 7.80 | h i j k |
| 29 | 11.77 | a b c d | 20 | 9.88 | a b c d e f g h i j k | 27 | 7.79 | h i j k |
| 19 | 11.76 | a b c d | 10 | 9.52 | b c d e f g h i j k | 37 | 7.75 | h i j k |
| 42 | 11.76 | a b c d | 28 | 9.36 | b c d e f g h i j k | 36 | 7.70 | h i j k |
| 3 | 11.60 | a b c d e | 17 | 9.33 | b c d e f g h i j k | 35 | 7.64 | h i j k |
| 14 | 11.49 | a b c d e f | 40 | 9.30 | b c d e f g h i j k | 21 | 7.37 | i j k |
| 23 | 11.45 | a b c d e f | 5 | 9.22 | c d e f g h i j k | 15 | 7.24 | j k |
| 43 | 11.43 | a b c d e f | 26 | 9.15 | c d e f g h i j k | 24 | 6.99 | k |
| 49 | 11.38 | a b c d e f | | | | | | |

$$LDL=10.00-3.31\sqrt{2.11(7^2-1)/(7^3+7^2)}=8.32$$

$$UDL=10.00+3.31\sqrt{2.11(7^2-1)/(7^3+7^2)}=11.68$$

3.31 value was found easily from table 8 in appendix B. Then, the varieties that were outside and within the decision lines were determined. Thus, varieties were divided into three groups as statistically superior, equal, and inferior to the overall average.

The ANOM method does not compare the average of varieties with each other. It compares the average of varieties with the overall average. Therefore, this situation should not be overlooked in the interpretations. When the ANOM chart was examined, it was seen that the varieties numbered 2, 12, 16, 19, 22, 29, 31, 33, 42, 44, 45 and 48 were statistically significantly higher than the overall average. Varieties numbered 7, 13, 15, 21, 24, 27, 35, 36, 37, 46 and 47were statistically significantly smaller than the overall average.

However, the differences of the other varieties from the overall average were not statistically significant. The ANOM chart provided information not only about statistical significance but also about practical significance. For example, it was seen that varieties numbered 3, 14, 23, 43 and relatively 49 were very close to UDL, and varieties numbered 8, 11, 30 and 34 to LDL. Therefore, the varieties in question were very close to statistical significance compared to other varieties. This situation can be of practical significance. As can be seen, the ANOM method has allowed statistical inferences in a very simple and understandable way.

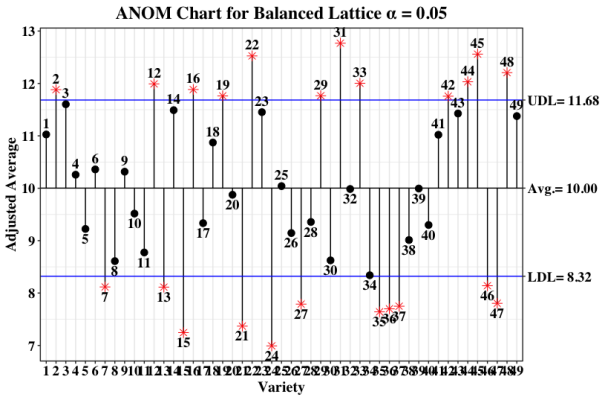


Figure 3. ANOM Chart for example of the balanced lattice design.

## Conclusion

Multiple comparison tests have an important place in comparing group averages. When the number of groups is small, it is easy and understandable pair-wise comparisons. However, the increase in the number of groups compared brings some problems. For example, Fisher's LSD test is widely used in practice. However, the LSD (Hayter, 1986) and Duncan tests (Hsu, 1996; Tukey, 1991) are very negatively affected by the increase in the number of groups. In the lattice designs, the number of groups is generally higher than the classical trial designs (completely randomized design, randomized block design, etc.). Simulation experiments in this study also, showed that the actual type I errors of LSD and Duncan tests were frighteningly high. This means that as the number of groups increases, the LSD and Duncan tests are more likely to find a result that there is a difference between the group averages while there is actually no difference between the group averages. For example, in every experiment set up in 8x8 balanced lattice design, the LSD test will certainly find that at least one group mean is different from the others, even though there is actually no difference between the group averages (Table 1). The Duncan test is almost exactly the same to the LSD test. Therefore, using the LSD and Duncan tests will cause misleading results. On the other hand, ANOM approach and Tukey test have almost excellent performance in terms of preserving the actual type I error rate. Regardless of the number of groups, both tests are very reliable. However, another problem is that arises with the increase in the number of groups is the difficulties in interpreting the results. When the number of groups increases, the number of pair-wise comparisons also increases significantly. The rapid increase in the number of pair-wise comparisons makes very complex and difficult to the interpretation of the results. At this point, it can be a very useful and reliable solution to compare the group averages with the overall average using the ANOM approach rather than comparing them with each other. Since the results of the ANOM can be presented graphically, both statistical and practical significance can be easily understood. As a result, the use of the ANOM test can be recommended when the number of groups compared is large so that researchers can easily understand reliable results.

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## *Appendices*

### *A. Plan and Data for 7×7 The Balanced Lattice Design*

Table 7. Plan and data for 7×7 balanced lattice design.

| Block | Replication I | | | | | | | Totals | Block | Replication II | | | | | | | Totals |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (1) | (1) | (2) | (3) | (4) | (5) | (6) | (7) | | (8) | (1) | (8) | (15) | (22) | (29) | (36) | (43) | |
| | 12 | 14 | 11 | 9 | 10 | 11 | 9 | 76 | | 11 | 8 | 7 | 13 | 11 | 6 | 10 | 66 |
| (2) | (8) | (9) | (10) | (11) | (12) | (13) | (14) | | (9) | (2) | (9) | (16) | (23) | (30) | (37) | (44) | |
| | 11 | 12 | 10 | 10 | 10 | 7 | 14 | 74 | | 12 | 8 | 12 | 12 | 10 | 7 | 14 | 75 |
| (3) | (15) | (16) | (17) | (18) | (19) | (20) | (21) | | (10) | (3) | (10) | (17) | (24) | (31) | (38) | (45) | |
| | 7 | 13 | 9 | 11 | 14 | 11 | 7 | 72 | | 11 | 8 | 8 | 5 | 12 | 10 | 14 | 68 |
| (4) | (22) | (23) | (24) | (25) | (26) | (27) | (28) | | (11) | (4) | (11) | (18) | (25) | (32) | (39) | (46) | |
| | 12 | 13 | 8 | 11 | 10 | 9 | 10 | 73 | | 11 | 9 | 12 | 9 | 11 | 9 | 8 | 69 |
| (5) | (29) | (30) | (31) | (32) | (33) | (34) | (35) | | (12) | (5) | (12) | (19) | (26) | (33) | (40) | (47) | |
| | 9 | 9 | 15 | 9 | 13 | 8 | 10 | 73 | | 7 | 11 | 11 | 11 | 11 | 6 | 7 | 64 |
| (6) | (36) | (37) | (38) | (39) | (40) | (41) | (42) | | (13) | (6) | (13) | (20) | (27) | (34) | (41) | (48) | |
| | 10 | 9 | 10 | 9 | 8 | 11 | 10 | 67 | | 8 | 9 | 8 | 6 | 10 | 14 | 13 | 68 |
| (7) | (43) | (44) | (45) | (46) | (47) | (48) | (49) | | (14) | (7) | (14) | (21) | (28) | (35) | (42) | (49) | |
| | 10 | 9 | 13 | 8 | 9 | 10 | 10 | 69 | | 6 | 10 | 7 | 9 | 9 | 13 | 12 | 66 |
| | | | | | | | | 504 | | | | | | | | | 476 |
| | Replication III | | | | | | | | | Replication IV | | | | | | | |
| (15) | (1) | (9) | (17) | (25) | (33) | (41) | (49) | | (22) | (1) | (37) | (24) | (11) | (47) | (34) | (21) | |
| | 11 | 10 | 12 | 10 | 15 | 11 | 13 | 82 | | 12 | 8 | 8 | 9 | 6 | 6 | 8 | 57 |
| (16) | (43) | (2) | (10) | (18) | (26) | (34) | (42) | | (23) | (15) | (2) | (38) | (25) | (12) | (48) | (35) | |
| | 11 | 12 | 10 | 10 | 8 | 6 | 13 | 70 | | 9 | 12 | 9 | 11 | 15 | 13 | 8 | 77 |
| (17) | (36) | (44) | (3) | (11) | (19) | (27) | (35) | | (24) | (29) | (16) | (3) | (39) | (26) | (13) | (49) | |
| | 7 | 10 | 9 | 10 | 11 | 10 | 8 | 65 | | 13 | 10 | 10 | 13 | 7 | 9 | 13 | 75 |
| (18) | (29) | (37) | (45) | (4) | (12) | (20) | (28) | | (25) | (43) | (30) | (17) | (4) | (40) | (27) | (14) | |
| | 11 | 7 | 12 | 10 | 13 | 10 | 8 | 71 | | 13 | 8 | 8 | 10 | 11 | 6 | 9 | 65 |
| (19) | (22) | (30) | (38) | (46) | (5) | (13) | (21) | | (26) | (8) | (44) | (31) | (18) | (5) | (41) | (28) | |
| | 13 | 8 | 11 | 9 | 10 | 7 | 5 | 63 | | 8 | 12 | 14 | 11 | 8 | 13 | 9 | 75 |
| (20) | (15) | (23) | (31) | (39) | (47) | (6) | (14) | | (27) | (22) | (9) | (45) | (32) | (19) | (6) | (42) | |
| | 8 | 11 | 12 | 10 | 7 | 13 | 11 | 72 | | 11 | 8 | 14 | 12 | 12 | 10 | 13 | 80 |
| (21) | (8) | (16) | (24) | (32) | (40) | (48) | (7) | | (28) | (36) | (23) | (10) | (46) | (33) | (20) | (7) | |
| | 7 | 13 | 7 | 12 | 11 | 11 | 8 | 69 | | 9 | 11 | 10 | 8 | 14 | 12 | 8 | 72 |
| | | | | | | | | 492 | | | | | | | | | 501 |
| | Replication V | | | | | | | | | Replication VI | | | | | | | |
| (29) | (1) | (30) | (10) | (39) | (19) | (48) | (28) | | (36) | (1) | (23) | (45) | (18) | (40) | (13) | (35) | |
| | 11 | 7 | 10 | 11 | 12 | 13 | 8 | 72 | | 10 | 10 | 12 | 11 | 12 | 6 | 7 | 68 |
| (30) | (22) | (2) | (31) | (11) | (40) | (20) | (49) | | (37) | (29) | (2) | (24) | (46) | (19) | (41) | (14) | |
| | 13 | 11 | 12 | 7 | 9 | 8 | 9 | 69 | | 12 | 12 | 8 | 7 | 11 | 9 | 13 | 72 |
| (31) | (43) | (23) | (3) | (32) | (12) | (41) | (21) | | (38) | (8) | (30) | (3) | (25) | (47) | (20) | (42) | |
| | 14 | 11 | 13 | 7 | 11 | 10 | 9 | 75 | | 9 | 8 | 13 | 11 | 10 | 10 | 12 | 73 |
| (32) | (15) | (44) | (24) | (4) | (33) | (13) | (42) | | (39) | (36) | (9) | (31) | (4) | (26) | (48) | (21) | |
| | 6 | 13 | 7 | 10 | 12 | 8 | 10 | 66 | | 9 | 13 | 15 | 10 | 10 | 13 | 7 | 77 |
| (33) | (36) | (16) | (45) | (25) | (5) | (34) | (14) | | (40) | (15) | (37) | (10) | (32) | (5) | (27) | (49) | |
| | 7 | 15 | 13 | 8 | 11 | 9 | 14 | 77 | | 7 | 9 | 11 | 9 | 10 | 8 | 10 | 64 |
| (34) | (8) | (37) | (17) | (46) | (26) | (6) | (35) | | (41) | (43) | (16) | (38) | (11) | (33) | (6) | (28) | |
| | 10 | 7 | 9 | 7 | 11 | 10 | 7 | 61 | | 9 | 13 | 9 | 9 | 11 | 10 | 10 | 71 |
| (35) | (29) | (9) | (38) | (18) | (47) | (27) | (7) | | (42) | (22) | (44) | (17) | (39) | (12) | (34) | (7) | |
| | 12 | 9 | 8 | 9 | 7 | 9 | 8 | 62 | | 14 | 12 | 11 | 8 | 12 | 9 | 10 | 76 |
| | | | | | | | | 482 | | | | | | | | | 501 |
| | Replication VII | | | | | | | | | Replication VIII | | | | | | | |
| (43) | (1) | (16) | (31) | (46) | (12) | (27) | (42) | | (50) | (1) | (44) | (38) | (32) | (26) | (20) | (14) | |
| | 11 | 10 | 10 | 10 | 12 | 7 | 10 | 70 | | 10 | 13 | 7 | 10 | 9 | 9 | 9 | 67 |
| (44) | (36) | (2) | (17) | (32) | (47) | (13) | (28) | | (51) | (8) | (2) | (45) | (39) | (33) | (27) | (21) | |
| | 8 | 12 | 7 | 10 | 8 | 11 | 12 | 68 | | 8 | 10 | 11 | 9 | 10 | 7 | 9 | 64 |
| (45) | (22) | (37) | (3) | (18) | (33) | (48) | (14) | | (52) | (15) | (9) | (3) | (46) | (40) | (34) | (28) | |
| | 14 | 7 | 15 | 11 | 10 | 12 | 12 | 81 | | 8 | 12 | 11 | 8 | 9 | 9 | 9 | 66 |
| (46) | (8) | (23) | (38) | (4) | (19) | (34) | (49) | | (53) | (22) | (16) | (10) | (4) | (47) | (41) | (35) | |
| | 8 | 13 | 8 | 12 | 11 | 10 | 12 | 74 | | 10 | 9 | 8 | 10 | 8 | 10 | 6 | 61 |
| (47) | (43) | (9) | (24) | (39) | (5) | (20) | (35) | | (54) | (29) | (23) | (17) | (11) | (5) | (48) | (42) | |
| | 13 | 11 | 5 | 11 | 10 | 11 | 6 | 67 | | 15 | 11 | 11 | 7 | 8 | 13 | 13 | 78 |
| (48) | (29) | (44) | (10) | (25) | (40) | (6) | (21) | | (55) | (36) | (30) | (24) | (18) | (12) | (6) | (49) | |
| | 11 | 13 | 9 | 12 | 8 | 10 | 7 | 70 | | 6 | 9 | 8 | 12 | 12 | 11 | 12 | 70 |
| (49) | (15) | (30) | (45) | (11) | (26) | (41) | (7) | | (56) | (43) | (37) | (31) | (25) | (19) | (13) | (7) | |
| | 6 | 10 | 11 | 9 | 7 | 10 | 7 | 60 | | 11 | 8 | 12 | 9 | 12 | 8 | 9 | 69 |
| | | | | | | | | 490 | | | | | | | | | 406 |

### *B. Critical Table Values For The Balanced, Simple And Triple Lattice Designs*

Table 8. Critical table values for the balanced lattice designs.

| $\alpha$ | Experimental Design (k×k) | | | | | |
|---|---|---|---|---|---|---|
| | 3×3 | 4×4 | 5×5 | 7×7 | 8×8 | 9×9 |
| 0.25 | 2.2751 | 2.4262 | 2.5611 | 2.7695 | 2.8517 | 2.9231 |
| 0.10 | 2.7853 | 2.8308 | 2.9230 | 3.0939 | 3.1655 | 3.2284 |
| 0.05 | 3.1405 | 3.1007 | 3.1631 | 3.3089 | 3.3743 | 3.4338 |
| 0.01 | 5.0886 | 3.6700 | 3.6618 | 3.7588 | 3.8135 | 3.8609 |
| 0.001 | 13.3087 | 4.4066 | 4.3034 | 4.3336 | 4.3662 | 4.4042 |

Table 9. Critical table values for the simple lattice designs.

| $\alpha$ | E×perimental Design (k×k) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 3×3 | 4×4 | 5×5 | 6×6 | 7×7 | 8×8 | 9×9 | 10×10 | 12×12 |
| 0.25 | 2.7140 | 2.6684 | 2.7264 | 2.7971 | 2.8668 | 2.9305 | 2.9896 | 3.0429 | 3.1374 |
| 0.10 | 3.7789 | 3.3168 | 3.2366 | 3.2391 | 3.2679 | 3.3035 | 3.3420 | 3.3807 | 3.4543 |
| 0.05 | 4.6933 | 3.7992 | 3.5929 | 3.5421 | 3.5368 | 3.5544 | 3.5804 | 3.6088 | 3.6682 |
| 0.01 | 7.3911 | 4.9533 | 4.3986 | 4.1980 | 4.1176 | 4.0866 | 4.0815 | 4.0877 | 4.1174 |
| 0.001 | 13.4869 | 6.8589 | 5.5596 | 5.0874 | 4.8944 | 4.7884 | 4.7355 | 4.7106 | 4.6985 |

Table 10. Critical table values for the simple lattice designs.

| $\alpha$ | E×perimental Design (k×k) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 3×3 | 4×4 | 5×5 | 6×6 | 7×7 | 8×8 | 9×9 | 10×10 | 12×12 |
| 0.25 | 2.3578 | 2.4961 | 2.6173 | 2.7200 | 2.8077 | 2.8839 | 2.9514 | 3.0111 | 3.1133 |
| 0.10 | 2.9586 | 2.9637 | 3.0266 | 3.0954 | 3.1611 | 3.2208 | 3.2754 | 3.2556 | 3.4138 |
| 0.05 | 3.4002 | 3.2869 | 3.3020 | 3.3458 | 3.3972 | 3.4467 | 3.4926 | 3.5369 | 3.6172 |
| 0.01 | 4.4253 | 3.9976 | 3.8894 | 3.8790 | 3.8919 | 3.9191 | 3.9509 | 3.9817 | 4.0433 |
| 0.001 | 6.0289 | 4.9666 | 4.6716 | 4.5706 | 4.5371 | 4.5312 | 4.5385 | 4.5519 | 4.5900 |

### *C. The R Code Of The 'ANOMLattice' Function*

```
ANOMLattice=function(block,treat,rep,response,alpha){
   nr=nlevels(factor(rep))
   k=nlevels(factor(block))/nr
   corr=matrix(NA,k^2,k^2)
   for (i in 1:k^2) {
      for(j in 1:k^2){
         corr[i,j]=1
         if (i!=j){corr[i,j]=-(1/((k^2)-1))}}}
y=response
sink("NUL")
output=PBIB.test(block,treat,rep,y,k=k,method = "VC")
sink()
nr=output$parameters$r
Eb=output$ANOVA$`Mean Sq`[3]
Ee=output$ANOVA$`Mean Sq`[4]

if(nr==(k+1)){
Mu=(Eb-Ee)/(k*k*Eb)
Ee1=Ee*(1+k*Mu)
title="ANOM Chart for Balanced Lattice"
v=(k-1)*((k^2)-1)}
else{Mu=(Eb-Ee)/(k*(nr-1)*Eb)
   Ee1=Ee*(1+(nr*k*Mu)/(k+1))
title="ANOM Chart for Partially Balanced Lattice"
v=(k-1)*(nr*k-k-1)}
Gmean=output$statistics$Mean
h=qmvt(1-alpha,df=v,corr=corr,tail = "both")$quantile
LDL=Gmean-h*sqrt(Ee1)*sqrt(((k^2)-1)/((k^2)*nr))
UDL=Gmean+h*sqrt(Ee1)*sqrt(((k^2)-1)/((k^2)*nr))
labeludl=paste('UDL=',format(round(UDL,2),nsmall=2))
labelavg=paste('Avg.=',format(round(Gmean,2),nsmall=2))
labelldl=paste('LDL=',format(round(LDL,2),nsmall=2))
a1=expression(alpha)
a2=paste("=",format(round(alpha,2),nsmall=2))
Xmean=output$means[,2]
color=NULL
label=NULL
alig=NULL
shape=NULL
XX=as.character(rep(1:k^2))
XX=factor(XX,levels = XX)
YY=Xmean[1:k^2]
ZZ=data.frame(XX,YY)
for (i in 1:k^2) {
   if ((Xmean[i]>=UDL)||(Xmean[i]<=LDL)){
      color[i]='red'
      shape[i]=8
   }else{color[i]='black';shape[i]=19}

   if (Xmean[i]>mean(Xmean)){alig[i]=-0.7}
   if (Xmean[i]<mean(Xmean)){alig[i]=1.5}
}
font='Times'
chart=ggplot(ZZ, aes(x=XX, y=Xmean)) +
   ylab("Adjusted Average")+
   xlab(substitute(treat))+
   geom_segment(aes(xend=XX), yend=Gmean, colour="gray50") +
   geom_hline(yintercept = c(LDL,UDL,Gmean),
```

```
            colour=c('blue','blue','black'))+
   scale_y_continuous(sec.axis = sec_axis(~ ., breaks = c(LDL,Gmean,UDL),
                    labels = c(labelldl,labelavg,labeludl)))+
   geom_point(shape=shape,size=1,color= color)+
   geom_text(size=3,aes(label=XX,family=font),vjust=alig)+
   theme_bw() +
   theme(panel.grid.major.y = element_blank(), # No horizontal grid lines
        legend.position=c(1, 0.55), # Put legend inside plot area
        legend.justification=c(1, 0.5),
        plot.margin = unit(c(0.2,0.2,0.2,0.2), "cm"),
        axis.title = element_text(family = font),
        axis.text.x = element_text(colour='black',size = 6,
                                   hjust = .5,vjust = .5,
                                   family = font),
        axis.text.y = element_text(colour='black',
                                   hjust = .5,vjust = .5,
                                   family = font))+
   ggtitle(label = paste(title,sep=" ","\u03b1",a2))+
   theme(plot.title = element_text(hjust=0.5,
      family = font))+
   coord_cartesian(clip = "off")
return(chart)
}
```

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# Effect of Kohlrabi Peel Powder as a Dietary Fibre Enrichment on Technological, Nutritional, and Sensory Properties of White Bread

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ABSTRACT

The addition of dietary fibres (DF) obtained from by-products into bread is an attractive way to enhance consumers' fibre consumption while simultaneously reducing waste from by-products. This study aimed to examine the viability of replacing kohlrabi peel powder (KPP) into white bread at levels ranging from 0% to 12%. The technological and nutritional characteristics of white breads were evaluated, and sensory analysis was conducted. The specific volume values of the samples ranged from 1.486 to 1.861 mL/g. The findings showed that the specific volume of the white bread samples decreased when KPP was used at concentrations higher than 3%. The sample coded N4 (9%) exhibited higher moisture levels in both the crumb and crust, compared to the all bread samples ($p<0.05$). The nutritional features of the bread samples varied as follows: ash content ranged from 0.573% to 0.588%, protein from 6.460% to 7.998%, fat from 3.200% to 4.200%, total DF from 0.250% to 3.214%, total carbohydrate content from 51.963% to 69.272%, and energy levels from 211.7 to 324.0 kcal. The sample coded N3 (6%) was approved by the panelists, however the other samples with a greater quantity of KBB were not favored. SEM images showed the gluten structure in the enriched bread samples coded N2, N3 and N4 were constituted by fibrous components that established a more open network. The study's results indicated that elevated amounts of KPP might enhance the DF content of white bread; however, consumers do not favor higher fibre content in the bread samples. Utilization of 6% KPP could enhance the technological, nutritional, and sensory characteristics of white bread.

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## Introduction

Obesity and associated disorders, including cardiovascular diseases, diabetes, and hypertension, have been increasing in recent years in both industrialized and low to middle-income countries. If this trend continues, almost 38% of the global adult population is projected to be overweight and 20% obese by 2030 (Kelly et al., 2008). Obesity is rising due to factors such as altered dietary habits and decreased physical activity resulting from globalization. Analysis of consumed products reveals a decline in the intake of fibre, whole grains, fruits, and vegetables, contrasted by an increase in the consumption of processed carbs and saturated fats (Popkin, 2006). Individuals who consume inadequate amounts of fruits and vegetables are prone to poor intake of several micronutrients, including folic acid and vitamin C, hence elevating the chances of DNA damage, such as cancer and other degenerative disorders. Moreover, deficiencies in micronutrients not predominantly sourced from fruits and vegetables, including zinc, iron, and vitamins E, niacin, B6, and B12, also elevate the risk of DNA damage (Maietti et al., 2021).

Despite the growing interest in whole grain breads for their superior fibre content, white bread remains the most prevalent variety globally. Among this popularity, fibre concentrates or high-fibre content products, such as banana flour, can be included into bread production (Agama-Acevedo et al., 2019). The primary factor is to enhance the dietary fibre content of breads by reformulating them with fibre-rich components while little altering the sensory attributes of the finished product.

Functional foods may be produced by using the by-products from fruits and vegetables as natural raw materials that are high in antioxidants and dietary fibre. A fruit or vegetable's main components include its flesh, peels, seeds, and stems, which are high in valuable chemicals, especially dietary fibres (DF), with a balanced ratio of soluble and insoluble fibre fractions as well as its nutritional profile, colour, texture, and volume characteristics (Torbica et al., 2019). The growing interest in transforming waste into value, particularly through the production of value-added products from organic waste, has led to an increase in study within this field. There have been previous studies on the use of chestnut

peels for breads (Alinovi et al., 2022; Mironeasa & Mironeasa, 2019), sausages (Choi et al., 2010), and cookies ( Joo & Choi, 2012) in an effort to decrease food waste and to enhance a variety of food. Also banana peel was used in the researches with same respect (Akhter et al., 2024). This study will be the first to evaluate Kohlrabi peel powder (KPP) as a bread ingredient and will serve as a good example of utilizing DF-rich food waste in food production.

Kohlrabi (*Brassica oleracea* var. gongylodes) is a bulbous, onion-like vegetable that is part of the Cruciferae (*Brassicaceae*) family. It grows in temperate nations and certain subtropical areas. Türkiye ranks as one of the significant cabbage-producing nations globally, with an output of roughly 500,000 tons. Vegetable species like cauliflower, Brussels sprouts, broccoli, green cabbage, and red cabbage, which are part of the *Brassicaceae* family, are extensively cultivated in our country. Kohlrabi, a member of the same family, is a cool-weather vegetable characterized by a fleshy, turnip-like stem that develops above the ground through nutrient storage (Yildirim et al., 2017). Kohlrabi is strongly advised for dietary inclusion due to its nutritional advantages and low caloric content (Marcinkowska et al., 2021). It is an abundant source of vitamin C, vitamin B6, fibre, and potassium (K) (Golob et al., 2020). Kohlrabi possesses anti-inflammatory, antibacterial, and antioxidant properties. Oxidative stress is diminished, cancer is inhibited, asthma, cancer, and Alzheimer's disease are prevented, Type II diabetes is managed, and cardiovascular health is enhanced (Marcinkowska et al., 2021).

Kohlrabi peel is less frequently used in research, while having equally amazing qualities as the flesh. A study that looked at kohlrabi peel found that it had higher levels of organic acid, vitamin C, minerals, essential amino acids, and total amino acids. It was also an excellent source of free sugar and unsaturated fatty acids (Seon-Suk Cha et al., 2013). The primary and secondary metabolites, comprising organic acids, amino acids, sugars, and an amine from Kohlrabi peel, were examined in a separate study (Park et al., 2017). They stated that kohlrabi peel is a valuable source of primary metabolites, which impart significant nutritional characteristics, and secondary metabolites, which are utilized as medicinal agents for human health (excluding anthocyanins).

Dietary fibre (DF) constitutes the indigestible component of fruits, vegetables, and grains. Fibre facilitates the softening of feces, hence avoiding constipation. DF is known to diminish the digestion rate of starchy foods owing to its significant water absorption capacity and enhanced food viscosity, leading to a reduced blood sugar response post-ingestion (Angioloni & Collar, 2011). Bread and bakery products are the primary sources of DF in overall food intake. High-fibre bread constitutes a cereal-based diet and is more effective than a low-carbohydrate diabetic diet in managing maturity-onset diabetes (Babiker, 2013). The incorporation of DF in bread production is attributable to their technological attributes. The water retention capacity of DFs, which inhibits bread staling and prolongs product shelf life, might be used as an example (Kurek, 2015). Furthermore, the incorporation of fibre enhances and modifies the textural and sensory attributes, as well as the shelf life of food products, owing to its gelling capabilities, fat mimicry, and texturizing and thickening effects (Sabanis et al., 2009). Numerous studies have demonstrated the possible enhancement of wheat-based cereal products through the incorporation of dietary fibre (Gómez et al., 2003; Rios et al., 2020; Rosell et al., 2006; Sabanis et al., 2009). Research on the conversion of food processing by-products into various valued goods, owing to their vital nutritional components such as fibre, vitamins, and minerals, is on the rise (Babiker, 2013). The choice of DFs in bread production is crucial. Meticulous selection of dietary fibres (DF) possessing suitable physicochemical characteristics that avert irreversible degradation of the protein matrix is essential for producing doughs with optimal workability, resulting in sensory-acceptable breads (Angioloni & Collar, 2011).

The purposes of this study were i) to develop a novel, healthy, and desirable bread recipe that is enriched with KPP in various amounts, and ii) to provide a practical example for assessing peel powder, which is a waste or by-product.

## Materials and Methods

### *Material*

The kohlrabi used in the study was purchased from a greengrocer in Sivas, Türkiye. The other ingredients of the breads given in Table 1 were obtained from local markets.

### *Manufacture of Kohlrabi Peel Powder*

Kohlrabi was manually peeled, cleaned of any wounds and damaged regions on the skin, and dehydrated in an oven at 55°C until it had attained a moisture content of 7%. The peels were pulverized into flour by a food processor and utilized in bread manufacturing after being filtered through a 150 μm sieve.

### *Preparation of Bread Dough*

The components and their respective ratios to be incorporated into the bread dough are presented in Table 1. The dough was produced following the method outlined by (Angioloni & Collar, 2011). All ingredients, except for oil, were added to the dough mixer (KitchenAid, USA) and mixed for 15 min. Thereafter, oil was added to the mixture and blended for an additional 5 min.

Table 1. Formulations of breads

| Sample | KP (%) | Formulation (g) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Flour | Yeast | Sugar | Salt | Water | Sunflower oil | Total |
| N-1 | 0 | 120 | 1.25 | 5 | 1.25 | 86 | 1.25 | 215 |
| N-2 | 3 | 117 | 1.25 | 5 | 1.25 | 86 | 1.25 | 215 |
| N-3 | 6 | 114 | 1.25 | 5 | 1.25 | 86 | 1.25 | 215 |
| N-4 | 9 | 111 | 1.25 | 5 | 1.25 | 86 | 1.25 | 215 |
| N-5 | 12 | 108 | 1.25 | 5 | 1.25 | 86 | 1.25 | 215 |

The container was sealed with stretch film, and the dough underwent fermentation for 2 hours in an incubator (Membert, Germany) at 28°C. At the end of the period, the dough (500 g) was transferred to a rectangle loaf pan and permitted to ferment under the same incubation conditions for 30 min. Upon completion of the allotted time, the dough was baked at 190°C for 30 min.

***Evaluation Technological Properties of Breads***

The weight (g) of the breads was measured, and subsequently their volume (ml) was estimated using rapeseed displacement recommended by AACC method 10-05.01 (2000). The specific volume (ml/g) was determined by dividing the volume by the weight.

The moisture content of crumb and crust was measured by using an infrared moisture analyser (Shimadzu, MOC63U).

The crust colour of baked samples were measured using the Chroma Meter CR-400 (Konica Minolta Co., Ltd., Osaka, Japan) in the CIE $L^*a^*b^*$ colour space. $L^*$ indicates whiteness (value 100) or blackness (value 0), *a* indicates redness (positive value) or greenness (negative value), and b* indicates yellowness (positive value) or blueness (negative value).* Colour was assessed at five spots on the bread crust after 2 h after baking.

***Evaluation of the Nutritional Composition of Breads***

The procedures established by the Association Official of Analytical Chemists (AOAC, 2000) for moisture (925.09), ash content (923.03), total lipid content (922.06), and protein content (920.87) were employed to ascertain the chemical composition of breads. The total carbohydrates (TC) were determined by the difference, as per the subsequent equation:

$$TC = [100 - (\text{moisture} + \text{ash} + \text{protein} + \text{fat})] \quad (1)$$

with data reported as g/100 g in wet weight.

The energy levels (kcal) were calculated by multiplying the amount of macronutrients by their corresponding conversion factors (4 kcal/g for protein, 9 kcal/g for fat, and 4 kcal/g for total carbohydrates) (Krupa-Kozak et al., 2021).

The total DF level of the samples was quantified using an enzymatic-gravimetric analysis with a commercial assay kit (K-TDFR-200A, Megazyme International Ireland Limited, Bray, Ireland). The procedure was executed following the AOAC (2000) Method 985.29 enzymatic-gravimetric method as outlined in the kit instructions. This method entails the hydrolysis of 1 g of dried, crushed, and sieved bread sample (0.5 mm mesh) using α-amylase, protease, and amyloglucosidase, succeeded by ethanol precipitation to get a residue for protein and ash analysis. The comprehensive DF calculations for the samples were performed with MegaCalc™ Excel®, which can be downloaded from the Megazyme website (www.megazyme.com) where the product is featured.

All nutritional analysis were conducted in duplicate.

***Sensory Analysis***

A total of forty panellists, consisting of both males and females aged between 25 and 55, were selected to evaluate the sensory characteristics of the bread samples. Following a concise briefing, participants became knowledgeable about the breads they had evaluated. The participants were instructed to assess each bread based on its colour, apperance, flavor, aroma, taste, and overall acceptability using a five-point hedonic scale. This scale, ranges from 1 (indicating extreme dislike) to 5 (indicating extreme liking) for each sensory characteristic. Breads were deemed satisfactory if their average ratings for general acceptance exceeded 3 (indicating a neutral opinion).

***SEM Analysis***

The pore structure of the bread samples was assessed using scanning electron microscopy (SEM) (TESCAN MIRA3 XMU, Brno – Czech Republic). The samples were attached to circular aluminum studs using double-sided sticky carbon tape and subsequently gold-plated for 100 seconds. The examination was conducted at room temperature using SEM mode at 10 kV voltage. The magnification ratio for the bread samples was set at x1000. These specific micrographs were selected because to their superior clarity and sharpness.

***Statistical Analysis***

The research investigated the impact of different levels of KPP as a fibre addition on the technological and nutritional properties of bread. The reported results are the mean of two replicates. The data were analysed using the analysis of variance technique (ANOVA), and the difference between the samples was assessed by the Fisher LSD method. Statistical significance was assessed at a *p*-value of less than 0.05. The statistical analyses were conducted using the MiniTab (20th Edition) software (MiniTab, State College, Pennsylvania, USA).

**Results and Discussions**

***Technological Properties of Breads***

Specific volume is a critical technological attribute since it affects both the appearance and sensory acceptance of bread. Table 2 indicates that the specific volume values of the white bread samples varied from 1.2488 to 1.861 mL/g. ANOVA test findings showed that the sample coded N1 (control) exhibited the maximum specific volume, also no statistically significant difference observed with the sample coded N2 ($p$ <0.05). The results indicated that the usage of KPP at concentrations over 3% resulted in a decrease in the quality of the white bread samples. Including fibre and fibre-rich sources into gluten-free dough and bread enhances the fluidity of liquid batter. Consequently, it enhances the gas retention ability during proofing and baking, thereby increasing the specific volume of the bread. Nonetheless, enhancing the consistency of a more rigid dough inhibits its rise and diminishes loaf volume (Begum et al., 2020). It has been established that the incorporation of higher level of fibre decreases the volume of bread due to the dilution of gluten in the flour mixtures (Koletta et al., 2014).

Moisture levels quantifies the freshness of bread. The enriched bread samples' crumb moisture level varied from 33.61% to 41.50%, and the control sample was 37.06%. The crust moisture level of the enhanced bread varied from 19.83% to 26.58%, whereas the control samples measured 23.40%. The highest crumb and crust moisture levels detected in the sample coded N4 among the bread samples

($p$ <0.05). Eshak (2016) obtained identical results in enriched wheat bread utilizing banana powder, and they reported that the incorporation of banana fibre into bread enhances its water absorption, as it is recognized as effective water binders, resulting in elevated moisture content in the bread. Nonetheless, the KPP levels both below and above 9% resulted in a reduction of moisture levels in the crumb and crust. A similar trend was noted by Begum et al. (2020), indicating that the incorporation of a DF source leads to an increase in moisture content up to a certain percentage of DF consumption; beyond this point, moisture content begins to decline. This attribute arises from gluten production and may lead to a fragile protein network in the dough. As indicated in a study, the incorporation of 4 g per 100 g of DF influences gluten production and may lead to a compromised protein network in the dough (Rosell et al., 2001).

Colour is an essential characteristic of bakery products, as it, together with texture and flavor, influences consumer acceptance. The colour is dependent on the properties of the dough and the baking conditions. The findings from the investigation of crust colour are displayed in Table 2. The $L^*$, $a^*$, and $b^*$ values of white breads samples, the values ranged from 46.700 to 68.160, 10.00 to 17.117, and 28.373 to 35.400, respectively. For the $L^*$, the sample coded N1 (control) had the lowest value, in contrast N5 (12%) had the highest value ($p$ <0.05). The $L*$ values, which denote lightness, rise with increased KPP concentration, leading to lighter crumbs. On the other hand, $a^*$ and $b^*$ values of bread crumbs were changed depend on the concentration of KPP used in the breads. The $a^*$ values of the samples increased until a concentration of 6%, after which they declined with increasing concentrations. On the other hand, the greatest $b^*$ value was recorded in the sample designated N2 (3% KPP). Same trend was observed by Sławińska et al. (2022) in the wheat bread samples supplemented with freeze-dried white and brown button mushrooms (2.5% and 5%). Djordjević et al. (2019) reported that the crust colouration is primarily due to Maillard and caramelization reactions occurring at elevated temperatures, likely caused by an increased concentration of soluble sugars and free amino acids in the enriched breads, which influences the $L^*$ and $a^*$ values of the breads.

***Nutritional Properties of Breads***

The ash levels in the white bread samples ranged from 0.573% to 0.588% (Table 3). The incorporation of KPP into the white bread dough led to an important increase in ash content, seen in samples N2 and N3 ($p<0.05$). The same results was observed in the study (Akhter et al., 2024) who searched enrichment of wheat bread with banana peel powder. The increase in ash level indicates the presence of minerals supplied by KPP in the enriched breads.

The protein levels ranged from 6.460% to 7.998%, whereas the fat content varied from 3.20% to 4.2% in the bread samples. No statistically significant difference was seen between the control and enriched bread samples for both contents ($p>0.05$). TC content of the samples varied from 51.963% to 69.272%. The addition of KPP to the samples resulted in significantly higher total carbohydrate levels, with the highest total carbohydrate value recorded in sample N3 (6% KPP) ($p<0.05$). The energy level of the samples was detected between 211.7 kcal and 324.0 kcal. The sample coded N5 has the lowest energy value between the samples ($p<0.05$), there was no significantly importance difference between control and enriched breads ($p>0.05$). This was due to the sample coded N5 has the lowest flour content. The elevated carbohydrate content enhances the baked qualities, including texture and structure, which are desirable in bakery products (Ezeocha et al., 2022).

The level of DF in enriched bread samples were measured between 0.347% and 3.214% (Table 3), whereas the control sample had only 0.25% DF content. Also it was detected that the DF content of enriched bread samples exhibited a statistically significant increase with greater KPP addition. The coded sample N5 had the highest KPP at 12%, resulting in an almost 12-fold increase in the DF of the white bread sample. Numerous studies on bread enrichment yielded findings consistent with our research. Gadallah (2017) manufactured wheat flour with pomegranate peel powder at varying ratios of 5%, 10%, 15%, and 20%, and detected a maximum increase of 6.2-fold in the samples. Omran et al. (2020) manufactured wheat flour with yellow onion peel powder (OPP) at concentrations of 1, 2, 3, 4, and 5%, and observed a maximum 3.2-fold increase, García et al. (2023) used 10% pomegranate peel powder, resulting in an approximate 3.9-fold increase, Akhter et al. (2024) incorporated 10% banana peel powder into wheat bread, resulting in an approximate 11.28-fold increase, in total DF levels. A product may be included as a "source of fibre" if it contains at least 3 g of fibre per 100 g or at least 1.5 g of fibre per 100 kcal, according to the declaration of Reg. (EC) No. 1924 (2006), which allows us to declare enriched breads coded N4 and N5 as sources of fibre.

Sensory analysis is an important criterion for assessing quality in the development of new products and for meeting consumer requirements (Chisa & Jonah, 2023). The sensory properties of the bread samples are shown in Figure 1.

Table 2 Technological properties of bread samples

| Sample code | Spesific Volume (mL/g) | Crumb moisture (%) | Crust Moisture (%) | $L^*$ | $a^*$ | $b^*$ |
|---|---|---|---|---|---|---|
| N1 | 1.8610±0.06 a | 37.060±0.71 b | 23.400±0.71 b | 46.700±0,678 d | 11.577±0,499 c | 28.373±0.755 c |
| N2 | 1.7318±0.04 ab | 36.162±0.00 b | 22.230±0.71 bc | 57,697±1,057 c | 16,533±0,127 a | 35.40±2.19 a |
| N3 | 1.6738±0.04 b | 33.610±0.71 c | 19.830±0.71 c | 64.990±01.356b | 17,117±0,842 a | 31.613±0.402 b |
| N4 | 1.4863±0.01 c | 41.510±0.71 a | 26.575±0.73 a | 65.793±1.542 b | 12,970±0,576 b | 30.897±0.440 b |
| N5 | 1.2488±0.04 d | 37.920±0.71 b | 24.370±0.71 ab | 68,160±1.163 a | 10,00±0,530 d | 30.393±1.442 bc |

Values are expressed as mean ± standard deviation from five replicates for $L^*$, $a^*$, $b^*$ measurements, and from two replicates for specific volume and moisture analysis. N1: Control, white bread with no addition; N2: White bread with 3% Kohlrabi peel powder addition, N3: White bread with 6% Kohlrabi peel powder addition, N4: White bread with 9% Kohlrabi peel powder addition, N5: White bread with 12% Kohlrabi peel powder addition

Table 3 Nutritional properties of bread samples

| Sample code | Ash (%) | Protein (%) | Lipid (%) | Dietary Fibre (%) | Carbonhydrate (%) | Energy (kcal) |
|---|---|---|---|---|---|---|
| N1 | 0.585±0.01 b | 7.998±1.25 a | 3.800±0.85 a | 0.250±0.006 c | 51.963±0.80 c | 274.04±9.41 ab |
| N2 | 0.647±0.02 a | 7.337±0.08 a | 3.400±0.57 a | 0.347±0.074 c | 52.455±0.66 c | 248.8±32.5 ab |
| N3 | 0.643±0.01 a | 6.555±0.25 a | 4.200±0.28 a | 1.395±0.020 b | 69.272±0.52 a | 324.0±25.6 a |
| N4 | 0.573±0.01 b | 6.946±0.17 a | 3.700±0.42 a | 2.509±0.618 a | 61.690±0.60 b | 292.5±23.7 ab |
| N5 | 0.588±0.01 b | 6.460±0.02 a | 3.200±0.28 a | 3.214±0.152 a | 42.641±0.30 d | 211.7±20.5 b |

All values are mean ± standard deviation of two replicates. a-d Means within a column with different letters are significantly different ($p<0.05$). N1: Control, white bread with no addition; N2: White bread with 3% Kohlrabi peel powder addition, N3: White bread with 6% Kohlrabi peel powder addition, N4: White bread with 9% Kohlrabi peel powder addition, N5: White bread with 12% Kohlrabi peel powder addition.

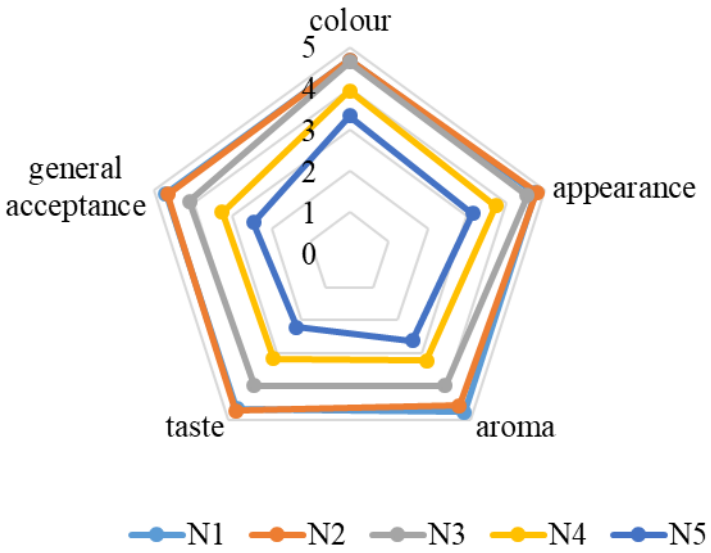


Figure 1 Sensory analysis results of bread samples

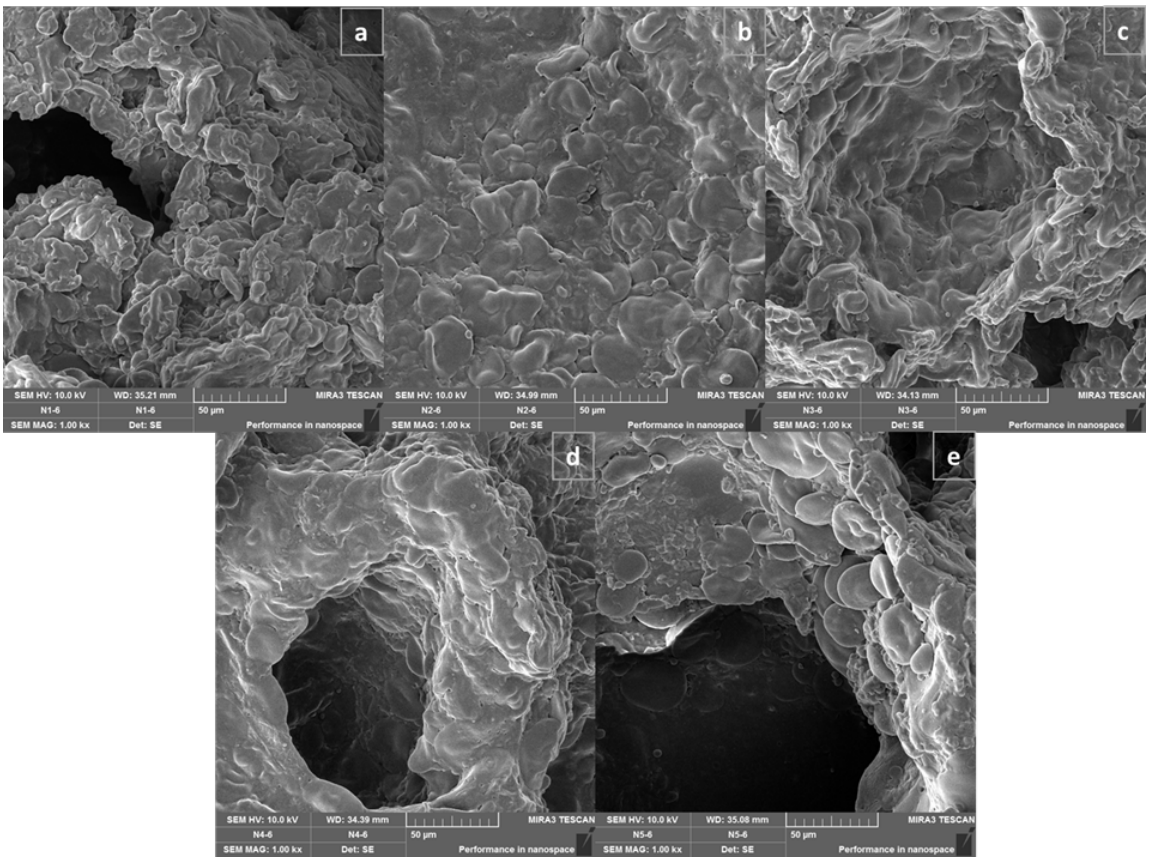


Figure 2. Scanning electron microscope images of bread samples. (a) control sample (b) sample coded N2 (c) sample coded N3 (d) sample coded N4 (e) sample coded N5

A radar map was created to visually represent the sensory profiles and variations among the samples for simple identification based on the results. Crust colour of the samples ranged from 3.35-4.7, aroma score of the samples ranged from 2.6-4.75, taste score of the samples ranged from 2.2-4.7, apperance score of the samples ranged fro 3.15-4.8, while the overall acceptability score of the samples ranged from 2.45-4.7. N1 (control) and N2 (3%) were the most favored bread samples. N3 (6%) was approved by the panelist, however the other samples with a greater quantity of KBB were not favored. The elevated fibre content in these samples disturbed the panelists.

The structure of the bread coded N1 (control) consisted of flattened and folded starch granules densely integrated inside a gluten matrix (Fig. 2a). The gluten structure in the enriched bread samples coded N2, N3 and N4 were constituted by fibrous components that established a more open network (Fig 2b,c,d). It was clearly seen that starch granules partially gelatinized in the enriched bread samples coded N2, N3, and N4. The microstructure of enrichment bread coded N5 exhibited a uniform distribution of big granules inside the gluten matrix. Significant structural alterations, manifested as voids, fissures, and fragmentation of starch granules, are evident in the microstructure of bread supplemented with KPP (Fig. 2e).

## Conclusion

The application of 6% KPP may improve the technological, nutritional, and sensory attributes of white bread. Based on these results, it can be inferred that the application of KPP may be viable for producing a fibre-enriched product that contributes to the advancement of more sustainable and efficient plant-based consumption. To conclude the assessment of this ingredient, additional research must be conducted to evaluate its impact on doughs with various dietary fibre sources, sensory acceptance, and potential variations in shelf life compared to standard and enriched breads.

## Declarations

### *Ethical Approval Certificate*

Before conducting sensory analysis, a decision was made by the ethics committee of the Sivas Cumhuriyet University Social Sciences Institute Scientific Research Proposal Ethics Evaluation Board (decision No. 2023/15 dated 25.12.2023).

### *Author Contribution Statement*

Please indicate how each author contributed to this work and at what stage. For example:

İrem Bilge TEK, and Suna Dilara AKTAS: Project administration, data collection, investigation, and formal analysis.

Hatice Aybuke KARAOGLAN: Supervision, conceptualization, methodology, writing, review and editing.

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### *Conflict of Interest*

The authors declare no conflict of interest.

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# Catalase, Superoxide Dismutase and Malondialdehyde Levels in Different Rambutan Fruit Extracts

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ABSTRACT

Vegetables, fruits and herbs are the main elements of a balanced diet. Rambutan is one of the tropical fruit species of *Nephelium lappaceum.* As in many different regions around the world, the tropical fruit Rambutan grows in the Mediterranean region of Turkey, especially in Antalya. There are more than 200 varieties of Rambutan, which is grown and consumed in tropical and humid geography. This fruit, which can be consumed both fresh and dried, has a slightly sour and predominantly sweet taste. This is the first study, we aimed to investigate the *in vitro* antioxidant capacity and oxidative stress in different solvents of fresh Rambutan fruit extracts and to storage conditions. Fresh Rambutan fruit supplied from Antalya province was used. Fresh Rambutan fruit was extracted and homogenized with 1.15% KCl (potassium chloride), 0.9% NaCl (sodium chloride) and 0.1 M (Molar) phosphate buffer. Antioxidant enzyme activity MDA (malondialdehyde), SOD (superoxide dismutase) and CAT (catalase) levels, which are indicators of oxidative stress, were measured in these plant homogenates. Furthermore, the storage conditions of Rambutan fruit extracts were investigated on 1th, 3th, 5th, 7th, 15th and 30 days at +4°C, -20°C and -70°C. MDA, SOD and CAT levels were measured as spectrophotometrically in these homogenates. We showed that the highest antioxidant capacity and the lowest MDA levels were found at fruit extracts with %1.15 KCI. However, the lowest antioxidant capacity and highest MDA levels, were found at fruit extract with phosphate buffer. Moreover, it was observed that Rambutan fruit maintained its activity for approximate 1-2 days at +4°C, 10 days at -20°C and 15 days at -70°C. Our results showed that Rambutan fruit has high antioxidant power and is a food with development potential.

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# Farklı Rambutan Meyve Ekstraktlarında Katalaz, Superoksit Dismutaz ve Malondialdehit Düzeyleri

MAKALE BİLGİSİ

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*Anahtar Kelimeler:*
*Nephelium lappaceum*
Saklama koşulları
Oksidatif stres biyobelirteçleri
Antioksidan kapasite
Enzim aktiviteleri

ÖZ

Sebzeler, meyveler ve otlar dengeli beslenmenin ana unsurlarıdır. Rambutan, *Nephelium lappaceum* tropical meyve türlerinden biridir. Dünyadaki bir çok değişik bölgelerde olduğu gibi Türkiye'de de Akdeniz bölgesinde özellikle de Antalya'da tropikal bir meyve olan Rambutan yetişmektedir. Tropik ve nemli coğrafyada yetiştirilip tüketilen Rambutanın 200'den fazla çeşiti vardır. Hem taze hem de kuru olarak tüketilmesi mümkün olan bu meyve, hafif ekşi ve ağırlıklı tatlı bir tada sahiptir. Bu ilk çalışma olup, taze Rambutan meyve ekstraktlarının farklı solventlerinde ve saklama koşullarında *in vitro* antioksidan kapasiteyi ve oksidatif stresi araştırmak amaçlandı. Antalya ilinden temin edilen taze Rambutan meyvesi kullanıldı. Taze Rambutan meyvesi %1,15 KCl (potasyum klorür), %0,9 NaCl (sodyum klorür) ve 0,1 M (Molar) fosfat tamponu ile ekstrakte edilip homojenleştirildi. Bu bitki homojenatlarında oksidatif stresin göstergesi olan antioksidan enzim aktivitesi MDA (malondialdehit), SOD (süperoksit dismutaz) ve CAT (katalaz) seviyeleri ölçüldü. Ayrıca rambutan meyve ekstraktlarının depolama koşulları 1., 3., 5., 7., 15. ve 30. günlerde +4°C,-20°C ve -70°C'de incelendi. MDA, SOD ve CAT düzeyleri bu homojenatlarda spektrofotometrik olarak ölçüldü. En yüksek antioksidan kapasitenin ve en düşük MDA düzeyinin %1,15 KCI içeren meyve ekstraktında bulundu. Ancak en düşük antioksidan kapasite ve en yüksek MDA düzeyi, fosfat tamponlu meyve ekstraktında bulundu. Ayrıca Rambutan meyvesinin +4°C'de yaklaşık 1-2 gün, -20°C'de yaklaşık 10 gün ve -70°C'de yaklaşık 15 gün boyunca aktivitesini koruduğu gözlemlendi. Sonuçlar, Rambutan meyvesinin yüksek düzeyde antioksidan güçte olup geliştirilme potansiyelinde bir gıda olduğunu göstermiştir.

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## Giriş

Sapindaceae familyasında bulunan *Nephelium lappaceum;* Rambutan ya da tüylü litchi adıyla bilinmektedir. Malezya'ya özgü bir bitkidir. Fakat artık Brezilya'da ve dünyanın birçok bölgesinde de bulunur (Oliveira ve ark., 2023). Tropikal bir meyve olan Rambutan meyveleri genellikle kırmızı renkli, yenilebilir, esnek tüylü, dikenli yapıda, kösele cilt dokusu formunda, küresel ve oval şekillidir (Afzaal ve ark., 2023). Egzotik bir meyve olan Rambutan Güneydoğu Asya'ya özgüdür (Hernández-Hernández ve ark., 2019). Rambutan meyvesi geleneksel tıp alanında giardiasis, cilt hastalıkları, ateş, ishal, dizanteri ve karın ağrısı tedavilerinde kullanılır. Antidiyabetik, antelmintik ve hazımsızlıkta gaz giderici aktivitelere de sahiptir (Oliveira ve ark., 2023). Rambutan meyvesi, önemli yapıdaki biyoaktif ve besinsel bileşimleri ile antidiyabetik, antikanser, antihiperglisemik, antiobezite, antihiperkolesterolemik, antimikrobiyal ve antialerjik gibi iyileştirici etkiler gösterir (Afzaal ve ark., 2023).

Fitokimyasal taramalar ve geliştirilmiş araştırmalar, Rambutan meyvesinin fonksiyonel gıdaların ve ilaçların hazırlanmalarında uygun bir seçenek olarak görülmektedir (Afzaal ve ark., 2023). Doğal antioksidanların serbest radikalleri temizleme yeteneği vardır. Bu etki de cilt yaşlanmasının gecikmesine, aterosklerozun ve kan basıncının azalmasına, beyin fonksiyon bozukluklarının, kanserin ve diyabetin engellenmesine ve antiinflamatuar etkilerin hafiflemesine sebep olur. Metal şelasyonu, serbest radikallerin temizlenmesi ve lipit peroksidasyonu inhibisyonu doğal gıda ürünlerinin antioksidan aktivitelerini inceleyen sistemler olarak geniş düzeyde araştırılmıştır (Bartsch ve Nair, 2006). Rambutan meyvesi, tohumları ve posası üzerine yapılan incelemeler bu meyvenin bir dizi biyoaktif bileşen bulundurduğunu ve ticari açıdan önemli bir meyve olduğunu kanıtladı (Afzaal ve ark., 2023). Chigurupati ve ark. (2019)'nın Rambutandaki besinsel bileşim üzerinde çalışmaları mevcuttur. Bu çalışma sonucunda rutindeki eşdeğerler ve gallik asit bakımından toplamda fenolik içerik ve flavonoit içeriğe rastlamışlar (Chigurupati ve ark., 2019). Yapılan incelemeler sonucunda Rambutanın kabuk içeriğinin de saponinlerce zengin olduğu tespit edilmiştir (Önel ve ark., 2021). Bu meyvenin her bölgesinde hidrosiyanik asit, saponin, oksalat, alkaloitler, tanenler, fitatlar ve fenoller bazında bileşenler saptanmıştır (Hernández-Hernández ve ark., 2019). Rambutan; mineraller, diyet lifleri, vitaminler ve antioksidanlar bakımından yüksek içeriğe sahiptir. Rambutan meyvesinde niasin, tiamin, riboflavin ve askorbik asit farklı düzeylerde mevcuttur. Ayrıca Rambutan meyvesinin hepatoprotektif ve kardiyoprotektif özelliklerde olduğu saptanmıştır. (Afzaal ve ark., 2023).

Geleneksel yöntemler genel olarak gayet uzun ekstraksiyon süresi ve düşük ekstraktif verimlilikleriyle karakterizedir. Bunun başlıca sebebi ise öncelikle örnek matristeki solventlerin sınırlanmış difüzyonu ve hedefteki bileşiklerin sınırlı olan çözünürlüğüdür. Bu sınırlamaların giderilmeleri için kantitatife yakın olan ve hızlı ekstraksiyona imkan sağlayan farklı ekstraksiyon yöntemleri mevcuttur (Torgbo ve ark., 2024).

Bitki ekstraksiyon yöntemlerinin en etkili olanı, ekstraksiyonda su kullanımının hemen ardından aseton-su kullanılmasıdır. Bu yöntemin çok miktarda totaldeki flavonoit ve fenolik içeriğe sahip numuneler oluşturduğu ve en yüksek oranda ekstraksiyon verimini oluşturduğu kanıtlanmıştır. Bu süreç etkindir ve rahatça ölçeklendirilebilir (Torgbo ve ark., 2024). Farklı literatürlerde ekstraksiyon verimleri değişkenlik gösterir. Bu durum da içerik tespit yöntemleri, içerik verimi hesaplama teknikleri ve farklı kültüre alınan rambutan çeşitlerinden kaynaklanabilir (Tingting ve ark., 2022).

Oksidatif stres; sağlıklı organizmanın homeostazında çok önemli görevi olan oksidan-antioksidan dengenin oksidanların lehine bozulmasıyla süperoksit radikallerin fazla birikimi ve lipid peroksidasyonu sonucunda hücrelerde hasar oluşması şeklinde tanımlanabilir. Lipid peroksidasyonu hücrelerde inflamatuar hasar oluşumunda anahtar mekanizmadır (Bartsch ve Nair, 2006). Antioksidanlar, serbest oksijen radikalleri (SOR)'nin hücredeki hasarların onarımından sorumludurlar (İkikardeş, 2024). Antioksidan aktivitenin; metal şelatlama aktivitesini ve redoks dengesini muhafaza ettiği, serbest radikal temizliği yaptığı, enzimatik olan ve olmayan aktiviteleri bloke ettiği ve sonrasında ise oksidatif stresi düzenlediği bilinir (Tingting ve ark., 2022). Antioksidanlar toksik maddelerin, karsinojenlerin ve ilaçların istenilmeyen etkilerinden hücreyi korurlar (İkikardeş ve Kurutaş, 2023).

İlk defa yapılan bu çalışmada, Antalya ilinden temin edilen taze Rambutan meyvesi kullanılmıştır. Bu çalışmanın birinci kısmında taze Rambutan meyvesinin farklı solventlerde hazırlanmış farklı ekstraktlardaki *in vitro* antioksidan kapasite ve oksidatif stres düzeylerinin araştırılması amaçlandı. Taze Rambutan meyvesi %1,15 KCl, %0,9 NaCl ve 0,1 M fosfat tamponu ile ekstrakte edilmiş ve daha sonra oksidatif stresin göstergeleri olan antioksidan enzim aktiviteleri (CAT ve SOD) ve MDA düzeyleri spektrofotometrik yöntemlerle belirlenmiştir. Çalışmanın ikinci kısmını ise taze Rambutan meyvesinin saklama koşulları oluşturmuştur.

## Materyal ve Metot

Bu çalışmada Antalya ilinden taze Rambutan meyvesi temin edilip farklı solventlerde farklı ekstraktları oluşturuldu. Taze Rambutan meyve ekstraktları %1,15 KCl, %0,9 NaCl ve 0,1 M fosfat tamponu ile homojenize edildi. Elde edilen homojenantlar 14.000 rpm'de 30 dk süre ile santrifüj (Hettich 420 R) edildi ve böylece elde edilen supernatantlarda antioksidan enzim SOD ve CAT aktiviteleri ve oksidatif stresin göstergesi olan MDA düzeyleri spektrofotometrik olarak ölçüldü. Ayrıca Rambutan meyve ekstraktlarının farklı saklama koşullarında (+4°C, -20°C ve -70°C sıcaklıklarda) 1., 3., 5., 7., 15. ve 30. günlerinde CAT, SOD ve MDA düzeyleri ölçüldü.

### *Lipit Peroksidasyonunun Belirlenmesi (MDA Analizi)*

Rambutan supernatantlarda MDA düzeyi Ohkawa yöntemiyle saptanmıştır. Bu metodun prensibi, aerobik şartlarda pH 3,40'da tiyobarbitürik asit (TBA) ile örneğin 90-95°C'de inkübasyonu sonucu oluşan lipit peroksidasyonunun sekonder ürünü olan MDA'nın TBA ile pembe renkli kompleks oluşturma esasına dayanmaktadır. Oluşan renk şiddeti ortamdaki MDA konsantrasyonu ile doğru orantılı olup 532 nm'de spektrofotometrik olarak değerlendirilmektedir (Ohkawa ve ark, 1979).

***SOD Aktivite Tayini***

Rambutan supernatantlarda SOD aktivitesi Fridovich yöntemine göre saptandı (Fridovich, 1974). Bu yöntemin temel prensibi; SOD, oksidatif enerji oluşumunda üretilen toksik süperoksit radikallerinin hidrojen peroksit ($H_2O_2$) ve moleküler oksijene dismutasyonunu hızlandırır. Bu metod, 2-[4-iyodofenil]-3-[4-nitrofenol]-5-feniltetrazoliyum klorid (piyodonitrotetra zoliyum viyolet: INT) ile XO ve ksantinin açığa çıkardığı süperoksit radikallerinin üretildiği kırmızı formazan boyasının 505 nm dalga boyunda oluşturduğu optik dansitenin (O.D) okunmasına dayanmaktadır (Fridovich, 1974).

***CAT Aktivite Tayini***

Rambutan supernatantlarda CAT aktivitesi Beutler yönteme göre belirlendi. Yöntemin esası, $H_2O_2$ substratının CAT ile enzimatik olarak yıkılmının 240 nm'de izlenmesi esasına dayanmaktadır (Beutler, 1984). CAT, $H_2O_2$'nin yıkımını katalize eder ve $H_2O_2$'nin CAT tarafından yıkım hızı, $H_2O_2$'nin 230 nm'de ışığı absorbe etmesinden yararlanılarak spektrofotometrik olarak ölçülmüştür.

***İstatiksel Analizler***

Çalışmamızın istatistiksel değerlendirmesinde SPSS10.0 paket programı kullanıldı. Verilerin normal dağılıma uygun olup olmadığını belirlemek için normalite testleri uygulanmıştır. Tüm datalar ortalama±standart sapma (SD) şeklinde kullanıldı. Bağımsız grupların aralarındaki ortalama değerleri kıyaslamak için ise unpaired t testi kullanılmıştır faydalanıldı. İstatistiksel anlamlılık $p<0,05$ olarak kabul edildi.

**Bulgular**

Analizlerimiz sonucu çalışmamızda en yüksek antioksidan kapasitenin (CAT ve SOD) ve en düşük MDA düzeyinin %1,15 KCI içeren meyve ekstraktında olduğu ($p<0,05$), ancak en düşük antioksidan kapasite ve en yüksek MDA düzeyi fosfat tamponlu meyve ekstraktında olduğu ($p<0,05$) bulunmuştur (Şekil 1, 2 ve 3). Ayrıca Rambutan meyvesinin +4°C'de yaklaşık 1-2 gün, -20°C'de yaklaşık 10 gün ve -70°C'de yaklaşık 15 gün boyunca aktivitesini koruduğu gözlenmiştir.

Rambutan meyvesinin farklı solventlerde homojenize edildiğindeki CAT, SOD ve MDA düzeyleri Tablo 1'de gösterilmiştir.

**Tartışma**

İlk defa yapılan bu çalışmada farklı solventlerde ve farklı saklanma koşullarında taze rambutan meyve ekstraktlarında *in vitro* antioksidan kapasite ve oksidatif stres düzeyleri araştırılması sonucu elde edilen veriler, taze rambutan meyvesinin ülke ekonomisine katkı sağlayabilecek ve sağlık açısında çok önemli antioksidan bir meyve olarak saklama koşulları hakkında bilgi vermesi bakımında çok değerlidir.

İlk defa yapılan bu çalışmada, Antalya ilinden temin edilen taze Rambutan meyvesi kullanılmıştır. Bu çalışmanın birinci kısmında taze Rambutan meyvesinin farklı solventlerde hazırlanmış farklı ekstraktlardaki *in vitro* antioksidan kapasite ve oksidatif stres düzeylerinin araştırılması amaçlandı. Taze Rambutan meyvesi %1,15 KCl, %0,9 NaCl ve 0,1 M fosfat tamponu ile ekstrakte edildi ve daha sonra oksidatif stresin göstergeleri olan antioksidan enzim aktiviteleri (CAT ve SOD) ve MDA düzeyleri spektrofotometrik yöntemlerle belirlendi. En yüksek antioksidan kapasite ve en düşük MDA düzeyi %1,15 KCI içeren meyve ekstraktında bulundu. Buna karşın, en düşük antioksidan kapasite ve en yüksek MDA düzeyi fosfat tamponlu meyve ekstraktında bulundu. Rambutanın etkili bir antioksidan etkiye sahip olduğu sonucuna varıldı. Bu çalışma ilk defa yapıldığından başka çalışmaların sonuçlarıyla kıyaslama yapılamadı. Ancak, Demirhan ve ark. (2021) yılında mazı bitkisinde farklı solventlerde çalışmış ve en yüksek antioksidan aktiviteyi ve en düşük MDA düzeyini KCI ile bulmuşlardır. Bunun sebebi, KCI'nin antioksidan enzimlerin aktivitesini artıran bir aktivatör olarak işlev görmesi olabilir.

Çizelge 1. Rambutan Meyvesinin Farklı Solventlerde Homojenize Edildiğinde CAT, SOD ve MDA Düzeyleri
*Table 1. CAT, SOD and MDA Levels of Rambutan Fruit When Homogenized in Different Solvents*

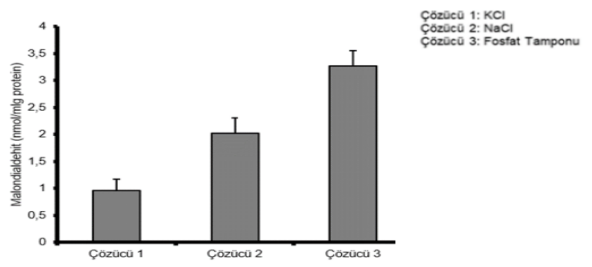
| Çözücüler | Katalaz (Ü/mg protein) | Superoksit Dismutaz (Ü/mg protein) | Malondialdehit (nmol/mg protein) |
|---|---|---|---|
| %1,15 KCI | 0,72±0,17* | 4,51±0,96* | 0,96±0,21* |
| %0,9 NaCI | 0,22±0,07 | 2,54±0,42 | 2,03±0,28 |
| 0,1 M Fosfat Tamponu | 0,14±0,05 | 1,52±0,39 | 3,27±0,28 |

Sonuçlar ortalama ve standart sapma olarak verilmiştir; *Rambutan meyve ekstresi %1,15 KCI çözücüsüyle homojenize edildiğinde CAT, SOD ve MDA düzeyleri diğerlerine göre oldukça yüksek çıkmıştır ($p<0,05$).

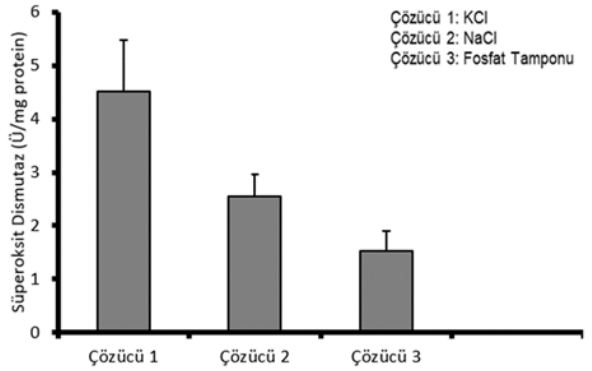
Çizelge 2. Rambutan Meyvesinde CAT, SOD ve MDA Düzeylerinin Farklı Saklanma Koşullarında Değişimi
*Table 2. Changes in CAT, SOD and MDA Levels in Rambutan Fruit under Different Storage Conditions*

| Günler | Katalaz | | | Süperoksit Dismutaz | | | Malondialdehit | | |
|---|---|---|---|---|---|---|---|---|---|
| | +4°C | -20°C | -70°C | +4°C | -20°C | -70°C | +4°C | -20°C | -70°C |
| 1. | 0,65 | 0,63 | 0,61 | 5,18 | 5,22 | 5,34 | 0,69 | 0,63 | 0,67 |
| 3. | 0,51 | 0,62 | 0,60 | 4,88 | 4,98 | 5,22 | 0,65 | 0,61 | 0,61 |
| 5 | 0,34 | 0,59 | 0,54 | 3,54 | 4,65 | 4,95 | 0,67 | 0,59 | 0,65 |
| 7. | 0,23 | 0,48 | 0,52 | 2,70 | 4,69 | 4,64 | 0,71 | 0,72 | 0,64 |
| 15. | 0,11 | 0,30 | 0,34 | 1,10 | 3,08 | 4,57 | 0,76 | 0,69 | 0,60 |
| 30. | 0,03 | 0,16 | 0,21 | 0,08 | 1,57 | 4,55 | 0,81 | 0,64 | 0,69 |

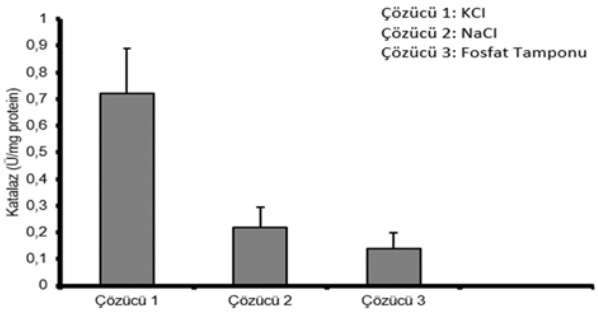
Not: CAT, SOD aktivite sonuçları Ü/mg protein, MDA düzeyleri nmol/mg protein olarak verilmiştir.




Şekil 1. Farklı Solventlerde MDA Düzeyinin Değişimi
*Figure 1. Changes in MDA Levels in Different Solvents*




Şekil 2. Farklı Solventlerde SOD Aktivitesinin Değişimi
*Figure 2. Variation of SOD Activity in Different Solvents*




Şekil 3. Farklı Solventlerde CAT Aktivitesinin Değişimi
*Figure 3. Variation of CAT Activity in Different Solvents*

Çalışmanın ikinci kısmını taze Rambutan meyvesinin saklama koşulları oluşturmuştur. Buna göre Rambutan meyvesinin -70°C'de 30 günde yaklaşık %50 oranında, -20°C'de %30 oranında ve +4°C'de ise %3 oranında antioksidan aktivitesini koruduğu görülmüştür. Bu veri, Rambutan meyvesinin -70°C'de yaklaşık 15 gün, -20°C'de yaklaşık 10 gün, +4°C'de ise yaklaşık 1-2 gün sonra bile rahatça kullanılabileceğini göstermektedir. Bulduğumuz tüm sonuçlar literatür çalışmalarını destekler niteliktedir.

Bazı bitkilerde aşırı sıcaktan ya da aşırı soğuktan dolayı stres oluşmaktadır. Bitkide oluşan stres, serbest radikallerin oluşumunu artırmakta ve antioksidan yetersizliğine ya da dengesizliğine neden olmaktadır. O yüzden, bu tip araştırmalar gerek ev ortamında gerekse ticari ortamlarda (manav, pazar gibi) saklanma koşulunun araştırılması ve bilinmesi, hem ulusal ekonomiye hem de sağlık açısından büyük önem arz etmektedir.

Literatür taramalarında bazı bitkilerin saklanma koşulları araştırılmıştır. Bahçe ürünlerinde hasat sonrası kayıpların önlenmesinde bitkisel uçucu yağların da içerisinde yer aldığı kimyasalların alternatif olarak uygulandığı çalışmalar mevcuttur. Bu çalışmalardan biri; Şener ve ark. (2022)'nın kullandıkları uçucu yağların 'Rubygem' çilek çeşidi meyvelerinin muhafaza süresi ve kalitesi üzerine etkileri incelenmiştir. Belirli nemde ve sıcaklıklarda meyveler depolanmıştır. Soğukta muhafaza sırasında kayısı çekirdeği ve okaliptüs yağları ağırlık kabını engellemede etkili bulunurken, manav koşullarında ise kayısı çekirdeği yağı daha başarılı bulunmuştur. Çiftci ve ark. (2022)'nın biberlerin muhafaza süresinin korunması ve raf ömrüne etkisini belirlemek amacıyla hasat sonrasında bitkisel uçucu yağların, modifiye atmosfer paketleri ile entegre kullanım olanakları değerlendirilmiştir. Yeşil olum döneminde hasat edilen biberler, belirli nem ve sıcaklıklarda depolanmıştır. Bitkisel uçucu yağlar emdirilmiş MAP-Y uygulamasının depolama ve raf ömrü sırasında karşılaşılan bozulmaların sınırlandırılmasında olumlu etki yaptığı belirlenmiştir. Başka bir çalışmada ise Yüksel ve ark. (2022), sera şartlarında yetiştirilen mısır hat ve hibritlerinin farklı polen muhafaza yöntemleri ile polen canlılığındaki değişimi izlemişlerdir. Çalışma sonuçlarında +4°C ve -20°C'de polen örneklerini doğrudan depolanması halinde 6. güne kadar canlılık durumunda önemli bir farkın olduğu, 9. günde ise canlılık ortalamaları bakımından var olan bu farkın kaybolduğu dikkat çekmiştir.

Çalışma sonuçlarımız ve literatür bilgileri göstermektedir ki, uygun solventler, uygun uçucu yağlar ve uygun sıcaklıklarda yani uygun ortam koşulları sağlandığında, besin değeri ve tazeliği korunmak istenen bitkilerin antioksidan değerinin korunması, oksidatif stresin önlenmesi, sağlıkla tüketilmesi bir süreye kadar mümkündür.

## Sonuç

Yaptığımız bu çalışmada analizlerimiz sonucunda taze Rambutan meyvesinde en yüksek antioksidan kapasitenin ve en düşük MDA düzeyinin %1,15 KCI içeren meyve ekstraktında olduğu bulundu. En düşük antioksidan kapasite ve en yüksek MDA düzeyi ise fosfat tamponlu meyve ekstraktında bulundu. Ayrıca rambutan meyve ekstraktlarının depolama koşulları 1., 3., 5., 7., 15. ve 30. günlerde +4°C,-20°C ve -70°C'de incelemeleri ve MDA, SOD ve CAT düzeyleri belirttiğimiz homojenatlarda spektrofotometrik olarak ölçümü sonucunda Rambutan meyvesinin +4°C'de yaklaşık 1-2 gün, -20°C'de yaklaşık 10 gün ve -70°C'de yaklaşık 15 gün boyunca aktivitesini koruduğu gözlenmiştir. İlk defa yapılan bu çalışmanın ileride yapılacak araştırmalara ve çalışmalara ışık tutacağı düşünülmektedir.

## Beyanlar

Bu çalışma 3. Uluslararası İpekyolu Bilimsel Araştırmalar Kongresi'nde (Özbekistan-Semerkand 6-8 Mart 2024) sunulmuştur.

***Teşekkür***

Bu çalışmaya desteklerinden dolayı biyokimya araştırma laboratuvarı personellerine teşekkürü borç bilirim.

***Çıkar Çatışması***

Yazarlar herhangi bir çıkar çatışması beyan etmemektedir.

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# Effects of Microwave Pretreatment on Color and Phenolic Component Profile of Orange Slices Dried Using the Vacuum Drying Method: Multivariate Analysis Approach

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ABSTRACT

This study investigates the effects of microwave pre-treatment (90 W, 30 min) on the quality characteristics of orange slices subjected to vacuum drying (VD) processes at various temperature (60, 70, and 80°C) and absolute pressure (15 and 30 kPa) combinations. The vacuum drying processes were carried out with (microwave-assisted vacuum drying, MAVD) and without (VD) the application of microwave pre-treatment under the same temperature and pressure conditions. The analysis encompassed polyphenolic compounds, including vanillic acid, chlorogenic acid, gallic acid, sinapic acid, *o*-coumaric acid, epicatechin, hesperidin, and naringenin. Additionally, visual quality attributes such as color parameters (*L*, a*, b**, and *ΔE*), browning index (*BI*), and whiteness index (*WI*) were evaluated. The vacuum drying conditions that best preserved the initial color characteristics and phenolic compound levels of fresh oranges as a result of the drying process were 80°C temperature and 15 kPa absolute pressure. Using principal component analysis (PCA) and hierarchical cluster analysis (HCA), the effects of drying conditions on color and polyphenolic component profiles were comprehensively evaluated. These analyses allowed the separation of chemical and polyphenolic profiles related to product quality.

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 13(3): 719-730, 2025

# Mikrodalga Ön İşlemli Vakumlu Kurutma Yönteminin Portakal Dilimlerinin Renk ve Fenolik Bileşen Profili Üzerine Etkileri: Çok Değişkenli Analiz Yaklaşımı

MAKALE BİLGİSİ

*Araştırma Makalesi*

Geliş : 20.11.2024
Kabul : 28.11.2024

*Anahtar Kelimeler:*
Portakal (*Citrus sinensis* L.)
Vakumlu kurutma
Polifenolik bileşenler
PCA
HCA

ÖZ

Bu çalışmada, portakal dilimlerinin kalite özellikleri üzerine farklı sıcaklık (60, 70 ve 80°C) ve mutlak basınç (15 ve 30 kPa) kombinasyonlarında gerçekleştirilen vakumlu kurutma (VK) işlemlerinde mikrodalga ön işleminin (90 W, 30 dk) etkileri incelenmiştir. Vakumlu kurutma işlemleri, aynı sıcaklık ve basınç koşullarında mikrodalga ön işlemi uygulanarak (MDVK) ve uygulanmadan (VK) gerçekleştirilmiştir. Çalışma kapsamında, polifenolik bileşikler (vanilik asit, klorojenik asit, gallik asit, sinapik asit, *o*-kumarik asit, epikateşin, hesperidin ve naringenin) analiz edilmiştir. Ek olarak, renk parametreleri (*L*, a*, b** ve *ΔE)*, esmerleşme indeksi (*BI*) ve beyazlatma indeksi (*WI*) gibi görsel kalite özellikleri de değerlendirilmiştir. Taze portakalların başlangıçta sahip olduğu renk özellikleri ve fenolik bileşen seviyelerini kurutma işlemi sonucunda en iyi koruyan vakumlu kurutma koşulları 80°C sıcaklık ve 15 kPa mutlak basınç olmuştur. Temel bileşen analizi (PCA) ve hiyerarşik kümeleme analizi (HCA) kullanılarak, kurutma koşullarının renk ve polifenolik bileşen profilleri üzerindeki etkileri kapsamlı bir şekilde değerlendirilmiştir. Bu analizler, ürün kalitesine ilişkin kimyasal ve polifenolik profillerin ayrıştırılmasına olanak sağlamıştır.

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## Giriş

Portakal (*Citrus sinensis* L.), *Rutaceae* familyasına ait, dünya çapında yaygın olarak yetiştirilen, ticareti yapılan ve tüketilen önemli bir turunçgil meyvesidir (; Özcan ve ark., 2021). Portakal, yüksek C vitamini içeriği ile güçlü antioksidan, antikanser ve antiinflamatuar özellikler taşır; bu sayede bağışıklık sistemini destekleyerek viral enfeksiyonlara karşı koruma sağlamada önemli bir rol oynamaktadır (Oikeh ve ark., 2020; Pacheco ve ark., 2020). Ayrıca, çekici rengi, aroması, kendine özgü ekşi-tatlı ve ferahlatıcı lezzeti tadının yanı sıra askorbik asit, diyet lifi, fenolik bileşikler, mineraller ve karotenoidler açısından zengin olması nedeniyle küresel pazarda değerli bir ürün olarak öne çıkmaktadır (Zou ve ark., 2016; Deng ve ark., 2020; Alibas & Yilmaz, 2022). 2022 yılı itibarıyla FAO verilerine göre, turunçgil üretimi yaklaşık 158 milyon tona ulaşmış olup, bu üretimin yaklaşık %55-60'ını portakal temsil ederek küresel pazarda en çok talep gören turunçgil türü konumundadır (FAO, 2024). Portakalın yalnızca besin değerleri değil, aynı zamanda mevsimsel olarak sınırlı hasadı da çeşitli muhafaza tekniklerinin kullanılmasını gerektirmektedir (O'Shea ve ark., 2012). Özellikle kurutma işlemi, portakalın yanı sıra diğer turunçgillerin muhafazasında giderek yaygın bir tercih haline gelmiştir (Alibas & Yilmaz, 2022; Özcan ve ark., 2021; Özkan-Karabacak ve ark., 2023; Silva-Espinoza ve ark., 2021).

Kurutma, tarımsal ürünlerdeki suyun ısı ve kütle transferi yoluyla buharlaştırılması işlemidir (Fratianni ve ark., 2013). Bu işlem, ürünlerin mikrobiyolojik aktivitesini azaltarak uzun süre muhafaza edilmesini sağlar ve dolayısıyla gıda güvenliği açısından kritik bir rol oynar. Ayrıca, su içeriği azalmış ürünler, depolama ve nakliye süreçlerinde önemli avantajlar sunarak maliyet tasarrufuna yardımcı olmaktadır (Alibas & Yilmaz, 2022; Bozkir, 2020).

Kurutma işlemi çeşitli yöntemlerle gerçekleştirilebilir. Vakumlu kurutma, ürünlerin düşük sıcaklıklarda ve atmosfer basıncının azaltıldığı ortamlarda suyun buharlaştırılmasıdır. Bu yöntem, besin değerlerinin yanı sıra aroma ve renk gibi kalite parametrelerinin korunmasında etkili bir yöntem olarak öne çıkmaktadır. Düşük sıcaklıklarda gerçekleştirildiği için kurutma süresi, konvektif kurutmaya göre daha kısadır; ancak yüksek başlangıç maliyetleri ve özel ekipman gerektirmesi gibi dezavantajları bulunmaktadır (Boris ve ark., 2018; Bozkir, 2020). Mikrodalga kurutma, malzemenin içinden dışarıya ısı ve kütle transferi sağlayarak hızlı bir kurutma süreci sunmaktadır. Bu yöntem, yüksek enerji verimliliği ve rehidrasyon kapasitesi ile dikkat çekmektedir; ancak yüksek güç seviyelerinde polarizasyona yol açarak yerel yanıklara neden olma riski taşımaktadır (Shu ve ark., 2020; Zielinska & Michalska, 2016). Bunun yanı sıra, mikrodalga kurutma (MW) tekniğinin herhangi bir başka kurutma yöntemi veya ön işlem uygulanmaksızın tek başına kullanımı, nihai ürün kalitesinde ciddi bozulmalara yol açabileceği gibi, ürünün tamamen tahrip olmasına da neden olabilmektedir (Zielinska & Michalska, 2016; Zielinska ve ark., 2019).

Mikrodalga ön işlemli vakumlu kurutma uygulaması, kurutma kinetiğinin yanı sıra nihai ürünün duyusal ve besin kalitesi üzerinde olumlu bir etkiye sahiptir. Elma küpleri (Chong ve ark., 2014), domates (Orikasa ve ark., 2018), yaban mersini (Zielinska & Michalska, 2016) ve narenciye kabukları (Shu ve ark., 2020) gibi ürünlerde, mikrodalga ön işlemli vakumlu kurutma sayesinde yüksek kaliteli kurutulmuş meyve ve sebze elde edilmesine yönelik araştırmalar yapılmıştır. Ancak, portakal dilimlerinin kurutulmasında mikrodalga ön işlemli vakumlu kurutma yönteminin polifenol özellikler ve renk parametreleri üzerindeki çoklu değişken analizine dayalı etkisini inceleyen çalışmalar literatürde sınırlıdır. (Gómez-Mejía ve ark., 2023; Rafiq ve ark., 2019; Wang ve ark., 2023). Rafiq ve ark. (2019), innow kabuklarına uygulanan tepsili, vakumlu ve dondurarak kurutma yönemlerinin renk ve fenolik bileşenler üzerine olan etkisini incelemiştir. Wang ve ark.(2023) portakal kabuklarına dondurarak kurutma, ısı pompalı kurutma, mikrodalga kurutma ve uzak kızılötesi kurutma yöntemlerini uygulayarak kurutmuş ve kurutma yöntemlerinin nihai üründe renk, fenolik bileşen profili ve antioksidan kapasite gibi kritik kalite parametreleri üzerindeki etkilerini değerlendirilmiştir. Bir diğer çalışmada, Gómez-Mejía ve ark. (2023) klementin ve limon kabuklarından elde edilen hidroetanol ekstraktlarının farklı kurutma tekniklerinin (fırın ve vakumlu kurutma dahil) fenolik bileşimleri ve antioksidan aktiviteleri üzerine olan etkilerini incelemiştir. Yapılan bu çalışmalar ürünlerin nihai özelliğini korunması için literatürde başarıyla uygulanan vakumlu kurutma yönteminin süre dezavantajını azaltılması için uygulanabilecek olan mikrodalga ön işlemini içermemektedir. Ayrıca mikrodalga önişlemi destekli uygulanan vakumlu kurutma işleminin sıcaklık ve basınç kombinasyonunun fenolik bileşenler üzerindeki etkileri incelememiştir. ve renk parametresi ile kurutma koşulları arasında olan ilişki ortaya konulmamıştır. Diğer taraftan, yapılan çalışmalarda elde edilen bulgular korelasyon analizi, temel bileşen analizi (PCA) ve kümeleme analizi (HCA) gibi ileri düzey kemometrik veri analizi yöntemleriyle kapsamlı ve anlamlı bir şekilde yorumlanmamıştır. Bu çalışmada kullanılan yenilikçi mikrodalga ön işlemli vakumlu kurutma (MDVK) yönteminin, özellikle fenolik bileşikler gibi çeşitli parametreler üzerindeki sinerjik etkilerinin araştırılması, tüketicilere yönelik yüksek kaliteli, besin açısından zenginleştirilmiş sağlıklı kurutulmuş portakal atıştırmalıkları formüle etmek ve üretmek için önemli bulgular sunacağı düşünülmektedir.

Bu çalışmanın amacı, mikrodalga ön işleminin (90 W, 30 dk) farklı kurutma sıcaklıkları ve mutlak basınç kombinasyonlarında (15 ve 30 kPa ile 60, 70 ve 80°C) gerçekleştirilen vakumlu kurutma üzerindeki etkilerini portakal dilimlerinin polifenol özellikleri ve bazı kalite nitelikleri açısından değerlendirmektir. Kurutulmuş portakal dilimlerinin polifenol özellikleri üzerindeki etkilerini değerlendirmek amacıyla gerçekleştirilen analizlerde, korelasyon analizi, temel bileşen analizi (PCA) ve kümeleme analizi yöntemlerinden yararlanılmıştır.

## Materyal ve Metot

### *Materyal*

Taze portakal örnekleri (*Citrus sinensis Valencia*), Antalya'da bir üretici tarafından hasat edilmiştir. Örnekler, plastik kutularda üç gün içerisinde laboratuvara ulaştırılmıştır. Nem ve kalite kaybını önlemek amacıyla, kurutma deneyleri tamamlanana kadar örnekler, 4 ± 0,5°C sıcaklık ve %90–95 bağıl nem koşullarında soğuk depoda muhafaza edilmiştir (Özkan-Karabacak ve ark., 2020). İlerideki kurutma deneyleri için ortalama çapı 80 ± 2 mm olan portakallar, kabukları soyulmadan uzunlamasına 4 ± 0,5 mm kalınlığında dilimlenmiştir. Portakal dilimlerinin başlangıç nem içeriği, numunelerin dijital nem tayin cihazı (MA150; Sartorius, Göttingen, Almanya) ile 5,33 g $H_2O$/g kuru madde olarak belirlenmiştir.

### *Metot*

*Kurutma İşlemleri*

Mikrodalga ön işlemi uygulanmaksızın, vakumlu kurutma (VK) yöntemi kontrol olarak kullanılarak farklı sıcaklık ve basınç kombinasyonları ile gerçekleştirilmiştir. Önceki çalışmamızda kurutma prosedürü detaylı olarak verilmiştir (Özkan-Karabacak ve ark., 2020). Deneylerde 60, 70 ve 80°C sıcaklıklar ile 15 ve 30 kPa mutlak basınç düzeyleri uygulanmış olup, bu işlemler 49 L hacme sahip bir vakumlu kurutucuda (Memmert VO400, Almanya) gerçekleştirilmiştir. Toplamda altı farklı proses kombinasyonu belirlenmiştir: 60°C-15 kPa, 60°C-30 kPa, 70°C-15 kPa, 70°C-30 kPa, 80°C-15 kPa ve 80°C-30 kPa. Deneysel çalışmalar kapsamında, yaklaşık 100 ± 5 g ağırlığındaki portakal dilimleri, yağlı kağıt üzerine düzenli bir şekilde yerleştirilmiştir. Kurutma süreci boyunca, dijital terazi (Mettler Toledo, MS3002S) kullanılarak her 30 dakikada bir portakal dilimlerinin ağırlıkları kaydedilmiştir. Bu ölçümler, ürünlerin kuruma süreçlerini izlemek amacıyla gerçekleştirilmiştir.

MDVK işlemi, vakumlu kurutma öncesinde 30 dakika boyunca 90 W gücünde bir mikrodalga ön işlem adımını içermektedir. Mikrodalga ön işlemi, 230 V, 50 Hz teknik özelliklere sahip bir ev tipi mikrodalga fırın (Bosch, HMT72G420, Münih, Almanya) ile uygulanmıştır. Literatürde yapılan çalışmalarla uyumlu bir şekilde bu çalışma kapsamında yapılan ön denemelerde, seçilen bu mikrodalga koşullarının üründe su salınmasına neden olmadığı,ve ürünün iç yapısını bozmayarak homojen bir kuruma sağladığı gözlenmiştir (Karabacak ve ark., 2018; Özkan-Karabacak ve ark., 2020; Özkan-Karabacak ve ark., 2023; Suna & Özkan-Karabacak, 2019). Ayrıca, 90 W gücü, portakal dilimlerinin en iyi kurutma özelliklerini elde etmek amacıyla belirlenmiştir. Bu güç düzeyi, ürünlerde yanma riskini en aza indirerek, kurutma sürecinin etkinliğini artırmıştır (Alibas & Yilmaz, 2022; Chen ve ark., 2021).

MDVK kurutulması sırasında taze portakal dilimleri, vakumlu kurutma uygulamaları sırasında olduğu gibi, eşit ağırlıkta yağlı kağıt üzerinde kurutulmuştur. Kurutma sürecinin izlenmesi amacıyla tartım aralıkları, sırasıyla mikrodalga ön işlem ve vakumlu kurutma için 5 dakika ve 30 dakika olarak belirlenmiştir. Deneysel tasarım, portakal dilimlerinin kuruma süreçlerini optimize etmek ve nihai ürün kalitesini artırmak amacıyla dikkatlice planlanmıştır. Farklı proses koşullarında, hem VK hem de MDVK yöntemleri kullanılarak portakal dilimleri, nem içeriği 0,09 g $H_2O$/g kuru madde seviyesine düşene kadar kurutulmuştur (Senevirathne ve ark., 2009). Tüm kurutma deneyleri, her bir koşul için üç tekrarlı olarak gerçekleştirilmiştir. Bu çalışma kapsamında gerçekleştirilecek analizler için portakal dilimi numuneleri, ışığa maruz kalmadan vakumlu poşetlerle paketlenmiş ve -18 ± 0,5°C'de dondurucuda muhafaza edilmiştir.

*Renk Parametreleri, Esmerleşme İndeksi ve Beyazlatma İndeksi*

Portakal dilimlerinin kolorimetrik değerleri, CR-5 Konica Minolta Chroma Meter (Osaka, Japonya) kullanılarak belirlenmiştir. Renk parametreleri olan $L^*$, $a^*$, $b^*$, Kroma ($C^*$) ve ton açısı ($h°$) için ölçümler daha önceki çalışmamızda belirtilmiştir (Özkan-Karabacak ve ark., 2020). Bu çalışmada, söz konusu ölçümlere ek olarak kahverengileşme indeksi (Browning Index, *BI*) ve beyazlık indeksi (Whiteness Index, *WI*) de hesaplanmıştır.

Aşağıdaki eşitlik ile *BI* tespit edilmiştir (Maskan, 2001).

$$C^*=(a^2+b^2)^{1/2}\ h°=\arctan(b/a) \quad (1)$$

$$BI = \left[100\left(\frac{a^*+1{,}75L^*}{5{,}645L^*+a^*-0{,}3012b^*}-0{,}31\right)\right]\div 0{,}17 \quad (2)$$

Portakal dilimlerinin kurutma sonrasında yüzey renklerinde meydana gelen kahverengileşme, ölçülen Hunter renk parametrelerinin *WI* değerlerine dönüştürülerek analiz edilmiştir (Cuccurullo ve ark., 2018).

$$WI = 100 - \sqrt{(100-L^*)^2+a^{*2}+b^{*2}} \quad (3)$$

Bu indeksler, portakal dilimlerinin renk değişimini ve genel kalitesini değerlendirmek için önemli veriler sağlamaktadır.

*Polifenollerin Ekstraksiyonu*

Taze ve kurutulmuş portakal dilimlerindeki polifenolik bileşiklerin ve antioksidan aktivitenin ekstraksiyonu, Kamiloglu & Capanoglu (2013) tarafından daha önce açıklanan yöntem kullanılarak gerçekleştirilmiştir. Numunenin 2 ± 0,01 g'ı, %75'lik su ile metanol karışımında 0,1% formik asit içeren 5 mL'lik bir ekstraksiyon çözücüsü ile ekstrakte edilmiştir. Ekstraksiyon prosedürü, 15 dakika boyunca soğutulmuş bir ultrasonik su banyosunda uygulanmıştır. Elde edilen karışım 2700 g' de 10 dakika süreyle santrifüj edilmiştir. Santrifüj işlemi sonrasında elde edilen üstteki sıvı toplanmış ve ekstraksiyon işlemi, numunenin kalan kısmına taze ekstraksiyon çözücüsü eklenerek iki kez daha tekrarlanmıştır. Üç ayrı üstteki sıvı birleştirilmiş ve son hacim 15 mL' ye ayarlanmıştır. Elde edilen son ekstraktlar, analiz yapılıncaya kadar -20°C'de saklanmıştır.

*HPLC ile Polifenolik Bileşiklerin Karakterizasyonu*

Polifenol miktarı, Capanoglu ve ark. (2008) tarafından. tanımlandığı üzere yüksek performanslı sıvı kromatografi-fotodiyot dizisi dedektörü (HPLC-PDA) kullanılarak belirlenmiştir. Ekstrakte edilen örnekler, 0,45 µm membran filtreler aracılığıyla süzülmüş ve yüksek basınçlı sıvı kromatografisi fotodiyot dizisi dedektörü (HPLC-

PDA) sistemine (Waters 2695, Amerika) enjekte edilmiştir. Analiz için, 25 cm uzunluğunda, 4,6 mm çapında ve 5 µm partikül boyutuna sahip bir C18 kolonu (Sigma-Aldrich, Almanya) kullanılmıştır. Spektral ölçümler, 280 nm, 312 nm ve 360 nm dalga boylarında gerçekleştirilmiş olup, akış hızı 1 mL/dakika ve enjeksiyon hacmi 10 µL olarak ayarlanmıştır. Mobil faz olarak, trifloroasetik asit (TFAA)/ultra saf su (1:1000, v/v; eluent A) ve TFAA/asetonitril (1:1000, v/v; eluent B) kullanılmıştır. Doğrusal gradyan akış profili şu şekilde belirlenmiştir: 0. dakikada %95 A ve %5 B; 45. dakikada %65 A ve %35 B; 47. dakikada %25 A ve %75 B; 49. dakikada %65 A ve %35 B; 50. dakikada ise %95 A ve %5 B. Kolon sıcaklığı 45°C'de sabitlenmiş, otomatik örnekleyicinin sıcaklığı ise 10°C'de tutulmuştur. Polifenollerin tanımlanmasında, kolonun tutulma süreleri ve karakteristik UV spektrumları kullanılmıştır.

### *İstatistiksel Analiz*

Tüm analizler üç tekrarlı olarak gerçekleştirilmiştir. İstatistiksel analizler IBM SPSS Statistics 28.0 yazılımı kullanılarak gerçekleştirilmiş ve sonuçlar ortalama ± standart sapma olarak ifade edilmiştir. Ölçümlerden elde edilen bulgular varyans analizine (tek yönlü ANOVA) tabi tutulmuştur. Ortalamalar arasında anlamlı farklılıkların ($p$<0,05) varlığı Duncan'ın çoklu karşılaştırma testleri kullanılarak belirlenmiştir. Polifenolik profiller ve renk parametreleri arasındaki ilişkileri incelemek için Pearson korelasyon katsayıları belirlenmiştir. Bu çalışmada, incelenen çeşitli grupları farklılaştıran polifenolik profili ve renk parametrelerini belirlemek için temel bileşen analizi (PCA) ve hiyerarşik kümeleme analizi (HCA) olarak bilinen çok değişkenli bir analiz yaklaşımı kullanılmıştır. Tüm istatistiksel analizler IBM SPSS yazılımı (versiyon 28, Şikago, IL, ABD) ve Minitab yazılımı (versiyon 21, Pensilvanya, ABD) kullanılarak gerçekleştirilmiştir.

## Bulgular ve Tartışma

### *Kurutma Tekniklerinin Renk Özelliklerine Etkisi*

Renk değişimi, kahverengileşme indeksi ve beyazlatma indeksi, ürünün ısı, sıcaklık, hava akışı gibi faktörlere maruz kalarak ne kadar renk kaybı yaşadığını anlamak açısından önemli göstergelerdir. Çizelge 1' de, taze portakal dilimlerine kıyasla VK ve MDVK yöntemleriyle kurutulan örneklerin renk parametreleri, kahverengileşme indeksi ve beyazlatma indeksi değerleri sunulmaktadır. Kurutma tekniklerinin, portakal dilimlerinin renk özellikleri üzerinde istatistiksel olarak anlamlı ($p<0,05$) bir etki gösterdiği belirlenmiştir. Yapılan analizler sonucunda taze örneklerin renk parametreleri $L^*$, $a^*$ ve $b^*$ sırasıyla 56,49, 15,67 ve 46,99 olarak belirlenmiştir (Çizelge 1). Bu değerler, kurutma süreci boyunca meydana gelen renk değişimlerinin başlangıç noktasını göstermekte olup, taze örneklere göre kurutulmuş örneklerde renk parametrelerinde anlamlı farklılıklar gözlenmiştir. Önceki çalışmada (Özkan-Karabacak ve ark., 2020) portakal örneklerinde kurutma işlemlerinin renk parametreleri üzerindeki etkileri detaylı bir şekilde ele alınmıştır. Ancak söz konusu çalışmada, kahverengileşme indeksi ve beyazlatma indeksi gibi bazı önemli parametrelere yer verilmemiştir. Ayrıca, renk parametreleri arasındaki korelasyonun kapsamlı bir analizine de değinilmemiştir. Bu çalışmada, mevcut literatürde yer alan verilerle birlikte portakal dilimlerinin renk parametreleri arasındaki ilişkileri ve kurutma tekniklerinin bu parametreler üzerindeki etkilerini daha ayrıntılı incelenerek literatürdeki bu boşlukların doldurması amaçlanmaktadır. Yeni bulguların, kurutma yöntemlerinin renk kalitesi üzerindeki etkilerini daha iyi anlaşılmasına katkı sağlayacağı düşünülmektedir.

Bu çalışmada, *BI* değeri kurutulmadan önceki taze numune 27,91 olarak tespit edilmiştir. VK yöntemi ile *BI* değerleri ise 28,88 ile 34,88 arasında; MDVK yöntemi ile 34,44 ile 37,94 arasında değişmiştir. İstatistiksel olarak, kurutma yöntemlerinin (VK ve MDVK) kahverengileşme indeksi (*BI*) üzerindeki istatiksel olarak etkisi önemli bir bulgu ($p$<0,05) olarak ortaya çıkmaktadır (Çizelge 1). Özellikle MDVK yönteminin, VK yöntemine kıyasla kahverengileşme indeksini artırırken beyazlatma indeksini (*WI*) azalttığı gözlemlenmiştir. Bu durum mikrodalga enerjisinin üründe kahverengi oluşumuna neden olmuştur. Ayrıca, kurutma işleminin genel olarak ürünün görünüm özelliklerini ve besin değerini etkileyebileceği düşünülmektedir; zira renk, tüketici kabulü ve algısı açısından kritik bir parametre olup, besin kalitesinin değerlendirilmesinde de ilk izlenim açısından önemli bir rol oynamaktadır (Pathare ve ark., 2013).

Beyazama indeksi (*WI*) incelendiğinde Çizelge 1' de, taze portakal dilimlerine ait *WI* değeri (34,07) ile en yakın değerler sırasıyla 80°C 15 kPa VK (34,96), 70°C 30 kPa VK (33,24) ve 60°C 30 kPa VK uygulamalarında elde edilmiştir. Taze ürünlere kıyasla en fazla renk kaybı ise 70°C 15 kPa MDVK (27,10), 70°C 15 kPa VK (29,58) ve 60°C 15 kPa MDVK (30,05) kurutma işlemlerinde gözlemlenmiştir. Bu kurutma işlemlerindeki renk kayıplarının, uzun kurutma süreleri nedeniyle üründe artan oksidasyondan kaynaklanabileceği düşünülmektedir (Alibas ve Yilmaz, 2022). *WI* ve *BI* değerleri açısından değerlendirildiğinde, 80°C 15 kPa VK tekniği, renk koruma açısından en etkili yöntem olarak öne çıkmaktadır.

Alibas & Yilmaz (2022) tarafından gerçekleştirilen çalışmada, portakal kabukları mikrodalga ve konvektif kurutma yöntemleri kullanılarak kurutulmuş ve bu numunelerin renk parametreleri ile *BI* ve *WI* değerleri incelenmiştir. Söz konusu araştırmadan elde edilen sonuçlar, bu çalışmadaki bulgularla uyum göstermektedir. Başka bir çalışmada, kinnow kabukları farklı kurutma teknikleri (tepsi, vakum ve dondurarak kurutma) ile kurutulmuştur. Çalışmada, taze numunelerde *BI* değerinin daha yüksek olduğu tespit edilirken, *WI* değerinin ise kurutma sonrası daha yüksek olduğu rapor edilmiştir. Ayrıca, tepsi ve vakumlu kurutmanın esmerleşme indeksinde artışa ve beyazlatma indeksinde azalmaya yol açtığı gözlemlenmiş; bu durumun, kurutma sırasında kahverengi bileşiklerin oluşumundan kaynaklandığı belirtilmiştir (Rafiq ve ark., 2019). Alibas & Yilmaz (2023) araştırmasında kişniş yapraklarının renk parametreleri, doğal, konvektif ve mikrodalga kurutma teknikleri kullanılarak incelenmiştir. Kahverengileşme indeksi açısından en yüksek değerler, 50°C'de konvektif kurutma yöntemi ile kurutulan örneklerde kaydedilmiştir. Taze ürüne en yakın kahverengileşme indeksi, 200 W mikrodalga kurutma yöntemi ile kurutulan örneklerde ölçülmüş; en düşük değerler ise doğal kurutma yöntemiyle

kurutulan örneklerde elde edilmiştir. Taze örneklere en yakın beyazlatma indeksi, 800 W'da mikrodalga kurutma yöntemi ile kurutulan örneklerde gözlenmiş; bu örnekleri sırasıyla 200 W'da mikrodalga kurutma ve doğal kurutma yöntemleri takip etmiştir. En düşük beyazlatma indeksi, yani en yüksek kararma oranı, 50°C'deki konvektif kurutma yöntemi ile elde edilmiştir. Elde edilen bulgular, bu çalışmadaki sonuçlarla uyumlu niteliktedir.

Elde edilen sonuçlar, kurutma yöntemlerinin portakal dilimlerinin hem organoleptik özellikleri hem de besin değerleri üzerinde belirgin etkiler yarattığını göstermektedir. Özellikle düşük sıcaklık ve kontrollü vakum koşulları, renk kaybını minimize ederek üründe istenen renk kalitesini koruma açısından avantaj sağlamaktadır. Çalışmamız, farklı kurutma tekniklerinin renk kalitesi üzerindeki etkilerini ayrıntılı biçimde ortaya koyarak, gelecekteki uygulamalarda kurutma yönteminin seçiminde yol gösterici bir kaynak olma potansiyeli taşımaktadır. Bu doğrultuda, gıda teknolojisi alanında daha fazla araştırma yapılması, kurutma yöntemlerinin ürün kalitesi üzerindeki etkilerinin daha iyi anlaşılmasına katkı sağlayacaktır.

***Kurutma Tekniklerinin Portakal Dilimlerinin Polifenol Profili Üzerindeki Etkisinin HPLC ile İncelenmesi***

VK ve MDVK tekniklerinin portakal dilimlerinin polifenol profili üzerindeki etkisi Çizelge 2' de sunulmuştur. Kurutulmuş portakal dilimlerinden 5 adet fenolik asit (vanilik asit, klorojenik asit, gallik asit, sinapik asit ve o-kumarik asit) ve 3 adet flavonoid (epikateşin, hesperidin ve naringenin) olmak üzere 8 ana bileşiğin HPLC ile tanımlanması ve miktar tayini belirlenmiştir. Şekil 1, 80°C 15 kPa VK ile kurutulmuş numunenin 280 ve 312 nm'de ilgili HPLC kromatogramını göstermektedir. Tanımlanan 8 ana bileşen arasında, farklı kurutma yöntemlerinin her bir bileşenin içeriği üzerindeki etkileri istatistiksel olarak anlamlı farklılık göstermektedir ($p<0,05$).

Bu çalışmada, farklı kurutma işlemlerinin taze portakal dilimlerindeki fenolik bileşikler üzerindeki etkileri incelenmiştir. Taze örneklerde belirlenen yüksek fenolik bileşik seviyeleri, uygulanan kurutma işlemleri sonucunda kayda değer oranlarda azalmıştır. Bu sonuçlar önceki çalışmada sunulan aynı örneklerin toplam fenolik madde içeriği verileri ile uyumludur (Özkan Karabacak ve ark., 2020).

Sonuçlar, taze ve kurutulmuş portakal dilimlerinden elde edilen ekstraktların başlıca fenolik asit bileşeninin vanilik asit olduğunu göstermektedir; bu bileşiğin miktarı 77,50–503,63 mg/g kuru madde aralığında değişiklik göstermiştir. Taze örnekte 503,63 mg/g olarak belirlenen vanilik asit miktarı, kurutma işlemleri sonrasında özellikle 70°C ve 15 kPa MDVK koşullarında 77,50 mg/g seviyesine düşerek %84,6'lık bir azalma göstermiştir. Benzer şekilde, başlangıçta 187,22 mg/g olarak ölçülen klorojenik asit miktarı, kurutma işlemleri sonrasında en düşük seviyeyi 22,53 mg/g (70°C 15 kPa MDVK) olarak göstermiştir ve bu durum %84'lük bir azalmayı ifade etmektedir. Gallik asit miktarı taze örnekte 181,30 mg/g olarak tespit edilmiş olup, 70°C 15 kPa MDVK işleminde 23,78 mg/g seviyesine düşerek %86,9 oranında bir azalma kaydedilmiştir. Sinapik asit, taze örnekte 254,56 mg/g iken, kurutma işlemi sonrasında 80°C 30 kPa MDVK işleminde 22,13 mg/g seviyesine kadar azalmış ve %91,3 oranında bir düşüş gözlenmiştir. Benzer şekilde, *o*-kumarik asit içeriği taze örnekte 169,21 mg/g olarak belirlenmiş olup, kurutma işlemi sonrasında en düşük seviyeye (3,59 mg/g, 80°C 30 kPa MDVK) ulaşarak %97,9 oranında bir azalma göstermiştir.

Çalışmanın sonuçlarından başlıca flavonoid bileşeninin hesperidin olduğu saptanmıştır. Hesperidin miktarı 1022,98–5592,13 mg/g kuru madde aralığında belirlenmiş ve %78,7 oranında bir azalma göstermiştir. Epikateşin miktarı taze örnekte 930,75 mg/g olarak tespit edilmiş olup, kurutma işlemi sonrasında en düşük seviyeye 99,20 mg/g (80°C 30 kPa MDVK) olarak inmiş ve %89,3 oranında bir azalma kaydedilmiştir. Naringenin miktarı taze örnekte 236,86 mg/g olarak ölçülmüş olup, kurutma işlemleri sonrasında en düşük değeri 41,76 mg/g (80°C 30 kPa MDVK) olarak gözlemlenmiş ve %82,4 oranında bir azalma kaydedilmiştir.

Çizelge 1. Farklı tekniklerle kurutulan portakal dilimlerin renk parametreleri
*Table 1. Color parameters of orange slices dried by different techniques*

| Kurutma İşlemleri | $L^{*,1}$ | $a^{*,1}$ | $b^{*,1}$ | $\Delta E^{*,1}_{ab}$ | BI[2] | WI[2] |
|---|---|---|---|---|---|---|
| Taze | 56,49 ± 0,07$^{a}$ | 15,67 ± 0,01$^{k}$ | 46,99 ± 0,08$^{c}$ | — | 27,91± 0,02$^{i}$ | 34,07±0,02$^{ab}$ |
| 60°C 15 kPa VK | 51,11 ± 0,05$^{e}$ | 18,82 ± 0,01$^{f}$ | 45,05 ± 0,01$^{f}$ | 6,52 ± 0,01$^{g}$ | 34,40±0,04$^{e}$ | 30,94±0,04$^{f}$ |
| 60°C 15 kPa MDVK | 51,99 ± 0,03$^{c}$ | 19,93 ± 0,02$^{c}$ | 46,81 ± 0,04$^{d}$ | 6,19 ± 0,01$^{i}$ | 35,62±0,03$^{c}$ | 30,05±0,05$^{g}$ |
| 60°C 30 kPa VK | 50,70 ± 0,23$^{f}$ | 18,18 ± 0,11$^{g}$ | 41,99 ± 0,19$^{i}$ | 8,05 ± 0,02$^{e}$ | 33,20±0,05$^{f}$ | 32,74±0,07$^{c}$ |
| 60°C 30 kPa MDVK | 50,22 ± 0,06$^{g}$ | 18,87 ± 0,06$^{f}$ | 42,17 ± 0,09$^{h}$ | 8,53 ± 0,02$^{d}$ | 34,44±0,04$^{e}$ | 32,09±0,08$^{d}$ |
| 70°C 15 kPa VK | 51,19 ± 0,01$^{de}$ | 18,15 ± 0,05$^{g}$ | 47,41 ± 0,08$^{b}$ | 5,87 ± 0,01$^{j}$ | 34,05±0,03$^{e}$ | 29,58±0,05$^{h}$ |
| 70°C 15 kPa MDVK | 51,57 ± 0,01$^{d}$ | 20,94 ± 0,01$^{b}$ | 50,31 ± 0,06$^{a}$ | 7,93 ± 0,02$^{f}$ | 37,94±0,06$^{a}$ | 27,10±0,04$^{i}$ |
| 70°C 30 kPa VK | 47,06 ± 0,07$^{i}$ | 17,82 ± 0,02$^{h}$ | 40,55 ± 0,08$^{k}$ | 11,62 ± 0,04$^{a}$ | 34,88±0,05$^{d}$ | 33,24±0,04$^{b}$ |
| 70°C 30 kPa MDVK | 51,36 ± 0,02$^{d}$ | 21,07 ± 0,04$^{a}$ | 40,58 ± 0,02$^{k}$ | 9,82 ± 0,03$^{b}$ | 36,09±0,06$^{b}$ | 30,97±0,02$^{f}$ |
| 80°C 15 kPa VK | 52,80 ± 0,06$^{b}$ | 15,76 ± 0,05$^{j}$ | 41,88 ± 0,06$^{ij}$ | 6,29 ± 0,01$^{h}$ | 28,88±0,03$^{h}$ | 34,96±0,07$^{a}$ |
| 80°C 15 kPa MDVK | 52,10 ± 0,03$^{c}$ | 19,36 ± 0,03$^{d}$ | 46,14 ± 0,02$^{e}$ | 5,80 ± 0,01$^{k}$ | 34,70±0,04$^{d}$ | 30,73±0,05$^{f}$ |
| 80°C 30 kPa VK | 49,20 ± 0,24$^{h}$ | 16,59 ± 0,11$^{i}$ | 41,74 ± 0,22$^{j}$ | 9,02 ± 0,03$^{c}$ | 32,04±0,03$^{g}$ | 32,19±0,03$^{d}$ |
| 80°C 30 kPa MDVK | 50,29 ± 0,08$^{g}$ | 19,13 ± 0,01$^{e}$ | 43,43 ± 0,03$^{f}$ | 7,94 ± 0,02$^{f}$ | 35,01±0,06$^{c}$ | 31,27±0,06$^{e}$ |

*Sütunlardaki farklı harfler istatistiksel olarak önemli farklılıkları temsil etmektedir ($p<0,05$); [1]Bu veriler bir önceki çalışmamızdan alıntılanmıştır (Ozkan-Karabacak ve ark., 2020); [2]BI: Kahverengileşme indeksi; WI: Beyazlama indeksi

Çizelge 2. Farklı tekniklerle kurutulan portakal dilimlerin bireysel polifenolik içerikler (mg/g kuru ağırlık)
*Table 2. Individual polyphenolic contents of orange slices dried by different techniques (mg/g dry weight)*

| Kurutma İşlemleri | Fenolik asitler | | | | |
|---|---|---|---|---|---|
| | Vanilik asit | Klorojenik asit | Gallik asit | Sinapik asit | *o*-kumarik asit |
| Taze | 503,63±71,16$^{a}$ | 187,22±18,98$^{a}$ | 181,30±22,00$^{a}$ | 254,56±11,52$^{a}$ | 169,21±32,00$^{a}$ |
| 60°C 15 kPa VK | 128,16±7,69$^{bc}$ | 37,70±1,84$^{efg}$ | 35,53±6,14$^{bcde}$ | 35,29±3,32$^{def}$ | 13,18±1,81$^{bc}$ |
| 60°C 15 kPa MDVK | 111,38±11,80$^{bc}$ | 29,89±1,15$^{fg}$ | 32,12±1,72$^{cde}$ | 31,53±1,96$^{efg}$ | 15,10±2,40$^{bc}$ |
| 60°C 30 kPa VK | 104,38±3,22$^{bc}$ | 64,56±6,33$^{bc}$ | 33,34±1,02$^{cde}$ | 56,16±2,61$^{b}$ | 27,77±1,08$^{b}$ |
| 60°C 30 kPa MDVK | 112,70±1,79$^{bc}$ | 49,95±5,08$^{cde}$ | 39,53±2,73$^{bcde}$ | 33,65±3,24$^{ef}$ | 8,48±1,39$^{bc}$ |
| 70°C 15 kPa VK | 86,55±3,49$^{bc}$ | 58,68±0,31$^{bcd}$ | 27,03±2,05$^{de}$ | 49,65±5,88$^{bc}$ | 10,59±2,94$^{bc}$ |
| 70°C 15 kPa MDVK | 77,50±0,31$^{c}$ | 22,53±0,84$^{g}$ | 23,78±0,93$^{e}$ | 36,46±1,80$^{def}$ | 18,74±0,50$^{bc}$ |
| 70°C 30 kPa VK | 92,15±3,79$^{bc}$ | 71,88±1,70$^{b}$ | 34,84±2,06$^{cde}$ | 38,42±6,15$^{def}$ | 11,37±0,76$^{bc}$ |
| 70°C 30 kPa MDVK | 134,21±4,58$^{b}$ | 37,52±0,86$^{efg}$ | 26,08±1,72$^{de}$ | 42,15±0,79$^{cde}$ | 19,50±1,19$^{bc}$ |
| 80°C 15 kPa VK | 80,69±0,64$^{c}$ | 41,47±13,25$^{def}$ | 33,07±3,74$^{cde}$ | 28,10±0,71$^{fg}$ | 12,61±1,11$^{bc}$ |
| 80°C 15 kPa MDVK | 78,75±4,96$^{c}$ | 64,24±6,82$^{bc}$ | 51,99±4,44$^{b}$ | 45,86±2,60$^{bcd}$ | 19,23±2,07$^{bc}$ |
| 80°C 30 kPa VK | 122,59±5,33$^{bc}$ | 60,70±3,10$^{bc}$ | 48,75±1,36$^{bc}$ | 38,73±1,59$^{cdef}$ | 11,51±1,54$^{bc}$ |
| 80°C 30 kPa MDVK | 116,57±3,63$^{bc}$ | 35,08±0,87$^{efg}$ | 41,50±1,44$^{bcd}$ | 22,13±0,37$^{g}$ | 3,59±0,55$^{c}$ |

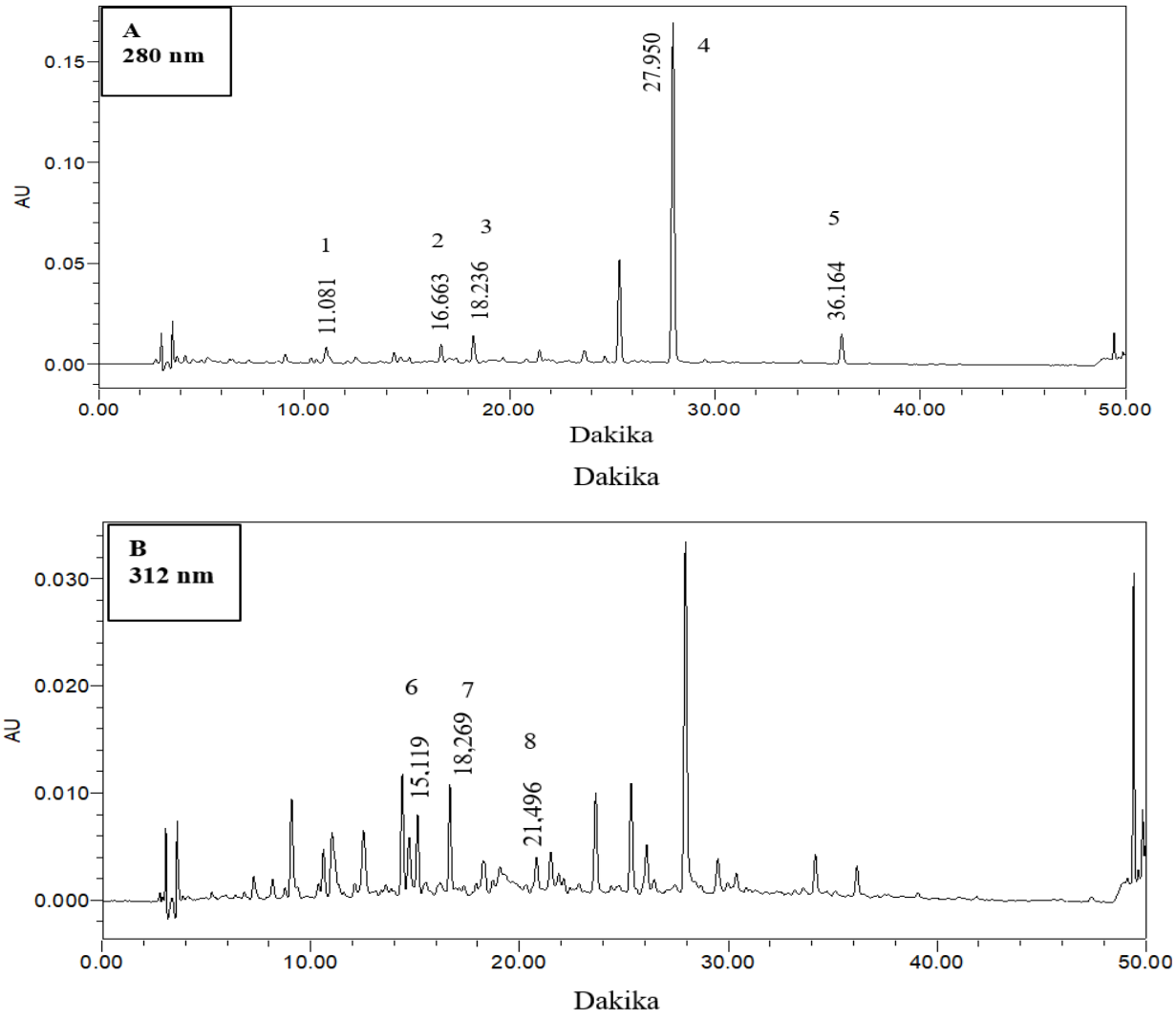
| Kurutma İşlemleri | Flavonoidler | | |
|---|---|---|---|
| | Epikateşin | Hesperidin | Naringenin |
| Taze | 930,75±39,44$^{a}$ | 5592,13±615,03$^{a}$ | 236,86±19,90$^{a}$ |
| 60°C 15 kPa VK | 275,80±25,92$^{bc}$ | 2255,99±149,65$^{c}$ | 81,25±13,71$^{ef}$ |
| 60°C 15 kPa MDVK | 129,92±14,49$^{ef}$ | 1091,93±144,63$^{e}$ | 67,15±2,87$^{ef}$ |
| 60°C 30 kPa VK | 141,67±23,27$^{ef}$ | 1280,88±48,02$^{de}$ | 65,95±1,75$^{efg}$ |
| 60°C 30 kPa MDVK | 167,59±12,92$^{de}$ | 2262,96±126,39$^{c}$ | 135,65±5,09$^{b}$ |
| 70°C 15 kPa VK | 181,17±21,15$^{de}$ | 1470,62±39,28$^{de}$ | 71,76±12,29$^{ef}$ |
| 70°C 15 kPa MDVK | 242,04±10,93$^{c}$ | 2468,07±56,17$^{bc}$ | 59,59±2,34$^{fg}$ |
| 70°C 30 kPa VK | 128,02±1,86$^{ef}$ | 1022,98±8,30$^{e}$ | 69,03±1,49$^{ef}$ |
| 70°C 30 kPa MDVK | 221,11±35,64$^{cd}$ | 2477,31±41,00$^{bc}$ | 143,40±3,03$^{b}$ |
| 80°C 15 kPa VK | 153,88±5,86$^{ef}$ | 1739,10±6,01$^{d}$ | 108,62±0,95$^{cd}$ |
| 80°C 15 kPa MDVK | 321,39±28,83$^{b}$ | 2837,76±159,08$^{b}$ | 127,12±2,45$^{bc}$ |
| 80°C 30 kPa VK | 167,57±10,73$^{de}$ | 1468,57±51,01$^{de}$ | 87,72±3,90$^{de}$ |
| 80°C 30 kPa MDVK | 99,20±20,93$^{f}$ | 1191,23±34,19$^{e}$ | 41,76±0,52$^{g}$ |

*Sütunlardaki farklı harfler istatistiksel olarak önemli farklılıkları temsil etmektedir ($p<0,05$)

Bu çalışma, yirmi bir çeşit turunçgil meyvesinin flavedo özütlerini inceleyen ve hesperidinin tüm flavedo özütlerinde en yüksek konsantrasyonlarda bulunduğunu bildiren Ramful ve ark. (2010)' un sonuçlarıyla güçlü bir şekilde korelasyon göstermektedir. Bir diğer çalışma, sıcak hava kurutma ve mikrodalga ön işlemi uygulanarak gerçekleştirilen sıcak hava kurutma yöntemleriyle elde edilen örneklerin analizleri yapılmıştır. Bu çalışmada, taze ve kurutulmuş portakal dilimlerinden elde edilen ekstraktların temel flavonoid ve fenolik asit bileşenleri sırasıyla hesperidin (1370,10–5592,13 mg/g km) ve vanilik asit (49,36–503,63 mg/g km) olarak belirlenmiştir. Elde edilen sonuçlar, hem sıcak hava kurutma hem de mikrodalga ön işlem uygulamalarının portakal dilimlerinin fenolik bileşen profilleri üzerindeki etkilerini net bir şekilde ortaya koymakta ve bu yöntemlerin ürün kalitesine olan etkisini vurgulamaktadır (Özkan-Karabacak ve ark., 2023). Bu çalışmanın bulguları, mevcut araştırmamızın sonuçlarıyla da uyum göstermektedir.

Safdar ve ark. (2017) HPLC yöntemini kullanarak gerçekleştirdikleri çalışmada, *Citrus reticulate* kabuğundaki en yüksek konsantrasyonlarda bulunan fenolik bileşikleri (gallik asit, klorojenik asit, hesperidin ve naringin) tespit etmiştir. Daha önce, Chun ve ark. (2007) flavonoidlerin bitkilerin antioksidan aktivitelerindeki rolüne dikkat çekmiştir. Portakalda bulunan başlıca flavanon bileşenleri hesperidin ve naringenin olup, bu bileşenler arasında hesperidin daha yüksek bir konsantrasyona sahiptir (Wang ve ark., 2008). Khan ve ark. (2010) tarafından yapılan bir çalışmada, portakal kabuğu ekstraktlarındaki flavanon bileşenleri olan hesperidin ve naringinin HPLC yöntemi ile sırasıyla 205,20 mg/100 g ve 70,30 mg/100 g taze ağırlık olarak ölçüldüğü rapor edilmiştir.

Kurutulmuş portakal dilimlerinde, taze portakal dilimlerine kıyasla fenolik bileşiklerde belirgin bir azalma gözlenmiştir. Bu bulgular, literatürde bildirilen termal işlemlerin fenolik ve flavonoid bileşikler üzerindeki bozucu etkileriyle uyumlu olup, özellikle yüksek sıcaklıkta yapılan kurutma işlemlerinin biyoaktif bileşiklerde kayıplara yol açabileceğini göstermektedir (Ahmed ve ark., 2024; M'hiri ve ark., 2017; Özkan-Karabacak ve ark., 2020). Literatürde fenolik bileşiklerin stabilitesini korumak amacıyla düşük sıcaklık ve vakum destekli kurutma tekniklerinin tercih edilmesi önerilmekte olup, bu çalışmada da görüldüğü gibi mikrodalga destekli vakumlu kurutma yönteminin uygun koşullarda uygulanması biyoaktif bileşiklerin daha iyi korunmasını sağlamaktadır (ElGamal ve ark., 2023; López ve ark., 2017; Papoutsis ve ark., 2017). Bu azalmanın, kurutma işlemi sırasında uygulanan ısının fenolik bileşiklerin yapısını bozmasıyla ilişkili olduğu düşünülmektedir. Ayrıca, fenolik içeriği etkileyen bu değişim, materyalin türü, yetiştirme koşulları, hasat zamanı, hasattan sonra portakalın dilimlenme şekli, uygulanan kurutma yöntemi, sıcaklık ve süre gibi çeşitli faktörlere de bağlı olabilir (Ahmed ve ark., 2024).




Şekil 1. 80°C 15 kPa VK kromatogramı A-280 nm (1) vanilik asit; (2) gallik asit; (3) epikateşin; (4) hesperidin; (5) naringenin; B-312 nm (6) sinapik asit; (7) klorojenik asit; (8) o-kumarik asit

*Figure 1. 80°C 15 kPa VK chromatogram A-280 nm (1) vanillic acid; (2) gallic acid; (3) epicatechin; (4) hesperidin; (5) naringenin; B-312 nm (6) sinapic acid; (7) chlorogenic acid; (8) o-coumaric acid*

***Portakal Dilimlerinin Kurutma Teknikleri ile Korelasyonunun, Temel Bileşen ve Hiyerarşik Kümeleme Analizleri Kullanılarak İncelenmesi***

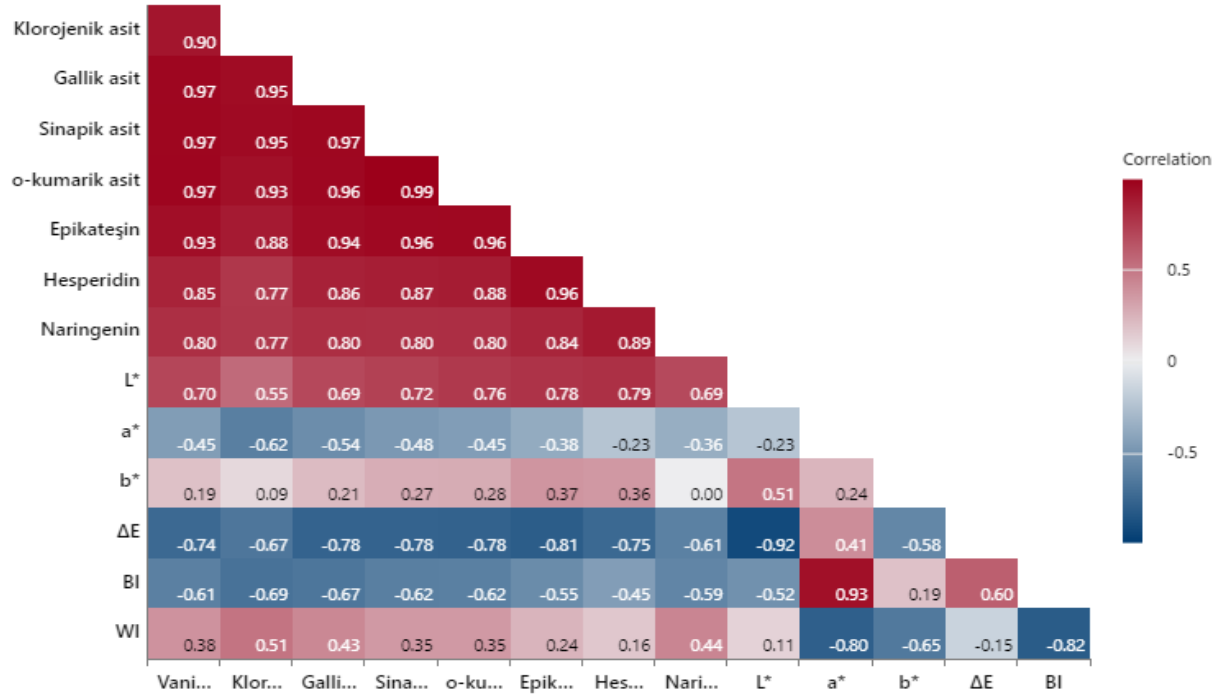
Korelasyon analizi, değişkenler arasındaki etkileşimleri anlamak ve dolaylı ilişkileri belirlemek açısından önemli bir rol oynamaktadır (Ratner, 2009). Elde edilen sonuçlar, kurutma yöntemlerinin portakal dilimlerinin renk özellikleri üzerindeki etkisini daha iyi kavramasına yardımcı olmaktadır.

Şekil 2, portakal dilimlerinin VK ve MDVK yöntemleriyle kurutulması sürecinde ölçülen ve hesaplanan parametreler arasındaki pozitif ve negatif doğrusal korelasyonları göstermektedir. Bu doğrultuda, renk parametreleri arasında birçok pozitif ve negatif anlamlı doğrusal korelasyonun varlığı saptanmıştır. En güçlü pozitif korelasyon, *BI* ile *a** ($R^2$=0,93) arasında gözlenmiştir; en güçlü negatif korelasyonlar ise *L** ile *ΔE** ($R^2$=-0,92), *BI* ile *WI* ($R^2$=-0,82) ve *a** ile *WI* ($R^2$=-0,80) arasında tespit edilmiştir. Ayrıca, *L** ile *b** ($R^2$=0,51) ve *BI* ile *ΔE** ($R^2$=0,60) arasında orta derecede pozitif korelasyon bulunurken, *WI* ile *b** ($R^2$=-0,65) ve *L** ile *BI* ($R^2$=-0,52) arasında orta derecede negatif korelasyon belirlenmiştir. Öte yandan, *ΔE** ile *a** ($R^2$=0,41) arasında zayıf pozitif bir korelasyon saptanırken, *ΔE** ile *b** ($R^2$=-0,58) arasında ise orta derecede negatif bir korelasyon saptanmıştır. Bu bulgular, Alibas & Yilmaz (2022) tarafından yapılan benzer bir çalışmada elde edilen sonuçlarla tutarlılık göstermektedir. Bir başka çalışmada ise; kinnow (*Citrus reticulata)* kabuklarının farklı kurutma teknikleri ile kurutulması sonucunda elde edilen renk parametreleri arasındaki korelasyon incelenmiştir. Elde edilen bulgular, beyazlatma indeksinin esmerleşme indeksi ile negatif bir korelasyon gösterdiğini ortaya koymaktadır. Ayrıca, *a** değeri ile *b** değeri arasında pozitif bir korelasyon gözlemlenirken, *a** değeri beyazlatma indeksi ile negatif bir korelasyon sergilemiştir (Rafiq ve ark., 2019). Bu sonuçlar, bu çalışmadan elde edilen bulgularla uyum göstermektedir.

Portakal dilimlerinin kurutulması sürecinde elde edilen veriler, polifenol bileşenleri ile renk parametreleri arasındaki ilişkilerin detaylı bir incelemesini sunmaktadır. Analiz sonuçlarına göre, *L** değeri ile polifenol bileşenleri arasında orta derecede pozitif korelasyon ($R^2$=0,55-0,79) bulunmuştur. Epikateşin bileşeni ile *ΔE** arasında yüksek

negatif korelasyon ($R^2$=-0,81) tespit edilmiştir. Diğer polifenol bileşenleri ile *ΔE** arasında ise orta derecede negatif korelasyon ($R^2$=-0,61 ila -0,78) saptanırken, *BI* ile polifenol bileşenleri arasında da benzer şekilde orta derecede negatif korelasyon ($R^2$=-0,52 ila -0,69) bulunmuştur. Çalışmalar, polifenol bileşenlerinin özellikle oksidatif reaksiyonlarla renk değişikliklerine katkıda bulunduğunu ve fenolik bileşenlerdeki azalmanın renk parametrelerini doğrudan etkilediğini göstermektedir (Pandey & Rizvi, 2009; Wojdylo ve ark., 2020). Örneğin, kurutma işlemi sırasında polifenollerin oksidasyonu, renk koyulaşmasına ve düşük *L** değerine yol açabilmektedir; bu durum, daha düşük parlaklık ile ilişkilendirilirken *b** ve *a** değerlerinde artışa neden olabilmektedir (Wojdylo ve ark., 2020). Ayrıca, BI değerindeki düşüş, polifenoloksidaz enziminin etkisiyle enzimatik esmerleşme reaksiyonlarının artışıylaa ilişkilendirilebilir; bu durum, genellikle biyoaktif bileşenlerin azalmasını ve renk koyulaşmasını yansıtmaktadır. Tüm polifenol bileşenleri kendi aralarında yüksek pozitif korelasyon ($R^2$=0,77-0,99) göstermiştir.

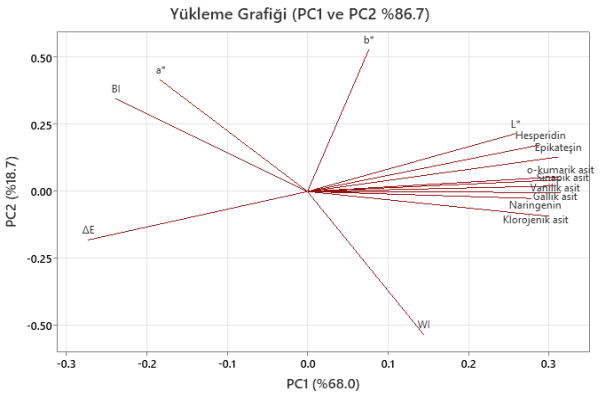
Temel bileşen analizi, büyük veri kümelerinin boyutluluğunu azaltmak ve sonuçların daha anlamlı bir şekilde yorumlanmasını kolaylaştırmak için kullanılmıştır. İstatistiksel analiz için tutulması gereken faktör sayısı, veri setinin yanı sıra açıklanan varyans miktarına da bağlıdır (Cozzolino ve ark., 2019).



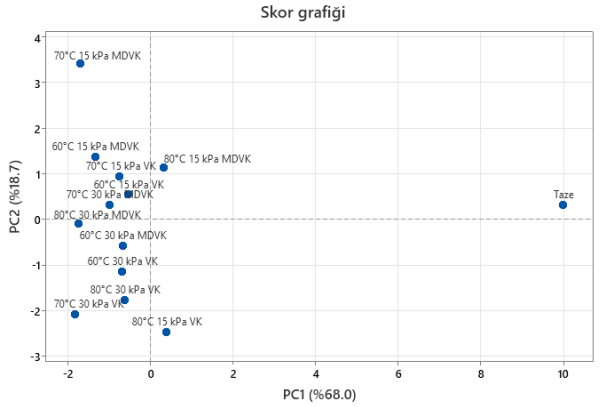

Şekil 2. Portakal dilimlerinin kurutma teknikleri ile korelasyonu
*Figure 2. Correlation of orange slices with drying techniques*

Çizelge 3. İki temel bileşene kadar açıklanan varyans oranları
*Table 3. Variance ratios explained up to two principal components*

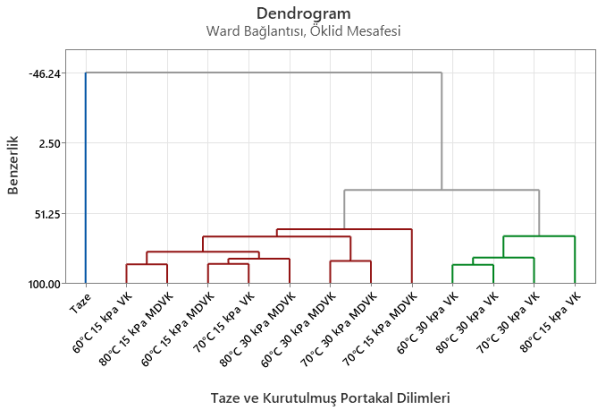
| Değişken | PC1 | PC2 |
|---|---|---|
| Özdeğer | 9,5211 | 2,6231 |
| Yüzde (%) | 68,0 | 18,7 |
| Kümülatif | 68,0 | 86,7 |
| Vanilik asit | 0,952 | 0,033 |
| Klorojenik asit | 0,929 | -0,150 |
| Gallik asit | 0,972 | -0,006 |
| Sinapik asit | 0,971 | 0,071 |
| o-kumarik asit | 0,971 | 0,089 |
| Epikateşin | 0,961 | 0,208 |
| Hesperidin | 0,893 | 0,282 |
| Naringenin | 0,857 | -0,042 |
| *L** | 0,797 | 0,349 |
| *a** | -0,567 | 0,675 |
| *b** | 0,236 | 0,857 |
| $\Delta E^*_{ab}$ | -0,841 | -0,292 |
| *BI* | -0,737 | 0,561 |
| *WI* | 0,448 | -0,866 |




Şekil 3. Portakal dilimlerin PCA-yükleme grafiği
*Figure 3. PCA-loading plot of orange slices*




Şekil 4. Portakal dilimlerin PCA-skor grafiği
*Figure 4. PCA-score plot of orange slices*




Şekil 5. Hiyerarşik kümeleme analizi
*Figure 5. Hierarchical clustering analysis*

Şekil 3'e göre, yükleme grafiğindeki her nokta, renk parametreleri ve polifenol bileşenleri gibi değişkenlerin skora katkısını temsil etmekte; skor grafiğindeki her nokta ise test edilen bir örneği ifade etmektedir. Temel bileşen analizinde, analizin güvenilirliğini sağlamak amacıyla özdeğerleri 1,00'den büyük olan bileşenler korunmuştur.

Çizelge 3'deki verilere göre, değişkenlerin PC1 ve PC2 bileşenlerine olan pozitif ve negatif katkıları, polifenolik bileşikler ve renk parametreleri açısından başlıca varyasyon kaynaklarını anlamaya yardımcı olmaktadır. PC1 bileşeni, toplam varyansın %68,0'ini açıklamakta olup, özellikle fenolik bileşiklerden vanilik asit (0,952), klorojenik asit (0,929), gallik asit (0,972), sinapik asit (0,971), *o*-kumarik asit (0,971), epikateşin (0,961), hesperidin (0,893) ve naringenin (0,857) yüksek pozitif yük değerleriyle dikkat çekmektedir. Bu durum, PC1'in polifenolik bileşenlerin etkisini yansıttığını göstermektedir. Renk parametrelerinden ise *L** (0,797) pozitif katkıda bulunurken, *ΔE** (-0,841) ve *BI* (-0,737) negatif yüklerle PC1'e katkı sağlamaktadır. Bu sonuçlar, PC1'in fenolik bileşiklerin yanı sıra renk değişiklikleriyle de ilişkili olduğunu işaret etmektedir.

PC2 bileşeni, toplam varyansın %18,7'sini açıklamakta ve renk parametrelerinden *b** (0,857), *a** (0,675) ve *BI* (0,561) değişkenleri yüksek pozitif yüklerle katkı sağlamaktadır. Buna karşın, PC2 bileşeninde *WI* (-0,866) ve *ΔE** (-0,292) değişkenlerinin negatif yükleri, bu bileşenin renk parametreleri üzerinde farklı yönlerde etkileri olduğunu göstermektedir.

Kümülatif olarak %86,7 oranında varyansı açıklayan bu iki bileşen, hem fenolik bileşiklerin hem de renk özelliklerinin değişiminde belirleyici olup, analiz edilen özelliklerin başlıca varyasyon kaynaklarını yeterince temsil etmektedir. Vektör yönünde ilerledikçe, ilgili niteliklerin değerlerinde bir artış gözlemlenmekte ve bu konumdaki örnekler söz konusu nitelikler ile tanımlanmaktadır. Vektörün ters yönü ise, bu nitelikler arasında negatif bir korelasyon bulunduğunu göstermektedir.

Şekil 4'te sunulan temel bileşen analizi (PCA) skor grafiği, portakal dilimlerinin PC1 ve PC2 eksenleri boyunca konumlanmalarını göstermektedir. Grafikteki verilere göre, PC1 boyunca taze portakal dilimleri yüksek bir korelasyon sergilemekte iken; 80°C 15 kPa MDVK örneği zayıf bir korelasyon göstermiştir. Polifenol bileşenleri ve *L** değeri, taze numunelerde yüksek değerlerle kümelenmiştir. Bununla birlikte, 60°C 30 kPa VK, 60°C 30 kPa MDVK, 70°C 30 kPa VK, 80°C 30 kPa VK ve 80°C 30 kPa MDVK örnekleri PC1'e negatif katkı sağlarken, özellikle *ΔE** parametresinin yüksek bir etki yarattığı gözlemlenmiştir. Ayrıca, 60°C 15 kPa VK, 60°C 15 kPa MDVK, 70°C 15 kPa VK, 70°C 15 kPa MDVK ve 70°C 30 kPa MDVK örnekleri PC2 ekseni boyunca pozitif bir katkı yaparken, 80°C 15 kPa VK numaralı örnek negatif bir etki göstermiştir. Ek olarak, 80°C 15 kPa VK örneği *WI* parametresinde yüksek değerlere sahip olduğu belirlenmiştir. Bu açıdan (Özkan-Karabacak ve ark., 2023) çalışmasıyla uyum göstermektedir. Bu bağlamda, önceki araştırmalar, farklı kurutma yöntemleri ve sıcaklıkların kurutulmuş ürünlerdeki biyoaktif bileşen seviyeleri üzerinde önemli etkileri olan temel faktörler olduğunu ortaya koymuş ve kurutma sürecinde meyvelerdeki kimyasal değişimlerin gerçekleştiğini desteklemiştir (Rafiq ve ark., 2019; Wojdylo ve ark., 2020).

Bu bulgular, elde edilen tüm değişkenler için gözlemlenen sonuçlarla tutarlılık göstermekte olup, önceki bölümlerde tartışılan verilerle uyum içindedir. PCA analizi, portakal dilimi örneklerinin kimyasal ve polifenol profillerini belirgin bir şekilde ayırt etmiş ve çeşitli parametrelerin dağılımı hakkında kapsamlı bir perspektif sunmuştur.

Hiyerarşik Kümeleme Analizi (HCA), incelenen örneklerdeki doğal gruplamaları tanımlamak amacıyla ölçülen özellikleri kullanarak yapılan sistematik bir sınıflandırma yöntemidir. Bu teknikte, öncelikle veriler standardize edilir ve ardından hiyerarşik kümeleme yöntemiyle, örnekler arasındaki benzerlik ve farklılıkları

yansıtan bir dendrogram oluşturulmaktadır (Patras ve ark., 2011). Şekil 5' te sunulan dendrogramda, taze ve kurutulmuş portakal dilimlerinin polifenolik içerikleri ve renk parametreleri, Ward yöntemi kullanılarak üç farklı grupta kümelenmiştir. Bu gruplama, kurutma işlemlerinin polifenolik içerik ve renk üzerinde belirgin bir etkiye sahip olduğunu ve kurutulmuş örneklerin taze örneklerden ayrıştığını göstermektedir. Hiyerarşik kümeleme analizi (HCA), yalnızca mesafelere dayandığı için Temel Bileşen Analizi'ne (PCA) kıyasla daha az bilgi kullanmaktadır. Yalnızca mesafelere dayanarak yapılabilecek sınıflandırma türlerinin gözlemlenmesi oldukça ilgi çekicidir (Patras ve ark., 2011).

Taze örnek, yüksek polifenol içeriği ile ilişkili olarak ilk kümede yer almakta ve diğer örneklerden belirgin bir şekilde ayrışmaktadır (Çizelge 2). Bu sonuç, taze örneğin PCA analizinde diğer örneklerden uzak bir konumda yer almasıyla uyum göstermektedir. İkinci küme, mikrodalga ön işlem uygulanan vakumlu kurutma teknikleri ile oluşturulmuş olup, bu kümeye ait 70°C 15 kPa MDVK uygulanan örnekler, renk parametreleri bakımından diğer örneklerden ayrılmaktadır (Çizelge 1). Bu farklılık, PCA analizinde de diğer örneklerden belirgin bir uzaklıkla konumlanan bu örnek için tutarlı sonuçlar sunmaktadır. Üçüncü küme ise yalnızca vakumlu kurutma teknikleri ile elde edilen örnekleri içermekte olup, kurutulmuş örneklerin polifenol içeriği ikinci kümeye kıyasla daha yüksektir (Çizelge 2). Bu durum, örneklerin ortak analitik özelliklerine göre doğal gruplama eğilimlerinin dendrogram analizinde açıkça gözlemlenmesiyle desteklenmektedir. Şekil 4' teki skor grafiği ile dendrogram karşılaştırıldığında, taze ve kurutulmuş örneklerin ayrıştığı, taze örneğin diğer örneklerden farklı bir davranış sergilediği görülmektedir. Bu bulgular, polifenolik bileşiklerin analizinde kullanılan HCA ve PCA tekniklerinin, örneklerin işlem koşullarına göre sınıflandırılmasında etkin bir araç olduğunu göstermektedir.

## Sonuç

Bu çalışma, belirli sıcaklık ve basınç koşullarında gerçekleştirilen vakumlu kurutma işlemi öncesinde uygulanan mikrodalga ön işleminin (90 W, 30 dk), portakal dilimlerinin kalite özellikleri üzerindeki etkisini ortaya koymaktadır. İncelenen koşullar arasında, 80°C ve 15 kPa vakumlu kurutma koşulu (mikrodalga ön işlem uygulanmadan) taze örneklere en yakın esmerleşme indeksi (*BI*) ve beyazlatma indeksi (*WI*) değerlerini sağlayarak portakal dilimlerinin doğal renk özelliklerinin korunmasında daha etkili olmuştur. Ayrıca, bu sıcaklık ve basınç koşulunda elde edilen polifenolik bileşik seviyeleri (örneğin, vanilik asit, klorojenik asit ve hesperidin), ürünün besin değerlerinin korunmasına yönelik önemli bir avantaj sağlamıştır.

Renk parametreleri ve polifenolik profil arasındaki ilişkilerin değerlendirilmesi için uygulanan PCA ve HCA, örneklerin kimyasal ve fenolik bileşen profillerinin ayrıştırılmasına olanak sağlamış ve 80°C ile 15 kPa koşulunun kalite parametreleri açısından en uygun koşul olduğunu vurgulamıştır. Portakal dilimlerinin renk ve besin değerlerinin korunması açısından en ideal yaklaşım olduğunu göstermektedir. Çalışma, narenciye kurutma işlemlerinde ürün kalitesini optimize etmeye yönelik önemli bulgular sunarak, endüstriyel işlem süreçleri ve tüketici sağlığı açısından önemli çıkarımlar sağlamaktadır. Gelecekte yapılacak araştırmalarda, farklı kurutma tekniklerinin (örneğin, ultrasonik veya osmotik ön işlemler) ve değişen sıcaklık ile basınç kombinasyonlarının çeşitli narenciye türlerinin yanısıra geniş bir ürün yelpazesinde biyokimyasal ve duyusal özellikleri üzerindeki etkilerini karşılaştırmalı olarak incelenmelidir. Özellikle uzun süreli depolama koşulları altında bu tür kurutulmuş ürünlerin kalite parametrelerinde meydana gelen değişimlerin incelenmesi, ürünün raf ömrü ve tüketici kabulü açısından değerli bilgiler sunacaktır. Ayrıca, kurutma sürecinde polifenolik bileşiklerin stabilitesini artırmak amacıyla farklı ön işleme yöntemleri veya alternatif kurutma koşulları ile entegre yaklaşımlar geliştirmek, ürün kalitesini daha etkin koruma stratejilerine katkı sağlayacaktır.

## Beyanlar

Makale yazarları olarak aramızda herhangi bir çıkar çatışması olmadığını beyan ederiz.

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# Effects of Pre-Harvest AVG (Aminoethoxyvinglycine) Applications on Fruit Quality and Cold Storage Time of 'Fuyu' Persimmon Cultivar

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ABSTRACT

In this study, the effects of AVG (Aminoethoxyvinglycine) applications at different doses (0, 75, 150 and 225 mg $l^{-1}$) 14 and 21 days before harvest on the fruits of the 'Fuyu' cultivar quality during storage were investigated. Fruits treated with AVG were packaged with modified atmosphere bags and stored at 0-1°C temperature and 85-90% relative humidity for 4 months. In the research, weight loss, total soluble solids content, titratable acidity, fruit firmness, ascorbic acid content, total phenolic content, total antioxidant content, respiration rate and chilling injury rates were examined. In the study, while fruit firmness decreased during storage, it was determined that the weight loss of fruits increased. In parallel with the delaying effect of AVG applications on fruit ripening, the occurrence of chilling injury was also delayed. The inhibitory effect of AVG on respiratory rate and loss of biochemical compounds became evident with increasing doses. As a result, 150 and 225 mg $l^{-1}$ AVG application applied before commercial harvest had a significant and positive effect on 'Fuyu' variety persimmon in terms of fruit quality during postharvest storage period.

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# Hasat Öncesi AVG (Aminoetoksivinilglisin) Uygulamalarının 'Fuyu' Trabzon Hurması Çeşidinin Meyve Kalitesi ve Soğukta Muhafaza Süresi Üzerine Etkileri

MAKALE BİLGİSİ

*Araştırma Makalesi*

Geliş : 22.01.2025
Kabul : 03.02.2025

*Anahtar Kelimeler:*
*Diospyros kaki*
Aminoethoxyvinglicine
Kalite
Depolama
Biyokimyasal bileşikler

ÖZ

Bu araştırmada 'Fuyu' çeşidi meyvelerine, hasattan 14 ve 21 gün önce farklı dozlarda (0, 75, 150 ve 225 mg $l^{-1}$) AVG (Aminoetoksivinilglisin) uygulamalarının depolama süresince meyve kalitesine etkileri incelenmiştir. AVG uygulaması yapılan meyveler modifiye atmosferli poşetler ile ambalajlanarak 0-1°C sıcaklık ve %85-90 oransal nemde 4 ay süreyle depolanmıştır. Araştırmada ağırlık kaybı, toplam suda çözünür kuru madde miktarı, titre edilebilir asit miktarı, meyve eti sertliği, askorbik asit miktarı, toplam fenolik madde miktarı, toplam antioksidan madde miktarı, solunum hızı ve üşüme zarar oranları incelenmiştir. Çalışmada depolama süresince meyve eti sertliği azalırken, meyvelerin ağırlık kayıplarında artışlar belirlenmiştir. AVG uygulamalarının meyve olgunlaşması üzerine olan geciktirici etkisine paralel olarak üşüme zararı oluşumu da gecikmiştir. Solumun hızı ve biyokimyasal bileşiklerin kaybı üzerine artan dozları ile AVG'nin engelleyici etkisi belirgin bir şekilde ortaya çıkmıştır. Sonuç olarak, ticari hasattan önce uygulanan 150 ve 225 mg $l^{-1}$ AVG uygulamasının, hasat sonrası depolama döneminde meyve kalitesi açısından Fuyu' çeşidi Trabzon hurması üzerinde önemli ve olumlu bir etkisi olmuştur.

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## Giriş

Trabzon hurması (*Diospyros kaki*), meyvelerin çekici turuncu rengi, kendine has tadı ve yapısı, antioksidan ve fenolik bileşikler bakımından zenginliği sebebi ile insanların ilgi odağı halindedir (Daood ve ark., 1992). Dünyada Trabzon hurması yetiştiriciliğinin, daha çok ılıman ve subtropikal bölgelerdeki ülkelerde yaygınlaştığı görülmektedir. Türkiye'de uzun senelerdir üretimi yapılan Trabzon hurması meyvesinin, 97500 tonluk üretim miktarı (TÜİK 2022) ile üretim ve pazarlama açısından henüz istenilen düzeyde olmadığı görülmektedir.

Klimakterik bir meyve olan Trabzon hurmasında, hasat sonrası dönemde ağırlık kaybı, üşüme zararı, meyve doku yumuşaması, kabuk ve et rengindeki değişimler gibi uğradıkları kalite kayıpları nedeni ile raf ömrü kısaltmakta ve pazar değerleri düşmektedir (Bal ve Zilci 2022). Meyvelerin olgunlaşmasını geciktirmek, su kaybını en aza indirmek ve hasat sonrası depolama ömrünü uzatmak amacıyla, hasat öncesi ve sonrası çeşitli uygulamalar yapılmaktadır. Düşük sıcaklıkta depolama, çoğu çabuk bozulabile üründe hasat sonrası kalite kayıplarını azaltmak ve pazarlama süresini uzatmak için kullanılan başlıca yöntemdir. Çalışmalar Trabzon hurması çeşitlerinin 2-4 ay soğukta muhafaza edilebildiğini bildirmektedir (Onur 1990; Koyuncu ve ark., 2005).

Ürünlerin hasat sonrası olgunlaşma ve yaşlanma süreçlerini etkileyen önemli unsurlardan birisi de etilendir. Etilen meyve olgunlaşmasını düzenleyen önemli bir hormondur. Dolayısıyla etilen üretiminin engellenmesi meyvenin hasat sonrası olgunlaşma sürecinin yavaşlatılmasında ve depolama ömrünün uzatılmasında önemli bir rol oynamaktadır. Aminoetoksivinilglisin (AVG), hasat sonrası aşamada meyve yumuşamasını önemli ölçüde geciktirebilen ve meyve raf ömrünü uzatabilen etilen sentezi engelleyicisidir. AVG birçok ülkede elma, armut, şeftali, erik ve nektarin için kullanılan insan ve çevre dostu organik bir üründür (Rath ve Prentice, 2004). AVG, S-adenosilmetionin'in etilen öncüsü 1-aminosiklopropan-1-karboksilik asite (ACC) dönüştürülmesinde rekabetçi bir inhibitör görevi görmektedir. Etilen biyosentez yolunu tersine çevrilebilir şekilde bloke etme kapasitesi nedeniyle, hasat öncesi ve sonrası AVG uygulamaları olgunlaşmayı geciktirmek ve klimakterik meyvelerin depolama potansiyelini arttırmak için kullanılmaktadır (Lara, 2013). Bazı klimakterik meyve türlerinde yapılan çalışmalarda hasattan önce uygulandığında içsel etilen seviyesini azaltarak klimakteriyumu geciktirmekte ve meyvelerin optimum olgunlukta daha uzun süre depolanmasını sağladığı belirlenmiştir (Butar, 2013; Koyuncu ve Çetinbaş, 2011; Öztürk ve ark., 2017; Öztürk ve ark., 2019). Ayrıca AVG'nin biyoaktif bileşikler üzerine etkilerinde çeşide, olgunluk seviyesine ve uygulama konsantrasyona bağlı olarak azalış veya artış şeklinde değişkenlikler de tespit edilmiştir (Yıldız ve ark., 2012; Karaman ve ark., 2013; Kucuker ve ark., 2015). Bu araştırmada hasat öncesi farklı dozda AVG uygulamalarının 'Fuyu' Trabzon hurmasının modifiye atmosferde muhafaza performansı ve meyve kalitesi üzerine olan etkisini belirlemek amaçlanmıştır.

## Materyal ve Yöntem

### *Bitki Materyali*

Denemede Tekirdağ ili Süleymanpaşa ilçesinde özel bir üretici bahçesinden 5x5 m aralıklarla dikilmiş 15 yaşındaki ağaçlardan hasat edilen 'Fuyu' (buruk olmayan, iri çekirdekli, sarı et rengi, iri ve basık meyve şekli, kabuk rengi sarı turuncu) Trabzon hurmaları (*Diospyros kaki* L.) kullanılmıştır.

### *Yöntem*

Meyvelere AVG'nin (ReTain, Valent BioScience) 0, 75, 150 ve 225 mg $l^{-1}$ dozları hasattan (tam çiçeklenmeden 150-160 gün sonra) 14 ve 21 gün önce ağaçlara püskürtme şeklinde, sabah erken ve rüzgarsız bir zaman diliminde sırt pompası ile uygulanmıştır. Denemede Fuyu' Trabzon hurması çeşidine ait toplam 24 adet ağaç kullanılmıştır. AVG püskürtmelerinde bileşiğin tesirini yükseltmek amacıyla, çözeltiye uygulanan yüzeyde gerilimi düşüren yayıcı yapıştırıcı (%0,1 Tween 20) ilave edilmiş ve kontrol ağaçlarına yalnızca yayıcı yapıştırıcı çözeltisi uygulanmıştır. Çalışmada kullanılan meyveler elle hasat edilip, belli bir standart oluşması için birbirlerine yakın irilikte ve üzerlerinde herhangi bir fiziksel ve fungal zarar belirtisi görülmeyen meyveler seçilmiştir. 4 gruba (0, 75, 150 ve 225 mg $l^{-1}$) ayrılan meyveler viyollere yerleştirilip MAP poşetleri (Fresh Plus) ile ambalajlanarak 0-1°C sıcaklık ve %85-90 nispi nem koşullarında 4 ay boyunca depolanmıştır. Depolama başlangıcında ve 30 gün aralıklarla depodan alınan meyvelerde bazı fiziksel ve kimyasal analizler gerçekleştirilmiştir.

*Ağırlık kaybı*

Ağırlık kaybı, muhafaza öncesinde ağırlıkları belirlenen meyve gruplarının, soğuk hava deposundan çıkarılmasının ardından, 0.01 g hassasiyet özelliğindeki terazi ile tartılıp yüzde (%) olarak belirlenmiştir.

*Suda çözünür toplam kuru madde miktarı (SÇKM)*

SÇKM tespiti meyvelerin sıkılarak sularının elde edilmesi ile el tipi refraktometre kullanılarak ölçülmüş ve sonuçlar % olarak hesaplanmıştır.

*Titre edilebilir asit miktarı (TEA)*

Titre edilebilir asit miktarı titrimetrik yöntemle meyvelerin suyu kullanılarak malik asit cinsinden mg 100 $ml^{-1}$ olarak tayin edilmiştir.

*Meyve eti sertliği*

Meyve eti sertliği tespiti Penetrometre (7.9 mm uç) kullanılarak kg olarak ölçülmüştür.

*Askorbik asit miktarı*

Meyve suyu içeriğindeki C vitamini miktarı 2,6-dikloroindofenol ile titrimetrik yöntem kullanılarak belirlenmiş, mg $kg^{-1}$ meyve suyu olarak ifade edilmiştir (Cemeroğlu, 2007).

*Toplam fenolik madde miktarı*

Meyve gruplarının depolanması boyunca toplam fenolik madde bileşimindeki farklılıkların tespit edilmesinde Slinkard ve Singleton (1977) tarafından geliştirilen spektrofotometrik metottan yararlanılmıştır. Örneklemelerde absorbans değerinin gallik asit (GA) cinsinden eşdeğeri olan fenolik bileşik miktarı mg GA 100 $g^{-1}$ cinsinden ifade edilmiştir.

*Toplam antioksidan miktarı*

Meyve ekstraksiyonları üzerine 0,1 mM DPPH (1,1-difenil 2-pikril hidrazil) metanolik çözeltisinden 1,95 ml ilave edilip ve karıştırma işlemi gerçekleştirilmiştir. Yapılan karışım oda sıcaklığında, karanlıkta 30 dk süre ile dinlendirilmesinin ardından absorbans değeri 517 nm dalga boyunda, spektrofotometrede okumalar yapılmıştır (Garzón ve Wrolstad, 2009). Örneğe ilişkin okuma değerleri mM (trolox eşdeğeri) $100g^{-1}$ olarak belirtilmiştir.

*Solunum hızı*

MAP içerisinde yer alan meyvelerin solunum hızı her analiz döneminde Systec Instrument Gaspace $CO_2$ analizatörü ile ölçülerek ortamda oluşan $CO_2$ düzeyi belirlenmiştir.

*Üşüme zararı*

Üşüme zararı değerleri 0-4 skalasına (0 = yok, 1 = hafif (esmerleşme yüzey alanının %25'inden az), 2 = orta (yüzey alanının %25 ile %50'si), 3 = şiddetli (yüzey alanının %50 ile %75'i) ve 4 = çok şiddetli (yüzey alanının %75'inden büyük) göre değerlendirilmiştir (Zhang ve ark., 2010).

Arazi uygulamaları 3 tekerrürlü ve her tekerrürde 2 ağaç olacak şekilde düzenlenmiştir. Depolama çalışmaları ise 'Tesadüf Parsellerinde Faktöriyel Düzen' deneme desenine göre planlanmıştır. Çalışmalar, 3 tekerrürlü ve her tekerrürde 8 meyve olacak şekilde düzenlenmiştir. Tüm istatistiksel analizler, SPSS 18 paket programında analiz edilmişlerdir. Varyasyon kaynaklarına ait ortalamaların karşılaştırılmasında LSD testi ($p<0.05$) kullanılmıştır.

## Bulgular ve Tartışma

### *Ağırlık Kaybı*

Trabzon hurması meyvesi, mumsu bir kutikula ile kaplı olması ve meyve yüzeyinde stoma veya lentisel bulunmadığından meyve gaz değişimi için kalikse bağımlıdır (Pérez-Munuera ve ark., 2009; Woolf ve Ben-Arie, 2011). Araştırmada modifiye atmosfer koşullarında soğukta muhafazası edilen Trabzon hurması meyvelerinin muhafaza süreleri arttıkça yüzdesel ağırlık kayıpları da zamanla artmıştır (Çizelge 1). Ancak depo çıkışı verilerinde muhafaza süresi x uygulama interaksiyon ve uygulama ortalamalarının istatistiksel olarak etkisi önemsiz görülürken, sadece zaman ortalamalarının ağırlık kayıpları üzerine etkileri istatistiksel olarak ($p<0.05$) önemli bulunmuştur. Muhafazanın 1. ayında ortalama %0,51 olan ağırlık kaybı, 3.ayda %2,56 ve 4.ay sonunda da %4,65 olarak belirlenmiştir. Yapılan gözlemler neticesinde ağırlık kaybının ticari açıdan önemli olabileceği kilit alanın kaliksin yeşil yapraklı dokusunu kuruması ve kahverengileşmesinin sonucu perakendeci veya tüketicinin tazelik algısını olumsuz etkileyebileceği bir görünüme sahip olması olduğu düşünülmektedir.

### *Suda Çözünür Toplam Kuru Madde Miktarı*

Araştırmada sadece zaman ortalama değerleri SÇKM miktarı üzerine etkileri istatistiksel olarak ($p<0.05$) seviyesinde önemli bulunmuştur (Çizelge 2). Hasattan sonra tüm uygulamalarda muhafazanın 1. ayında SÇKM'de artış, diğer aylarda orantılı olarak azalış gözlemlenmiştir. Hasat zamanında ortalama %19,26 olan SÇKM miktarı, 1. ayda %19,91, 2. ayda %18,63, 3 ayda %17,80 ve 4. ayda da %17,06 olarak saptanmıştır. Meyvelerde genel olarak olgunlaşmaya bağlı SÇKM değeri yükselmektedir. Yapılan farklı çalışmalarda AVG'nin meyvede olgunlaşmayı yavaşlatıp SÇKM içeriğini düşük düzeyde tuttuğu belirtilmiştir (Öztürk ve ark., 2013; Yaldız, 2014). Çalışmada AVG uygulamalarının SÇKM miktarı üzerine etkileri istatistiksel olarak önemsiz olsa da hasat döneminde AVG uygulamalarının kontrole göre daha düşük seviyede olduğu belirlenmiştir.

### *Titre Edilebilir Asit Miktarı*

Çalışmada muhafaza süresi ve AVG dozları TEA üzerine etkili olurken ($p<0.05$), muhafaza süresi x uygulama interaksiyonu istatistiksel olarak önemli bulunmamıştır (Çizelge 3).

Çizelge 1. Fuyu Trabzon hurması çeşidinde AVG uygulamaların ağırlık kaybı üzerine etkileri (%)
*Table 1. Effects of AVG applications on weight loss in Fuyu persimmon cultivar*

| Uygulamalar | Muhafaza süresi | | | | Uygulama ort. |
|---|---|---|---|---|---|
| | 1.ay | 2.ay | 3.ay | 4.ay | |
| Kontrol | 0,55 | 1,15 | 2,72 | 4,64 | 2,26 |
| AVG 75 | 0,44 | 1,25 | 2,66 | 4,97 | 2,33 |
| AVG 150 | 0,58 | 1,30 | 2,50 | 4,53 | 2,23 |
| AVG 225 | 0,47 | 1,04 | 2,36 | 4,48 | 2,09 |
| Zaman ort. | 0,51A | 1,18B | 2,56C | 4,65D | |

Büyük harfler muhafaza süreleri, küçük italik harfler uygulamalar ve küçük harfler de uygulama x muhafaza süresi interaksiyonu arasındaki farklılıkları ($p<0.05$) göstermektedir. LSD zaman:0,254

Çizelge 2. Fuyu Trabzon hurması çeşidinde AVG uygulamaların SÇKM üzerine etkileri (%)
*Table 2. Effects of AVG applications on TSS in Fuyu persimmon cultivar*

| Uygulamalar | Muhafaza Süresi | | | | | Uygulama ort. |
|---|---|---|---|---|---|---|
| | Hasat | 1.ay | 2.ay | 3.ay | 4.ay | |
| Kontrol | 19,86 | 20,46 | 17,86 | 17,53 | 16,66 | 18,47 |
| AVG 75 | 19,13 | 19,86 | 18,60 | 17,46 | 16,86 | 18,38 |
| AVG 150 | 19,33 | 19,66 | 19,20 | 17,96 | 17,20 | 18,67 |
| AVG 225 | 18,73 | 19,66 | 18,86 | 18,26 | 17,53 | 18,61 |
| Zaman ort. | 19,26AB | 19,91A | 18,63BC | 17,80CD | 17,06D | |

Büyük harfler muhafaza süreleri, küçük italik harfler uygulamalar ve küçük harfler de uygulama x muhafaza süresi interaksiyonu arasındaki farklılıkları ($p<0.05$) göstermektedir. LSD zaman:0,656

Çizelge 3. Fuyu Trabzon hurması çeşidinde AVG uygulamaların TEA üzerine etkileri (g $100g^{-1}$)
*Table 3. Effects of AVG applications on TA in Fuyu persimmon cultivar*

| Uygulamalar | Muhafaza Süresi | | | | | Uygulama ort. |
|---|---|---|---|---|---|---|
| | Hasat | 1.ay | 2.ay | 3.ay | 4.ay | |
| Kontrol | 0,26 | 0,24 | 0,22 | 0,17 | 0,15 | 0,21c |
| AVG 75 | 0,27 | 0,26 | 0,24 | 0,20 | 0,14 | 0,22bc |
| AVG 150 | 0,30 | 0,29 | 0,25 | 0,18 | 0,17 | 0,24ab |
| AVG 225 | 0,31 | 0,28 | 0,25 | 0,20 | 0,19 | 0,24a |
| Zaman ort. | 0,28A | 0,27A | 0,24B | 0,19C | 0,16C | |

Büyük harfler muhafaza süreleri, küçük italik harfler uygulamalar ve küçük harfler de uygulama x muhafaza süresi interaksiyonu arasındaki farklılıkları ($p<0.05$) göstermektedir. LSD uygulama:0,016 LSD zaman:0,018

Çizelge 4. Fuyu Trabzon hurması çeşidinde AVG uygulamaların meyve eti sertliği üzerine etkileri (kg)
*Table 4. Effects of AVG applications on fruit firmness in Fuyu persimmon variety*

| Uygulamalar | Muhafaza Süresi | | | | | Uygulama ort. |
|---|---|---|---|---|---|---|
| | Hasat | 1.ay | 2.ay | 3.ay | 4.ay | |
| Kontrol | 5,16bc | 4,20e | 1,93g | 1,16ı | 0j | 2,49b |
| AVG 75 | 5,23bc | 4,60de | 1,64gh | 1,20hı | 0j | 2,54b |
| AVG 150 | 5,56ab | 4,80cd | 2,80f | 2,06g | 0j | 3,04a |
| AVG 225 | 5,73a | 4,90cd | 3,06f | 1,96g | 0j | 3,13a |
| Zaman ort. | 5,42A | 4,62B | 2,36C | 1,60D | 0E | |

Büyük harfler muhafaza süreleri, küçük italik harfler uygulamalar ve küçük harfler de uygulama x muhafaza süresi interaksiyonu arasındaki farklılıkları ($p<0.05$) göstermektedir. LSD uygulama:0,280 LSD zaman:0,283 LSD uygulama x zaman:0,478

Çizelge 5. Fuyu Trabzon hurması çeşidinde AVG uygulamaların askorbik asit üzerine etkileri (mg $kg^{-1}$)
*Table 5. Effects of AVG applications on ascorbic acid in Fuyu persimmon cultivar*

| Uygulamalar | Muhafaza Süresi | | | | | Uygulama ort. |
|---|---|---|---|---|---|---|
| | Hasat | 1.ay | 2.ay | 3.ay | 4.ay | |
| Kontrol | 225,3 | 208,6 | 167,0 | 153,6 | 116,6 | 174,2 |
| AVG 75 | 236,3 | 216,6 | 156,0 | 151,3 | 122,3 | 176,5 |
| AVG 150 | 215,6 | 226,3 | 171,0 | 166,0 | 136,3 | 183,0 |
| AVG 225 | 220,3 | 231,0 | 173,6 | 162,6 | 130,6 | 183,6 |
| Zaman ort. | 224,4A | 220,6A | 166,9B | 158,4B | 126,5C | |

Büyük harfler muhafaza süreleri, küçük italik harfler uygulamalar ve küçük harfler de uygulama x muhafaza süresi interaksiyonu arasındaki farklılıkları ($p<0.05$) göstermektedir. LSD zaman:1,.43

Hasat döneminde AVG uygulanmış meyvelerde TEA değerleri nisbi olarak daha yüksek olduğu tespit edilmekle birlikte, depolama süresince tüm uygulamalarda başlangıç seviyesine göre düşüşler belirlenmiştir. Hasat döneminde ortalama %0,28 olan TEA değeri 4. ay sonunda %0,16 düşmüştür. Ayrıca uygulamaların ortalamalarına bakıldığında en yüksek değer %0,24 ile AVG 225 uygulamasında görülürken, en düşük değer %0,21 ile kontrol grubunda saptanmıştır. AVG uygulamalarının olgunlaşmayı geciktirerek asitlik kaybını yavaşlattığı düşünülmektedir. Benzer şekilde Öztürk ve ark. (2014) araştırmalarında elma meyvesinde yükselen AVG dozajlarının TEA bileşimini artırdığını belirtmektedir. Aynı zamanda, Çetinbaş ve ark. (2012) 'Monroe' çeşidi şeftalilerde yürüttükleri araştırmada, AVG püskürtülen meyvelerin TEA içeriklerinin depolama koşulunda kontrol meyvelerinkinden daha yüksek olduğunu tespit etmiştir.

***Meyve Eti Sertliği***

Araştırmada tüm uygulamalarda soğukta muhafaza süresince meyve sertliğinde doğrusal bir azalma kaydedilmiştir. AVG uygulamalarının hem uygulama ve muhafaza süresi üzerine etkilerini hem de bunlar arasındaki interaksiyon istatistiki açıdan önemli ($p<0.05$) olduğu tespit edilmiştir (Çizelge 4). Ancak AVG 150 ve AVG 225 uygulamalarıyla karşılaştırıldığında kontrol ve AVG 75 uygulamalarındaki düşüşler daha hızlı gerçekleşmiştir. Hasat döneminde ortalama meyve eti sertliği 5,42 kg iken 4. ay sonunda tüm meyvelerde aşırı doku yumuşaması nedeniyle ölçüm yapılamamıştır. Araştırmada uygulama ortalama değerleri incelendiğinde en düşük meyve eti sertliğinin 2,49 kg ile kontrol grubu meyvelerinde, en yüksek meyve eti sertliğinin 3,13 kg ile AVG 225 grubu meyvelerinde olduğu gözlemlenmiştir. Bulgularımıza benzer şekilde, AVG uygulamasının elma, erik ve şeftali meyvelerinin olgunlaşmasını geciktirdiği ve et sertliği kaybını yavaşlattığı ifade edilmiştir (Eisermann ve ark., 2008; Koyuncu ve Çetinbaş, 2011; Karaman ve ark. 2013; Öztürk ve ark., 2017). Jobling ve ark. (2003) ise, 'Tegan Blue' erik çeşidinde meyve sertliğinin korunmasının nedenini AVG uygulamalarıyla meyvelerde etilen üretiminin baskılanması olarak bildirmişlerdir.

***Askorbik Asit Miktarı***

Muhafaza süresi boyunca sadece zaman ortalama değerleri askorbik asit içeriğine etkisi önemli bulunmuştur ($p<0.05$). Genel olarak, meyvelerin askorbik asit içeriği AVG uygulamalarından bağımsız olarak depolama süresinin artmasıyla birlikte doğrusal bir azalma sergilemiştir. Başlangıçta kontrol grubunda 225,3 mg $kg^{-1}$, AVG 75 uygulamasında 236,3 mg $kg^{-1}$, AVG 150 uygulamasında 215,6 mg $kg^{-1}$, AVG 225 uygulamasında 220,3 mg $kg^{-1}$olan askorbik asit miktarı 4 aylık depolama sonucunda ise aynı sırayla 116,6 mg $kg^{-1}$, 122,3 mg $kg^{-1}$, 136,3 mg $kg^{-1}$ ve 130,6 mg $kg^{-1}$ olarak belirlenmiştir.

Çizelge 6. Fuyu Trabzon hurması çeşidinde AVG uygulamaların toplam fenolik bileşikler üzerine etkileri (mg $100g^{-1}$)
*Table 6. Effects of AVG applications on total phenolic compounds in Fuyu persimmon cultivar*

| Uygulamalar | Muhafaza Süresi | | | | | Uygulama ort. |
|---|---|---|---|---|---|---|
| | Hasat | 1.ay | 2.ay | 3.ay | 4.ay | |
| Kontrol | 36,50ab | 34,46a-d | 30,00e-h | 26,10hıj | 21,53k | 29,72b |
| AVG 75 | 35,46abc | 35,10abc | 32,83b-e | 28,06f-ı | 22,66jk | 30,82ab |
| AVG 150 | 32,66b-e | 37,10a | 34,43a-d | 30,93d-g | 25,93ıj | 32,21a |
| AVG 225 | 32,00c-f | 36,40ab | 35,20abc | 32,60b-e | 27,76ghı | 32,79a |
| Zaman ort. | 34,15A | 35,76A | 33,11A | 29,42B | 24,47C | |

Büyük harfler muhafaza süreleri, küçük italik harfler uygulamalar ve küçük harfler de uygulama x muhafaza süresi interaksiyonu arasındaki farklılıkları ($p<0.05$) göstermektedir. LSD uygulama:1,44 LSD zaman:1,61 LSD uygulama zaman:4,02

Askorbik asit bileşiği, meyveleri depolama ve işleme esnasında oksidasyonundan kaynaklı bozulmalara karşı oldukça duyarlıdır (Veltman ve ark., 2000). Elde edilen sonuçlara benzer şekilde Kuzucu ve Kaynaş (2004)'da Trabzon hurması ile yapmış oldukları bir araştırmada askorbik asit içeriğinin yükselme ve düşüş olarak dalgalanmalar gösterdiği depolama süresi arttıkça da askorbik asit içeriğinin azaldığını belirlemişlerdir.

***Toplam Fenolik Madde Miktarı***

Araştırmada muhafaza süresi başlangıcında, toplam fenolik madde içeriğinde artış azalış şeklinde dalgalanmalar tespit edilirken, muhafazanın 2. ayından itibaren tüm meyve gruplarında düşüş tespit edilmiştir (Çizelge 6). Ghasemnezhad ve ark. (2010) kayısı meyvesinde yaptıkları bir çalışmada soğukta muhafaza boyunca toplam fenolik madde içeriğinin düşmesinin, olgunlaşma fizyolojisi veya hücre yapısının bozulması ile ilişkilendirilebileceğini bildirmiştir. Araştırmada hasat sırasında yapılan ölçümlerde AVG uygulanan meyvelerde kontrol meyvesine göre daha düşük toplam fenolik bileşik içeriği tespit edilmiştir. Benzer şekilde Kılıç (2013) ve Güler ve ark. (2019) kiraz meyvelerinde AVG uygulamalarının fenolik madde oluşumunu geciktirdiğini de bildirmiştir. Muhafaza süresince yapılan ölçümlerde kontrol grubu meyvelerinin en yüksek toplam fenolik madde bileşimine sahipken (36,50 mg $100g^{-1}$), AVG 225 grubu meyvelerin ise en düşük toplam fenolik madde içeriğine sahip olduğu (32,00 mg $100g^{-1}$) tespit edilmiştir. Ancak olgunlaşmanın ilerlemesiyle AVG uygulanmış meyveler fenolik bileşik içeriklerini daha iyi korumuştur. Araştırmanın uygulama ortalamaları incelendiğinde kontrol grubu meyvelerinin en düşük toplam fenolik madde içeriğine sahip (29,72 mg $100g^{-1}$), AVG 225 grubu meyvelerin ise en yüksek içeriğe (32,79 mg $100g^{-1}$) sahip olduğu görülmüştür.

***Toplam Antioksidan Miktarı***

Büyüme düzenleyicilerin hasat öncesi uygulanması, fenolik bileşiklerin profilini, toplam antioksidan aktiviteyi ve meyvenin kalitesini değiştirebilmektedir. Çalışmada muhafaza süresi x uygulama interaksiyonu, uygulama ve zaman ortalama değerleri istatistiki açıdan %5 düzeyinde önemli tespit edilmiştir (Çizelge 7). Fuyu Trabzon hurmasında hasat dönemi en düşük antioksidan içeriği 244,0 mM $100g^{-1}$ değeri ile AVG 75 grubu meyvelerinde, en yüksek antioksidan içeriği 272,6 mM $100g^{-1}$ değeri ile AVG 225 grubu meyvelerinde ölçülürken, kontrol grubu meyvelerinde 261,0 mM $100g^{-1}$ değerinde ölçülmüştür. Uygulama ortalama değerleri ele alındığında en yüksek antioksidan değeri 286,3 mM $100g^{-1}$ olarak AVG 225 grubu meyvelerinde, en düşük antioksidan değeri ise 256,52 mM $100g^{-1}$ olarak AVG 75 grubu meyvelerde tespit edilmiştir. Benzer şekilde Güler ve ark. (2019) ile Öztürk ve ark. (2019) kiraz ve kivi meyvelerinde AVG ve MAP uygulamalarının kontrole göre önemli ölçüde daha yüksek C vitamini, toplam fenolikler ve antioksidan aktivite değerlerine sahip olduğunu bildirmiştir. Bununla birlikte, Karaman ve ark. (2013) ile Kucuker ve ark. (2015), erik meyvelerinde hasat öncesi AVG uygulamaları yaparak yürüttükleri araştırmalarda, hasat döneminde kontrol grubuna göre AVG uygulaması yapılmış meyvelerde antioksidan içerikleri daha yüksek bulunmasına rağmen, depolama sürecinde artan AVG dozlarının toplam fenolik ve antioksidan aktivitede azalmaya neden olduğunu rapor etmişlerdir. Bu durum AVG'nin etkisinin meyve türü ve çeşidi ile uygulama dozu ve süresine bağlı olarak değişebileceği düşüncesiyle açıklanabilir.

***Solunum Hızı***

Araştırmada solunum hızı değerleri başlangıç değerlerine göre tüm uygulamalarda artış olduğu ve AVG uygulamalarının solunum hızı kontrol grubuna göre daha düşük olduğu tespit edilmiştir (Çizelge 8). Hasattan önce uygulanan AVG ve 1-MCP gibi büyüme düzenleyiciler, meyvede klimakterik artışı geciktirerek solunum hızını sınırlandırdığı bildirilmiştir (Ordoñez ve ark., 2023). Yapılan çalışmada hasat döneminde solunum hızı değerleri AVG uygulanmış meyvelerde kontrol grubuna göre daha düşük seviyede olduğu gözlemlenmiş ve 4.ay ölçümlerine kadar devam etmiştir. Uygulama ortalama değerleri içerisinde en yüksek solunum hızı değeri kontrol grubu uygulamasında (27,54 ml $CO_2$ $kg^{-1}$ $h^{-1}$), en düşük solunum hızı değeri ise (25,55 ml $CO_2$ $kg^{-1}$ $h^{-1}$) AVG 225 grubu meyvelerinde ölçülmüştür. Bulgularımıza benzer şekilde, Kim ve ark., (2004) ile Koyuncu ve Çetinbaş, (2011) AVG uygulamasının şeftali meyvelerinde etilen üretimini ve solunum hızını azalttığını tespit etmişlerdir. Birçok araştırmada da AVG'nin uygulanan meyvelerde depolama boyunca kontrol örneklerine göre daha düşük bir solunum hızını koruduğu tespit edilmiştir ( Butar, 2013; Ünsal, 2017; Bal, 2019; Bal, 2020).

***Üşüme Zararı***

Araştırmada muhafaza süresi arttıkça üşüme zararının da yüzdesel olarak arttığı ve üşüme zararına bağlı olarak meyvelerin genel görünümleri de bozulma tespit edilmiştir. Trabzon hurması meyvelerinde üşüme zararının meyve etinin yumuşaması ve siyahımsı jel oluşumu şeklinde gözlemlendiği bildirilmiştir (Macrae, 1987).

Çizelge 7. Fuyu Trabzon hurması çeşidinde AVG uygulamaların toplam antioksidan üzerine etkileri (mM $100g^{-1}$)
*Table 7. Effects of AVG applications on total antioxidant in Fuyu persimmon cultivar*

| Uygulamalar | Muhafaza Süresi | | | | | Uygulama ort. |
|---|---|---|---|---|---|---|
| | Hasat | 1.ay | 2.ay | 3.ay | 4.ay | |
| Kontrol | 261,0fgh | 287,6a-e | 291,6a-d | 265,6e-h | 229,6ij | 267,1bc |
| AVG 75 | 244,0hı | 271,3d-g | 277,3b-g | 270,6d-g | 217,6j | 256,52c |
| AVG 150 | 255,6gh | 281,3a-f | 272,6c-g | 292,6a-d | 275,6b-g | 275,6ab |
| AVG 225 | 272,6c-g | 296,6abc | 304,6a | 297,0ab | 260,6fgh | 286,3a |
| Zaman ort. | 258,3B | 284,2A | 286,5A | 281,5A | 245,29B | |

Büyük harfler muhafaza süreleri, küçük italik harfler uygulamalar ve küçük harfler de uygulama x muhafaza süresi interaksiyonu arasındaki farklılıkları ($p<0.05$) göstermektedir. LSD uygulama:11,03 LSD zaman:12,34 LSD uygulama x zaman:24,95

Çizelge 8. Fuyu Trabzon hurması çeşidinde AVG uygulamaların solunum hızı üzerine etkileri (ml $CO_2$ $kg^{-1}$ $h^{-1}$)
*Table 8. Effects of AVG applications on respiration rate in Fuyu persimmon cultivar*

| Uygulamalar | Muhafaza süresi | | | | Uygulama ort. |
|---|---|---|---|---|---|
| | 1.ay | 2.ay | 3.ay | 4.ay | |
| Kontrol | 26,23 | 27,73 | 29,60 | 26,60 | 27,54b |
| AVG 75 | 24,90 | 27,20 | 28,36 | 26,30 | 26,69ab |
| AVG 150 | 23,00 | 25,66 | 27,33 | 27,16 | 25,79a |
| AVG 225 | 23,43 | 24,43 | 26,40 | 27,93 | 25,55a |
| Zaman ort. | 24,39A | 26,25B | 27,00B | 27,92B | |

Büyük harfler muhafaza süreleri, küçük italik harfler uygulamalar ve küçük harfler de uygulama x muhafaza süresi interaksiyonu arasındaki farklılıkları ($p<0.05$) göstermektedir. LSD uygulama:2,43 LSD zaman:2,95

Çizelge 9. Fuyu Trabzon hurması çeşidinde AVG uygulamaların üşüme zararı üzerine etkileri (0-4)
*Table 9. Effects of AVG applications on chilling injury in Fuyu persimmon cultivar*

| Uygulamalar | Muhafaza süresi | | | | Uygulama ort. |
|---|---|---|---|---|---|
| | 1.ay | 2.ay | 3.ay | 4.ay | |
| Kontrol | 0 | 0,50 | 1,50 | 3,16 | 1,29bc |
| AVG 75 | 0 | 0,66 | 2,00 | 3,26 | 1,48c |
| AVG 150 | 0 | 0,33 | 1,06 | 2,16 | 0,89ab |
| AVG 225 | 0 | 0 | 1,00 | 1,50 | 0,62a |
| Zaman ort. | 0A | 0,37A | 1,39B | 2,52C | |

Büyük harfler muhafaza süreleri, küçük italik harfler uygulamalar ve küçük harfler de uygulama x muhafaza süresi interaksiyonu arasındaki farklılıkları ($p<0.05$) göstermektedir. LSD uygulama:0,644

Muhafazanın 1. ayında tüm gruplarda üşüme zararı görülmezken, muhafazanın 2. ayında kontrol, AVG 75 ve AVG 150 grubu meyvelerde üşüme zararı başlamıştır. AVG 225 grubu Trabzon hurması meyvelerinde ise muhafazanın 3.ayından itibaren üşüme zararına maruz kaldıkları belirlenmiştir (Çizelge 9). Elde edilen sonuçlara benzer şekilde Özdemir ve ark. (2012)'de depolama süresi uzadıkça üşüme zararından kaynaklanan kabuk kararması, meyve et rengi kararması ve sulanması şeklinde görülen fizyolojik bozulmalarda artışlar olduğu belirlenmiştir. Araştırmada Trabzon hurması meyvelerinin soğukta muhafazasında uygulama ortalamaları incelendiğinde en fazla üşüme zararı AVG 75 grubu (%1,48) ile kontrol grubu (%1,29) meyvelerinde, en az üşüme zararı ise %0,62 ile AVG 225 grubu meyvelerinde tespit edilmiştir. Fizyolojik bozuklukların gelişmesi ve ağırlaşması genellikle soğuk depolama sırasındaki üşüme zararından kaynaklandığından, yapılan çalışmalarda depolama bozukluklarının görülme sıklığı ve şiddeti antioksidan metabolizma kapasitesiyle yakından ilişki olduğu ifade edilmiştir (Salvador ve ark., 2004; Leisso ve ark., 2015). Bu nedenle, antioksidan metabolizmanın, soğukta depolanan hurmalarında fizyolojik bozuklukların ve meyve kalitesi özelliklerinin gelişimi ile bağlantılı olması mümkündür. Çalışmada da AVG 150 ve AVG 225 uygulamaları meyve antioksidan içeriğini korumanın yanında üşüme zararını hafiflettiği ve çürüme oranını azalttığı tespit edilmiştir. Ancak AVG 75 dozunun kontrol grubuna yakın değerlere sahip olması nedeniyle etkili olmadığı belirlenmiştir. Bu konunun daha iyi anlaşılması için Trabzon hurması meyvesinde AVG'nin üşüme zararı şiddetini hafifletilmesinin mekanizması hakkında daha fazla araştırma yapılması son derece gereklidir.

## Sonuç

Araştırmada elde edilen tüm bulgular dikkate alındığında, 0-1°C sıcaklık ve %85-90 oransal nem koşullarında 'Fuyu' Trabzon hurması çeşidi meyve kalitesini korumada 3 aya kadar 150 ve 225 mg $l^{-1}$ AVG dozlarının etkili olduğu tespit edilmiştir. Ancak 'Fuyu' Trabzon hurması çeşidinde muhafaza süresi sonunda artan kayıplar nedeniyle 4 aylık depolama süresinin uzun olduğu, bu yüzden depolama süreleri ve farklı konsantrasyonlar ile detaylı çalışmaların yürütülmesi önerilmektedir.

## Beyanlar

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# The Determination of Body Defects, Hatching and Chick Quality Traits in Partridge (Alectoris Chukar) Parents

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ABSTRACT

The aim of this study was to determine the relationships between body defects, egg and chick quality and hatching results at different age periods of the laying period of partridges raised in production conditions (cage system). The study was based on three different periods. The first period is the pre-peak egg production period (<200 eggs), the second period is the peak egg production period (>200 eggs) and the third period is the post-peak egg production period (<200 eggs). These periods also represent the age of the animals. As material, 432 female and 216 male partridges in their first egg production year were used. The study was organized according to the random plots experimental plan. There were changes in body defects and other health parameters depending on egg production periods. Foot-pad dermatitis and elbow burns increased with increasing age. Parent partridges had better head, neck, back, chest and tail feather condition before peak egg production. Egg weight increased with advancing age. Hatching egg characteristics such as excessively pointed eggs and eggs with calcium deposits decreased in the peak and post-peak yield period. Eggshell transparency increased with advancing age. Fertility was higher in the pre-peak egg yield period compared to other periods. Hatchability was lower after the peak period. Tona score of chicks decreased with age. There were weak negative and positive phenotypic correlations between many traits. Practices that prevent body injuries in the early egg production period and deteriorating feather condition with advancing age, decreasing shell opacity, deterioration in hatching performance and chick quality traits in partridges will be beneficial in terms of increasing both production performance and animal welfare.

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 13(3): 739-749, 2025

# Kınalı Keklik (Alectoris Chukar) Ebeveynlerinde Vücut Kusurlarının, Kuluçka ve Civciv Kalitesi Özelliklerinin Belirlenmesi

MAKALE BİLGİSİ

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*Anahtar Kelimeler:*
Keklik
Yumurta
Vücut kusurları
Kuluçka
Tona skoru

ÖZ

Bu çalışmanın amacı, üretim şartlarında (kafes sisteminde) yetiştirilen kekliklerin yumurtlama döneminin farklı yaş periyotlarındaki vücut kusurları, yumurta ve civciv kalitesi ile kuluçka sonuçları arasındaki ilişkilerin belirlenmesidir. Çalışma üç farklı dönemi baz almıştır. Birinci dönem pik yumurta verim öncesi dönem (<200 yumurta), ikinci dönem pik yumurta verim dönemi (>200 yumurta) ve üçüncü dönem pik yumurta verim sonrası dönemdir (<200 yumurta). Bu dönemler aynı zamanda hayvanların yaşını da ifade etmektedir. Materyal olarak ilk yumurta verim yılında olan 432 dişi ve 216 erkek kınalı keklik kullanılmıştır. Çalışma tesadüf parselleri deneme desenine göre düzenlenmiştir. Yumurta verim dönemlerine bağlı olarak vücut kusurlarında ve diğer sağlık parametrelerinde değişimler meydana gelmiştir. Yaş ilerledikçe foot-pad dermatitis ve dirsek yanıklığı artmıştır. Ebeveyn kekliklerde pik yumurta verim öncesi dönemde daha iyi baş, boyun, sırt, göğüs ve kuyruk bölgesi tüy kondisyonu elde edilmiştir. Yumurta ağırlığı yaş ilerledikçe artmıştır. Kuluçkalık yumurta özelliklerinden aşırı sivri, kalsiyum birikmesi olan yumurtalar pik ve pik sonrası verim döneminde azalma göstermiştir. Yumurta kabuğu saydamlığı yaş ilerledikçe yükselmiştir. Döllülük pik öncesi yumurta verim döneminde diğer dönemlere göre daha yüksek tespit edilmiştir. Çıkış gücü pik dönem sonrasında daha düşük belirlenmiştir. Civcivlerde tona skoru dönemlere bağlı olarak azalma göstermiştir. Ele alınan birçok özellik arasında zayıf düzeyde negatif ve pozitif fenotipik korelasyonlar tespit edilmiştir. Kekliklerde erken yumurta verim döneminde vücut yaralanmaları ile ilerleyen yaşla birlikte kötüleşen tüy kondisyonunu, kabuk opaklığında azalmayı, kuluçka performansı ve civciv kalite özelliklerinde kötüleşmeyi önleyici uygulamalar hem üretim performansının hem de hayvan refahının artırılması bakımından faydalı olacaktır.

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## Giriş

Keklikler, Phasianidae familyasından Perdiciane alt familyasına ait bir türdür. Doğal yaşama alanlarında bulunan ve evcilleştirilmiş olan birçok keklik türü bulunmaktadır. Kekliklerde ticari olarak Kınalı Keklik (Alectoris chukar), Avrupa Kınalı Kekliği (Alectoris rufa), Kuzey Afrika Kınalı Kekliği (Alectoris barbara) ve Çil Kekliği (Perdix perdix) yetiştirilmektedir (Sarıca ve ark., 2003).

Türkiye'de Yozgat, Gaziantep, Afyonkarahisar, Kahramanmaraş ve Malatya illerinde Tarım ve Orman Bakanlığı, Doğa Koruma ve Milli Parklar Genel Müdürlüğü'ne bağlı üretim istasyonlarında kınalı keklikler üretilmekte ve belirli alanlara salınmaktadır. Üretim istasyonlarında veya özel işletmelerde ebeveyn olarak kınalı keklikler genellikle kafes sisteminde yetiştirilerek yumurta ve civciv elde edilmektedir. Yetiştiricilikte kafes sisteminin tercih edilme amacı ise kekliklerde henüz tamamlanmayan evcilleşme süreci şeklinde açıklanabilir. Çünkü keklikler hemen hemen her üretim ortamında hırçın ve agresif davranışlar göstermektedir. Bu nedenle kontrol edilebilirlik ve temiz yumurta üretiminin sağlanabilmesi açısından en iyi yetiştirme sistemi kafeslerdir. Hayvan refahı açısından değerlendirildiğinde, kafes sistemlerindeki kısıtlı yaşam alanı hayvanların günlük aktivitelerini ve doğal davranışlarını sergileyebilmelerine engel olmaktadır. Ayrıca kafes tellerinden kaynaklı tırnak ve gaga uzaması nedeniyle hayvanlar kendilerine, birbirlerine, bakıcılara zarar vermekte ve bu durum özellikle çiftleşme sırasında yaralanmalara ve tüy kayıplarına yol açmaktadır. Bu nedenle vücutta meydana gelen kusurların yumurta ve civciv kalitesi ile kuluçka sonuçları üzerine etkilerinin belirlenmesi önemlidir.

Keklikler kafes veya yer sistemlerinde yetiştirildiklerinde 1 erkek ile 3-4 dişi birlikte barındırılarak döllü yumurta elde edilebilmektedir. Kafeste yetiştiriciliğe göre yer sisteminden daha iyi kuluçka sonuçları elde edildiği (%75 kuluçka randımanı), ancak yine de bu oranın oldukça değişken olduğu bildirilmiştir (Sarıca ve ark., 2003).

Yumurtacı tavuklar ve bazı alternatif kanatlı hayvan türlerinin barındırılmasında yaygın olarak kullanılan kafes sistemleri gagalama, tüy çekme gibi istenmeyen davranışlara neden olabilmektedir. Kanibalizm yaralanmalara ve hatta ölümlere yol açmakta, önlenmesi için de hayvanlarda gaga kesimi yapılmaktadır (Riber & Hinrichsen, 2017).

Kanatlı hayvan yetiştiricileri, vücut ağırlığı, tüy skoru ve foot-pad dermatitis (FPD) gibi vücut özellikleri arasında var olan ilişkiyi kurmaya çalışmışlardır. Çünkü bu bilgiler hem yemden yararlanma hem de hayvanın performansına yansımaktadır. Ayrıca bu özellikler maksimum üreme ve et verimi için ıslah programlarında da kullanılabilmektedir (Yahaya ve ark., 2012). Maksimum ekonomik getiri sağlanabilmesi için vücut ağırlığı, konformasyon ve vücut kusurları gibi özelliklerin iyi durumda olduğu hayvanlar gereklidir (Okon ve ark., 1997).

Kanatlılarda kuluçka performansı, yumurta ve civciv kalite özelliklerinin hayvanın yaşından etkilendiği birçok araştırmacı tarafından bildirilmiştir (Okur, 2008; Şeremet, 2012; Durmuş & Kutlu, 2019). Kanatlı hayvanların yumurta kalitesi ile ilgili yapılan birçok araştırmada özellikler ve bu özellikler arasındaki genetik ve fenotipik ilişkilerin önemli olduğu belirtilmiştir. Kanatlılarda yapılan birçok çalışmada yaşın artmasıyla birlikte, yumurta ağırlığının da arttığı bildirilmektedir. Yaş artışı ile ortaya çıkan bu değişimin yumurta sarısında, akında ve kabuk ağırlığında meydana gelen değişimlerden kaynaklandığı belirtilmektedir (Rizzi & Chiericato, 2005; Tumova & Ledvinka, 2009; Onbaşılar & Avcılar, 2011).

Kanatlılarda civcivlerin kalitesi farklı gözlemsel veya sayısal kriterler baz alınarak kalitatif veya kantitatif olarak skorlama ile belirlenebilmektedir. Bu skorlama işlemleri özellikle etlik piliçlerin büyüme performansını değerlendirmede ve tahmin etmede kullanılmaktadır (Decuypere & Bruggeman, 2007).

Bu çalışma ile kafes sisteminde yetiştirilen kekliklerin yumurta verim döneminin üç farklı yaşına bağlı olarak vücut kusurları, yumurta ve civciv kalitesi ile kuluçka özellikleri arasındaki ilişkilerin belirlenmesi amaçlanmıştır.

## Materyal ve Yöntem

Bu çalışma, T.C. Tarım ve Orman Bakanlığı, Doğa Koruma ve Milli Parklar Genel Müdürlüğü'nün 2022 yılı ve "E-21264211-288.04-7698011" sayılı izni ile yönetmeliklere uygun olarak gerçekleştirilmiştir.

### *Hayvan ve Kümes Materyali ile Bakım-Besleme Özellikleri*

Çalışma, T.C. Tarım ve Orman Bakanlığı, Doğa Koruma ve Milli Parklar Genel Müdürlüğü, Yozgat Doğa Koruma ve Milli Parklar Şube Müdürlüğü'ne bağlı "Çalatlı Keklik Üretme İstasyonu'nda yürütülmüştür. Çalışmalar istasyonun rutin işlemleri aksatılmadan gerçekleştirilmiştir. Materyal olarak ilk yumurta verim yılında olan 432 dişi ve 216 erkek kınalı (toplam 648) keklik kullanılmıştır. Keklikler; 4 katlı ve 3 gözlü toplam 18 üniteden oluşan ve iki dişi bir erkek kekliğin bulunduğu 216 kafes gözünde yetiştirilmiştir. Her bir kafes gözü tekerrür olarak değerlendirilmiştir ve ölçüleri 32x45x35 cm'dir.

Çalışmada vücut kusurları, yumurta kalite özellikleri, kuluçka özellikleri ve civciv kalitesi için 3 farklı dönem (yaş) baz alınmıştır;

- Pik öncesi yumurta verim dönemi (<200 yumurta; 35-46 haftalar arası)
- Pik yumurta verim dönemi (>200 yumurta; 47-51 haftalar arası)
- Pik sonrası yumurta verim dönemi (<200 yumurta; 52-59 haftalar arası)

Keklik üretim istasyonunda son 4 yıla ait yumurta verim kayıtları incelenmiş ve buna bağlı olarak bu verim dönemleri belirlenmiştir. İşletmede ortalama olarak Ocak ayında başlayan yumurta verimi Mart ayında pike ulaşmakta ve Nisan ayı sonunda yumurta verimi düşmektedir. Bu çalışmada ilk yumurtaların görülme tarihi 01.01.2023 olarak belirlenmiştir (35. hafta). İlk yumurtalardan 30 gün sonra (30.01.2023) pik öncesi verim dönemi için veriler alınmaya başlamıştır. Daha önceki yıl verileri de dikkate alınarak günlük 200 ve üzeri yumurta verimi 3 gün peş peşe tespit edildikten sonra (08.03.2023) pik yumurta verim dönemi çalışmaları başlatılmıştır (47.

hafta). Daha sonra günlük yumurta verimi 200 ve altına düştüğünde pik sonrası yumurta verim dönemi (06.04.2023) çalışmaları gerçekleştirilmiştir (52. hafta).

Her bir dönemde yeterli sayıda civciv elde edebilmek için en az 5 gün süresince her bir kafes gözünden yumurtalar kodlanarak toplanmıştır. Çalışmada pik öncesi, pik ve pik sonrası dönemde sırasıyla 730, 698 ve 656 adet kuluçkalık yumurta kullanılmıştır. Toplanan yumurtalarda dış kalite özellikleri olarak her bir dönem için yumurta ağırlığı (g), yumurta eni ve boyu (mm), şekil indeksi (%) ile kırık-çatlak, sivri-küt, Kalsiyum (Ca) birikmesi, kabuksuz ve kirlilik durumları değerlendirilmiştir.

Her bir dönem için gerekli yumurtaların toplanma işlemi bittiğinde kafeslerdeki dişi ve erkek kekliklerin canlı ağırlıkları 0,1 g hassasiyetli terazi ile bireysel olarak belirlenmiştir. Kekliklerin pik yumurta verim döneme ait canlı ağırlıkları hayvanlarda stresten kaynaklı yumurta verim düşüklüğüne neden olmamak için alınmamıştır. Pik sonrası yumurta verim dönemi için daha önce numaralandırılan her kafesteki dişi ve erkek kekliklerin canlı ağırlıkları belirlenmiştir. Belirlenen dönemlerde yine kafes numaraları baz alınarak bireysel olarak vücut kusur skorları tespit edilmiştir (Çizelge 1; Çizelge 2; Sarıca ve ark., 2010; Yamak & Sarıca, 2012; Li ve ark., 2017; Noubandiguim, 2021). Keklikler çok ürkek ve stresli davranışlar gösterdiğinden, canlı ağırlık ve vücut kusurlarının belirlenme işlemleri deneme için gerekli yumurtaların toplama işlemi tamamlandıktan sonra yapılmıştır.

Sürü içerisinde yaralanma ve diğer nedenlere bağlı olarak toplamda 10 dişi ve 2 erkek ebeveyn keklik ölmüştür. Bu kekliklerin yerine yedek sürüden değişim gerçekleştirilmiştir. Değişim sonrasında kafesteki diğer keklikler tarafından kabul görmeyen 2 dişi keklik üretim dışı bırakılmıştır. Ebeveyn keklikler kafes sistemine alınmadan önce uzun gaga ve tırnağa sahip olanların, uygun yöntemlerle gaga ve tırnakları kesilmiştir. Bu işlem aylık olarak ihtiyaca göre devam etmiştir.

Yumurta eni ve boyu (uzunluğu) 0,01 mm hassasiyetli dijital kumpas ile belirlenmiş (Fideco, Türkiye) ve bu değerlerden şekil indeksi (Şİ)

$$\text{Şİ: (yumurta eni/yumurta boyu)} \times 100$$

hesaplanmıştır (Alkan ve ark., 2010). Yumurta kabuklarında opaklık (saydamlık) durumu yumurtanın ışığa küt ve sivri uç tarafından tutularak 1 ila 4 puan arasında skorlanması ile belirlenmiştir (Şekil 1; 1: opak, 4: saydam). Skorlama işlemi aynı kişi tarafından gerçekleştirilmiştir (Wang ve ark., 2017; Zhao ve ark., 2021; Ren ve ark., 2023).

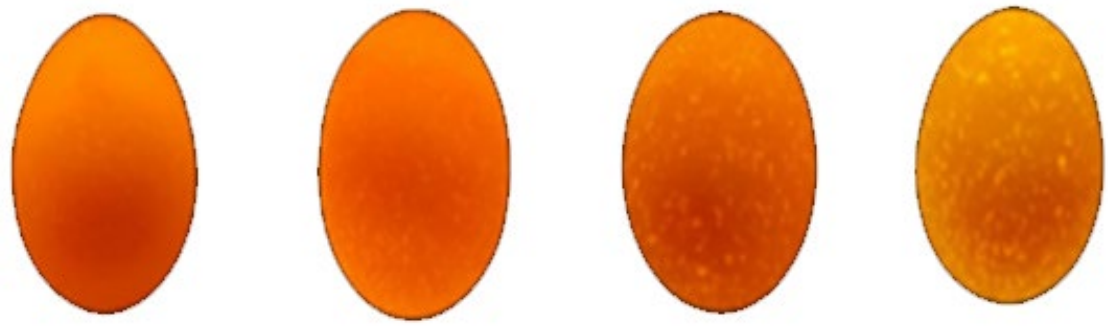
Diğer dış kalite özellikleri de her dönemde aynı kişi tarafından tespit edilmiştir. Veriler alındıktan sonra ilk toplanan yumurtalar 10, son toplananlar ise en az 1 gün depolanmıştır. Saklama kabinlerinde depolanan yumurtalara 16-18°C sıcaklık ve %60-70 nem koşulları sağlanmıştır. Daha sonra yumurtalar gelişim makinesine koyulmuştur.

Çizelge 1. Vücut kusurlarının belirlenmesinde kullanılan skorlama yöntemleri
*Table 1. Scoring methods used in identifying body defects*

| Skor | Ayak tabanı dermatiti (FPD) | Dirsek yanıklığı | Göğüs yanıklığı | Parmak bükülmeleri | Yaralanma Sırt | Yaralanma Boyun | Yaralanma Baş |
|---|---|---|---|---|---|---|---|
| 0 | Lezyon ve renk değişimi yok | Lezyon ve renk değişimi yok | Deformasyon yok | Tüm parmaklar sağlam | Yaralanma yok | Yaralanma yok | Yaralanma yok |
| 1 | Noktasal lezyonlar ve renk değişimi var | Noktasal lezyonlar ve renk değişimi var | Hafif kırmızılık ve tüy kaybı | 1-2 parmak çarpık | Noktasal yaralar ve renk değişimi var | Noktasal yaralar ve renk değişimi var | Noktasal yaralar ve renk değişimi var |
| 2 | Ayak tabanının %50'sinde lezyon var | Dirseğin %50'sinde lezyon var | Orta derece kırmızılık ve tüy kaybı | 3-4 parmak çarpık | Sırtta %50 yara var | Boyunda %50 yara var | Başta %50 yara var |
| 3 | Ayak tabanının %75'inde lezyon var | Dirseğin %75'inde lezyon var | Şiddetli kırmızılık ve tüy kaybı | 5-6 parmak çarpık | Sırtta %75 yara var | Boyunda %75 yara var | Başta %75 yara var |
| 4 | Ayak tabanının tamamında şiddetli lezyon var | Dirseğin tamamında şiddetli lezyon var | Göğüste aşırı yanık var | 7-8 parmak çarpık (mahmuz) | Sırtta ciddi çok fazla yara var | Boyunda ciddi çok fazla yara var | Başta ciddi çok fazla yara var |

Çizelge 2. Vücut tüy skorlarının belirlenmesinde kullanılan yöntem
*Table 2. Method used in determining body feather scores*

| Skor | Göğüs tüy skoru | Boyun tüy skoru | Baş tüy skoru | Sırt tüy skoru | Kuyruk tüy skoru |
|---|---|---|---|---|---|
| 0 | Tüylerle tam kaplı | Tüylerle tam kaplı | Tüylerle tam kaplı | Tüylerle tam kaplı | Tüylerle tam kaplı |
| 1 | Tüylerde hafif düzensizlik var | Tüylerde hafif düzensizlik var | Tüylerde hafif düzensizlik var | Tüylerde hafif düzensizlik var | Tüylerde hafif düzensizlik var |
| 2 | Göğüste hafif açılmalar var | Göğüste hafif açılmalar var | Göğüste hafif açılmalar var | Göğüste hafif açılmalar var | Göğüste hafif açılmalar var |
| 3 | Göğüste belirgin açılmalar var | Göğüste belirgin açılmalar var | Göğüste belirgin açılmalar var | Göğüste belirgin açılmalar var | Göğüste belirgin açılmalar var |
| 4 | Göğüs tamamen tüysüz durumda | Göğüs tamamen tüysüz durumda | Göğüs tamamen tüysüz durumda | Göğüs tamamen tüysüz durumda | Göğüs tamamen tüysüz durumda |



Şekil 1. Yumurta kabuğunda opaklık-saydamlık (soldan sağa; opak: skor 1, yarı opak: skor 2; yarı saydam: skor 3; saydam: skor 4) (Ren ve ark., 2023)
Figure 1. Opacity-transparency in eggshell (from left to right; opaque: score 1, semi-opaque: score 2; translucent: score 3; transparent: score 4) (Ren et al., 2023)

Çizelge 3. Tona Skoru belirlenmesinde kullanılan skorlama yöntemleri
*Table 3. Scoring methods used in determining the Tone Score*

| Parametre | Açıklama | Skor |
|---|---|---|
| Aktivite (2 sn ters dönme) | İyi | 6 |
| | Zayıf | 0 |
| Tüyler ve Görünüş | Temiz ve kuru | 10 |
| | Islak | 8 |
| | Kirli ve ıslak | 0 |
| Karın Boşluğuna Çekilen Yumurta Sarısı | Normal | 12 |
| | Büyük ve sert yumurta sarısı | 0 |
| Gözler | Açık ve parlak | 16 |
| | Parlak değil | 8 |
| | Kapalı | 0 |
| Bacaklar | Normal ayak ve tırnaklar | 16 |
| | Tek bacak enfekte | 8 |
| | Her iki bacak enfekte | 0 |
| Göbek (Yumuşaklık-Sertlik) | Tamamen kapalı ve temiz | 12 |
| | Kapalı değil ve koyu renkli | 6 |
| | Açık ve bozuk renkli | 0 |
| Kalan Membran (Zar) | Membran yok | 12 |
| | Küçük membran | 8 |
| | Büyük membran | 4 |
| | Çok büyük membran | 0 |
| Kalan Yumurta Sarısı | Yumurta sarısı yok | 16 |
| | Küçük yumurta sarısı | 12 |
| | Orta yumurta sarısı | 8 |
| | Büyük yumurta sarısı | 0 |

Gelişim makinesi koşulları 37,5 °C sıcaklık ve %60 nem olarak uygulanmıştır. Yumurtalar embriyonik gelişimin 21. gününde çıkım makinesine alınmış olup, son 3 günde (çıkım) makine koşulları 37,2 °C sıcaklık ve %70 nem şeklinde uygulanmıştır. Döllülük kontrolü, çıkım makinesine alınırken lamba kontrolüyle yapılmış olup, kuluçka bitiminde de yumurtalar kırılarak, döllülük ve embriyo ölümleri tespit edilmiştir. İlk döllülük kontrolünde dölsüz olarak belirlenen yumurtalar da kırılarak döllülük (döllü veya değil) ve embriyo ölümleri belirlenmiştir. Kuluçkanın bitiminde her kafes gözünü temsil edecek yumurtalar dikkate alınarak her dönemde 300 yumurtada (toplamda 900 bireysel çıkım) bireysel çıkış alınmıştır. Bireysel çıkış olmayan yumurtaların da üzerinde kafes numaraları olduğu için döllülük, çıkış gücü ve embriyo ölümleri (erken embriyo ölümü, orta dönem embriyo ölümü, geç dönem embriyo ölümü, kabuk altı embriyo ölümü) tespit edilebilmiştir. Bireysel çıkış alınan civcivlerde çıkış canlı ağırlığı ve kalite özellikleri belirlenmiştir (Tona ve ark., 2003; Çizelge 3). Döllülük (döllü veya değil), çıkış gücü (çıktı veya çıkmadı) ve embriyonik ölüm (erken, orta, geç ve kabuk altı dönemlerde ölü veya değil) bireysel olarak her yumurta için belirlenmiştir.

Yumurtalarda döllülük oranı; 21. gün döllülük kontrolü sonucunda, kuluçka makinesine koyulan yumurtaların içerisinde döllü tespit edilenlerinin toplam koyulan yumurta sayısındaki oranına göre tespit edilmiştir (Elibol, 2018).

Çizelge 4. Çalışmada kullanılan ebeveynlerin tükettiği yeme ait besin madde içeriği
*Table 4. Nutrient content of the food consumed by the parents used in the study*

| Besin maddeleri | Yumurta dönem yemi (38. hafta - bitiş) |
|---|---|
| Ham protein (%) | 17,50 |
| Metabolik enerji (Kkal/kg) | 2750 |
| Ham selüloz (%) | 5,50 |
| Ham kül (%) | 11,50 |
| Kalsiyum (%) | 3,80 |
| Yararlanılabilir fosfor (%) | 0,70 |
| Metiyonin (%) | 0,42 |
| Lisin (%) | 0,90 |

Çıkış gücü ise; çıkış yapan canlı civcivlerin kontroller sonucunda döllülüğü belirlenen yumurtaların sayısına oranlanmasıyla bulunmuştur (Elibol, 2018). Embriyo ölümleri makineye koyulan yumurtalarda meydana gelmiş olan ölümlerin döllü yumurta sayısına oranı olarak belirlenmiştir. Erken, orta ve geç embriyonik ölümler sırasıyla 0-6 gün, 7-21 gün ve 22-24 günlük embriyonik gelişim dönemlerine göre ayrılmıştır. Kabuk altı ölümler, kabuğu çatlatmış ancak yumurtadan çıkamayan embriyolar olarak kaydedilmiştir (Boz, 2015; Elibol, 2018; Erensoy & Sarıca, 2022).

Yumurtaların elde edildiği ebeveyn kekliklere yumurtlama döneminde yem ve su serbest olarak verilmiştir. Yemler ticari bir firmadan temin edilmiştir ve besin madde içeriği Çizelge 4'te verilmiştir.

Kekliklerde aydınlatma ve havalandırma işlemleri ebeveynlerin bulunduğu kafes odasındaki pencereler ile yapılmıştır. Her gün kafes altı gübre temizliği yapılmış ve oda temizlenmiştir. Aydınlatma programı yumurta verim döneminde 16 saat aydınlık 8 saat karanlık şeklinde uygulanmıştır. Yumurtalar her gün saat 8.30-10.30 arasında kafes numarası kodlanarak toplanmıştır.

***İstatistik analizler***

Çalışma tesadüf parselleri deneme planına göre değerlendirilmiştir. İstatistik analizler SPSS 21.0 yazılımı (SPSS Inc., Chicago, IL) kullanılarak yapılmıştır. Tüm veriler Kolmogorov-Smirnov testi kullanılarak normal dağılıma uygunluğu test edilmiştir. Yumurta ağırlığı, yumurta eni, yumurta boyu, şekil indeksi, civciv ağırlığı ve canlı ağırlıklar normal dağılış göstermiş ve one-way ANOVA prosedürü kullanılarak varyans analizine tabi tutulmuştur (Düzgüneş ve ark, 1987; Özdamar, 2002). Yumurta kalite özelliklerinden aşırı küt, aşırı sivri, çift sarılı, küçük, kırık, kalsiyum birikimi, boğumlu, çatlak, gizli çatlak ve opaklık; kuluçka özelliklerinden döllülük, çıkış gücü, embriyonik ölümler, civciv kalite ve vücut kusur özellikleri kesikli (skor) veri yapısına sahip olmuştur. Bu verilerden iki seviyeli olanlar binomial logit-link, ikiden fazla seviyeliler ise multinomial logit-link fonksiyonu ile Generalized Linear Model (GLM) prosedürü kullanılarak analiz edilmiştir. Yumurta kalitesi, kuluçka özellikleri ve civciv kalitesinin istatistik analizinde yumurtlama dönemi modele sabit etki olarak dahil edilirken, vücut kusur özelliklerinde ise yumurtlama dönemine ek olarak cinsiyet te modele dahil edilmiş ve ilgili yaştaki canlı ağırlık kovaryet olarak alınmıştır. Muameleler arasında $P<0,05$ önemlilik düzeyinde farklılık belirlendiğinde ortalamaların karşılaştırılması Tukey çoklu karşılaştırma testi ile yapılmıştır. Çeşitli vücut kusuru özellikleri, yumurta kalitesi ile kuluçka ve civciv özelliklerine ait parametreler arasındaki ilişkiler Pearson korelasyon katsayıları ($r$) hesaplanarak analiz edilmiştir. Korelasyonların göreceli gücünü tanımlamak için çok zayıf ($r<0,20$), zayıf ($r=0,20–0,39$), orta ($r=0,40–0,59$), güçlü ($r=0,60–0,79$) ve çok güçlü ($r=0,80–0,99$) olacak şekilde sınıflandırmalar yapılmıştır (Bowker & Zhuang, 2019).

## Bulgular

Çalışmada ebeveyn kekliklerde yumurtlama dönemine göre belirlenen vücut kusuru özellikleri Çizelge 5'te verilmiştir. Pik öncesi yumurta verim döneminde ebeveyn ağırlıkları 536,80 g iken pik verim sonrası 559,28 g olarak belirlenmiştir ($P<0,05$). Deneme süresinin ortalaması olarak dişiler 527,01 g, erkekler ise 588,80 g canlı ağırlığa sahip olmuştur ($P<0,001$). Ebeveynlerde ayak tabanı dermatiti (FPD) ($P<0,001$) ile dirsek yanıklığı düzeyi ($P<0,019$) pik yumurta verim döneminde ve sonrasında pik öncesi döneme göre daha yüksek bulunmuştur. Erkek ebeveyn kekliklerde dişilere göre daha yüksek dirsek yanıklığı belirlenmiştir ($P<0,001$). Vücut kusuru özelliklerinden göğüs yanıklığı ($P<0,05$), ayak-bacak problemi ($P<0,05$), baş bölgesinde yaralanma ($P<0,001$), boyun bölgesinde yaralanma ($P<0,001$) ve sırt bölgesinde yaralanma ($P<0,05$) pik öncesi verim döneminde daha yüksek bulunmuştur. Göğüs yanıklığı dişi kekliklerde daha yüksek olurken ($P<0,05$), benzer şekilde baş bölgesinde yaralanma, boyun bölgesinde yaralanma ve sırt bölgesinde yaralanma değerleri de dişilerde daha yüksek bulunmuştur ($P<0,001$).

Baş, boyun, sırt, göğüs ve kuyruk tüy kondisyonu pik öncesi dönemde daha iyi olarak tespit edilmiştir (Çizelge 6; $P<0,001$). Yine aynı bölgelerdeki tüy kondisyonları erkeklerde dişilerden daha iyi bulunmuştur ($P<0,001$).

Yumurta ağırlığı, yumurta eni, yumurta boyu ve şekil indeksi yaş faktöründen önemli düzeyde etkilenmiştir (Çizelge 7; $P<0,001$). Yumurtlama dönemine bağlı olarak, yaş ilerledikçe yumurta ağırlığı ile yumurta eni ve boyu artmıştır. Pik öncesi döneme göre pik ve pik sonrası dönemde şekil indeksi azalmıştır ($P<0,05$).

Ebeveyn kekliklerden elde edilen aşırı küt ve aşırı sivri yumurta ile kalsiyum birikmesi ve opaklık durumu muamele gruplarına göre istatistiki olarak farklılık göstermiştir (Çizelge 8; $P<0,05$). Pik yumurta verim döneminde aşırı küt yumurta oranı azalmıştır. Pik öncesi yumurta verim döneminde ise aşırı sivri, kalsiyum birikmesi olan yumurta oranı daha yüksek bulunmuştur. Yumurtalardaki opak görünüm yaş ilerledikçe artmıştır.

Çizelge 5. Kınalı kekliklerde yumurtlama dönemine bağlı olarak vücut kusuru özelliklerindeki değişimler
*Table 5. The changes in body defect traits in partridges depending on the laying period*

| Özellikler | Yumurtlama dönemleri | | | Cinsiyet | | OSH | Yumurtlama döneminin etkisi (P) | Cinsiyetin etkisi (P) |
|---|---|---|---|---|---|---|---|---|
| | Pik öncesi | Pik | Pik sonrası | Dişi | Erkek | | | |
| Canlı ağırlık (g) | 536,80 | - | 559,28 | 527,01 | 588,80 | 4,391 | 0,022 | <0,001 |
| FPD | 0,12[b] | 0,22[a] | 0,23[a] | 0,19 | 0,18 | 0,012 | <0,001 | 0,622 |
| Dirsek yanıklığı | 0,02[b] | 0,04[a] | 0,05[a] | 0,02 | 0,07 | 0,005 | 0,019 | <0,001 |
| Göğüs yanıklığı | 0,06[a] | 0,00[b] | 0,00[b] | 0,03 | 0,00 | 0,005 | 0,001 | 0,036 |
| Ayak-bacak problemi | 0,04[a] | 0,01[b] | 0,01[b] | 0,02 | 0,02 | 0,005 | 0,005 | 0,911 |
| Parmak çarpıklığı | 0,01 | 0,00 | 0,00 | 0,00 | 0,01 | 0,001 | 0,999 | 0,433 |
| Baş bölgesinde yaralanma | 0,49[a] | 0,26[b] | 0,30[b] | 0,44 | 0,17 | 0,018 | <0,001 | <0,001 |
| Boyun bölgesinde yaralanma | 0,23[a] | 0,05[b] | 0,06[b] | 0,15 | 0,04 | 0,010 | <0,001 | <0,001 |
| Sırt bölgesinde yaralanma | 0,09[a] | 0,04[b] | 0,04[b] | 0,08 | 0,01 | 0,007 | 0,001 | <0,001 |

OSH: Ortalamanın standart hatası; [a,b:] Aynı satırda farklı harflerle gösterilen ortalamalar arasındaki farklar önemlidir (P<0,05).

Çizelge 6. Kınalı kekliklerde yumurtlama dönemine bağlı olarak vücut tüy skoru özelliklerindeki değişimler
*Table 6. The changes in body feather score characteristics in partridges depending on the laying period*

| Özellikler | Yumurtlama dönemleri | | | Cinsiyet | | OSH | Yumurtlama döneminin etkisi (P) | Cinsiyetin etkisi (P) |
|---|---|---|---|---|---|---|---|---|
| | Pik öncesi | Pik | Pik sonrası | Dişi | Erkek | | | |
| Baş bölgesi tüy skoru | 0,93[b] | 1,42[a] | 1,54[a] | 1,58 | 0,72 | 0,026 | <0,001 | <0,001 |
| Boyun bölgesi tüy skoru | 1,02[c] | 1,48[b] | 1,64[a] | 1,67 | 0,80 | 0,029 | <0,001 | <0,001 |
| Sırt bölgesi tüy skoru | 0,70[c] | 1,54[b] | 1,70[a] | 1,59 | 0,74 | 0,027 | <0,001 | <0,001 |
| Göğüs bölgesi tüy skoru | 0,38[b] | 0,97[a] | 1,05[a] | 0,95 | 0,49 | 0,022 | <0,001 | <0,001 |
| Kuyruk bölgesi tüy skoru | 0,60[b] | 1,60[a] | 1,65[a] | 1,48 | 0,89 | 0,028 | <0,001 | <0,001 |

OSH: Ortalamanın standart hatası; [a-c:] Aynı satırda farklı harflerle gösterilen ortalamalar arasındaki farklar önemlidir (P<0,05).

Çizelge 7. Kınalı kekliklerde yumurtlama dönemine bağlı olarak yumurta kalite özelliklerindeki değişimler
*Table 7. The changes in egg quality characteristics in partridges depending on the laying period*

| Özellikler | Pik öncesi | Pik | Pik sonrası | s.d. | F değeri | P değeri |
|---|---|---|---|---|---|---|
| Yumurta ağırlığı (g) | 20,16±1,677[c] | 20,60±0,061[b] | 20,91±0,063[a] | 2 | 37,281 | <0,001 |
| Yumurta eni (mm) | 30,38±0,045[b] | 30,46±0,041[b] | 30,80±0,034[a] | 2 | 30,185 | <0,001 |
| Yumurta boyu (mm) | 40,34±0,062[c] | 40,98±0,065[b] | 41,29±0,069[a] | 2 | 55,680 | <0,001 |
| Şekil indeksi (%) | 75,18±0,103[a] | 74,31±0,113[b] | 74,62±0,109[b] | 2 | 17,259 | <0,001 |

OSH: Ortalamanın standart hatası; s.d.: Serbestlik derecesi; [a-c:] Aynı satırda farklı harflerle gösterilen ortalamalar arasındaki farklar önemlidir (P<0,05).

Çizelge 8. Kınalı kekliklerde yumurtlama dönemine bağlı olarak kuluçkalık yumurta özelliklerindeki değişimler
*Table 8. The changes in hatching egg characteristics in partridges depending on the laying period*

| Özellikler | Pik öncesi | Pik | Pik sonrası | s.d. | F değeri | P değeri |
|---|---|---|---|---|---|---|
| Aşırı küt | 0,05±0,008[a] | 0,01±0,001[b] | 0,04±0,007[a] | 2 | 13,370 | 0,001 |
| Aşırı sivri | 0,03±0,006[a] | 0,00±0,003[b] | 0,01±0,004[b] | 2 | 12,960 | 0,002 |
| Çift sarılı | 0,00±0,000 | 0,01±0,003 | 0,01±0,002 | 2 | 0,860 | 0,668 |
| Küçük | 0,01±0,004 | 0,00±0,000 | 0,00±0,000 | 2 | 0,000 | 1,000 |
| Kırık | 0,01±0,003 | 0,01±0,003 | 0,00±0,000 | 2 | 0,410 | 0,815 |
| Kalsiyum birikimi | 0,02±0,005[a] | 0,01±0,001[b] | 0,00±0,000[b] | 2 | 6,352 | 0,042 |
| Boğumlu | 0,00±0,000 | 0,01±0,002 | 0,02±0,005 | 2 | 4,712 | 0,095 |
| Çatlak | 0,01±0,002 | 0,00±0,000 | 0,00±0,000 | 2 | 0,000 | 1,000 |
| Gizli çatlak | 0,05±0,008 | 0,03±0,007 | 0,04±0,008 | 2 | 1,979 | 0,732 |
| Diğer | 0,02±0,005 | 0,01±0,001 | 0,00±0,000 | 2 | 5,128 | 0,072 |
| Opaklık | 1,46±0,024[c] | 1,84±0,028[b] | 2,01±0,034[a] | 2 | 177,873 | <0,001 |

OSH: Ortalamanın standart hatası; s.d.: Serbestlik derecesi; [a-c:] Aynı satırda farklı harflerle gösterilen ortalamalar arasındaki farklar önemlidir (P<0,05).

Döllülük oranı pik öncesi yumurta verim döneminde daha yüksek olmakla birlikte yaş ilerledikçe azalmıştır (Çizelge 9; P<0,001). Çıkış gücü pik öncesi ve pik yumurta verim dönemlerinde benzer iken, pik sonrası verim döneminde azalmıştır (P<0,05). Erken embriyo ve kabuk altı ölümleri üzerine yumurta verim döneminin etkisi önemsiz iken, pik öncesi yumurta verim döneminde orta ve geç dönem embriyo ölümleri daha yüksek tespit edilmiştir (P<0,05).

Civciv ağırlığı yumurta verim dönemlerine göre farklılık göstermemiştir (Çizelge 10; P>0,05). Civciv kalitesi (Tona skoru) ilerleyen ebeveyn yaşı ile birlikte azalmıştır (P<0,001). Civcivlerde çıkışta belirlenen aktivite skoru ve tüyler ile görünüş skoru pik sonrası yumurta verim döneminde daha düşük bulunmuştur (P<0,001). Civcivlerdeki göbek sorunları ebeveyn yaşı ilerledikçe azalmıştır (P<0,001).

Çizelge 9. Kınalı kekliklerde yumurtlama dönemine bağlı olarak kuluçka özelliklerindeki değişimler
*Table 9. The changes in hatching characteristics in partridges depending on the laying period*

| Özellikler | Pik öncesi | Pik | Pik sonrası | s.d. | F değeri | P değeri |
|---|---|---|---|---|---|---|
| Döllülük (%) | 85,47±1,318[a] | 75,43±1,638[b] | 70,49±1,785[b] | 2 | 44,610 | <0,001 |
| Çıkış gücü (%) | 70,81±1,700[a] | 68,79±1,763[a] | 62,23±1,897[b] | 2 | 12,294 | 0,002 |
| EEÖ (%) | 5,31±0,840 | 2,89±0,637 | 3,82±0,750 | 2 | 5,330 | 0,070 |
| OEÖ (%) | 1,40±0,439[a] | 0,14±0,145[b] | 0,46±0,264[ab] | 2 | 6,671 | 0,036 |
| GEÖ (%) | 6,70±0,935[a] | 2,89±0,637[b] | 2,75±0,640[b] | 2 | 16,609 | <0,001 |
| KAÖ (%) | 0,98±0,368 | 0,72±0,322 | 1,22±0,430 | 2 | 0,863 | 0,649 |

OSH: Ortalamanın standart hatası; s.d.: Serbestlik derecesi; [a-b:] Aynı satırda farklı harflerle gösterilen ortalamalar arasındaki farklar önemlidir (P<0,05); EEÖ: Erken dönem embriyo ölümleri, OEÖ: orta dönem embriyo ölümleri, GEÖ: Geç dönem embriyo ölümleri, KAÖ: Kabuk altı embriyo ölümleri

Çizelge 10. Kınalı kekliklerde yumurtlama dönemine bağlı olarak civciv kalite özelliklerindeki değişimler
*Table 10. The changes in chick quality characteristics in partridges depending on the laying period*

| Özellikler[1] | Pik öncesi | Pik | Pik sonrası | s.d. | F değeri | P değeri |
|---|---|---|---|---|---|---|
| Civciv ağırlığı (g) | 13,79±0,076 | 13,92±0,067 | 13,79±0,074 | 2 | 1,218 | 0,296 |
| Aktivite | 5,61±0,087[a] | 5,43±0,100[a] | 4,97±0,133[b] | 2 | 17,089 | <0,001 |
| Tüyler ve görünüş | 9,99±0,007[a] | 9,94±0,020[a] | 9,81±0,034[b] | 2 | 18,127 | <0,001 |
| Karın boşluğuna çekilen yumurta sarısı | - | - | - | 2 | - | - |
| Gözler | - | - | - | 2 | - | - |
| Bacaklar | - | - | - | 2 | - | - |
| Göbek | 11,90±0,046[a] | 11,43±0,100[b] | 10,84±0,140[c] | 2 | 35,908 | <0,001 |
| Kalan membran | - | - | - | 2 | - | - |
| Kalan yumurta sarısı | - | - | - | 2 | - | - |
| Tona skoru | 99,50±0,102[a] | 98,79±0,141[b] | 97,60±0,202[c] | 2 | 70,533 | <0,001 |

OSH: Ortalamanın standart hatası; s.d.: Serbestlik derecesi; [a-c:] Aynı satırda farklı harflerle gösterilen ortalamalar arasındaki farklar önemlidir (P<0,05); [1]: Karın boşluğuna çekilen yumurta sarısı, Gözler, Bacaklar, Kalan membran, Kalan yumurta sarısı değerleri tüm civcivlere aynı değeri göstermiştir. Bu nedenle tabloda belirtilmemiştir.

Ebeveyn kekliklerin canlı ağırlıkları ile ayak-bacak problemleri (r=-0,177) ve toplam yaralanma skorları arasında zayıf düzeyde negatif (r=-0,156), toplam tüy skoru ile arasında zayıf düzeyde pozitif (r=0,150), civciv tona skoru ile zayıf düzeyde pozitif (r=0,211) ve istatistiki olarak önemli fenotipik korelasyonlar tespit edilmiştir (Çizelge 11; P<0,05). Ebeveyn kekliklerin baş, boyun ve sırt, göğüs ve kuyruk bölgelerinden skorlanan toplam tüy skoru ile yumurta ağırlığı (r=0,131) ve boğumlu özellikteki yumurtalar (r=0,118) arasında zayıf, yumurta kabuğu opaklığı (r=0,268) ve tona skoru (r=0,310) arasında ise zayıf düzeyde pozitif ve istatistiki olarak önemli ilişkiler belirlenmiştir (P<0,01). Yine toplam tüy skoru ile kalsiyum birikmesi olan yumurtalar arasında (r=- 0,125) zayıf düzeyde negatif ve istatistiki olarak önemli ilişki bulunmuştur (P<0,01). Çalışmada yumurta ağırlığı ile civciv ağırlığı arasında güçlü düzeyde pozitif ve istatistiki olarak önemli bir fenotipik korelasyon bulunmuştur (r=0,738; P<0,01).

## Tartışma

Kanatlı hayvanlarda dişilerin canlı ağırlığı, yumurta ağırlığını etkileyen önemli bir faktördür ve aralarında pozitif bir ilişki vardır (Sevim & Aktan, 2020). Bununla beraber erkeklerin vücut ağırlığı da döllülük üzerine etkili olan önemli bir faktördür (İpek ve ark., 2003). Sevim & Aktan (2020), dişi bıldırcınlarda, vücut ağırlığı ile kuluçkalık yumurta ağırlığı arasında önemli bir korelasyon (0,49) olduğunu bildirmiştir. Aynı çalışmada dişi ebeveyn ağırlığı ile kuluçkalık yumurtaların ağırlığı ve civciv çıkış ağırlığı arasında da önemli korelasyonlar bulunmuştur. Sarıca & Soley (1995) de çalışmasında bu durumu tespit etmiştir. Bildirişlerden farklı olarak, çalışmamızda ebeveyn ağırlığı ile yumurta ve civciv ağırlığı arasında önemli bir korelasyon tespit edilmemiştir. Literatüre benzer olarak yumurta ağırlığı ile civciv ağırlığı arasında önemli ve güçlü bir pozitif ilişki tespit edilmiştir.

Ebeveyn olarak kullanılan kanatlı hayvanlarda yaşın artması ile birlikte yumurta boyutunun da artacağı ve büyüme performansı baz alınarak kuluçka sonuçları ve civciv kalitesinde olumlu sonuçlar alınabileceği bildirilmektedir (Moran, 1990; Hill, 2001). Bununla birlikte kanatlı hayvanlarda genç ebeveynlere göre yaşlılarda özellikle depolama işlemi nedeniyle yumurta ak oranındaki azalmanın embriyo ölümleri ve sakat-ölü civciv sayısını artırdığı bildirilmiştir (Lapao ve ark., 1999). Yaşlı ebeveynlerde özellikle depolama ile birlikte kuluçkalık yumurtaların Haugh birimi ve civciv kalitesi azalmaktadır. Genç yaştaki ebeveynlerde ise yumurta ağırlığı daha düşük olmasına rağmen, yumurtalar iyi bir ak kalitesine sahip olduğundan kuluçka sonuçları ve civciv kalitesi daha iyi olmaktadır (Tona ve ark., 2003; Tona ve ark., 2004). Çalışmamızda yumurta verim dönemleri ilerledikçe yumurta ağırlığı artmış, döllülük ve çıkış gücü ise düşmüştür. Benzer olarak civciv kalitesinde kullanılan tona skoru da azalma göstermiştir. Lapao ve ark. (1999)'nın bildirişinden farklı olarak bizim çalışmada erken yaş döneminde özellikle orta ve geç dönem embriyo ölümleri daha yüksektir. Çalışmamızda Tona ve ark. (2003) ve (2004)'e göre ise benzer olarak erken yaş döneminde kuluçka sonuçları ve civciv kalitesi diğer dönemlerden yüksek bulunmuştur. Bu durum erken embriyo ölümlerinin ebeveynlerin genotip, bakım, besleme, yetiştirme şartları ve hayvanların vücut kusurlarından kaynaklı olabileceğini düşündürmektedir. Çünkü sağlıklı bir vücut yapısı iyi bir performans için gereklidir.

Çizelge 11. Kınalı kekliklerde vücut kusurları, yumurta kalitesi, kuluçka ve civciv özellikleri arasındaki korelasyonlar
*Table 11. The correlations between body defects, egg quality, hatchability and chick characteristics in partridges*

| Ö | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 2 | -0,150** | | | | | | | | | |
| 3 | -0,049 | -0,042 | | | | | | | | |
| 4 | -0,050 | 0,058 | 0,073 | | | | | | | |
| 5 | -0,023 | -0,024 | -0,017 | -0,023 | | | | | | |
| 6 | 0,037 | -0,001 | -0,007 | -0,010 | -0,008 | | | | | |
| 7 | 0,103* | -0,043 | 0,093* | 0,029 | -0,011 | -0,023 | | | | |
| 8 | 0,272** | -0,172** | 0,069 | -0,031 | 0,053 | 0,003 | 0,223** | | | |
| 9 | 0,059 | 0,043 | -0,008 | 0,016 | -0,005 | 0,045 | 0,041 | -0,021 | | |
| 10 | -0,093* | 0,125** | -0,071 | 0,045 | 0,113** | -0,035 | 0,092* | -0,058 | 0,136** | |
| 11 | 0,089* | -0,013 | 0,040 | -0,016 | -0,083* | 0,061 | -0,007 | 0,015 | 0,806** | -0,394** |
| 12 | 0,738** | -0,105* | 0,036 | 0,089 | -0,084 | 0,078 | 0,104* | 0,151** | 0,046 | -0,005 |
| 13 | 0,032 | -0,079 | -0,040 | -0,082 | 0,069 | 0,018 | 0,003 | 0,131** | 0,066 | -0,054 |
| 14 | 0,084 | 0,065 | 0,001 | -0,071 | 0,116* | -0,043 | -0,081 | -0,008 | -0,010 | -0,042 |
| 15 | 0,089* | -0,039 | 0,068 | -0,047 | 0,002 | 0,103* | 0,033 | 0,050 | -0,067 | -0,036 |
| 16 | 0,026 | -0,069 | -0,022 | -0,030 | -0,026 | -0,011 | -0,041 | 0,001 | 0,018 | 0,038 |
| 17 | -0,007 | -0,022 | 0,073 | 0,039 | -0,024 | 0,173** | -0,003 | -0,071 | -0,001 | 0,045 |
| 18 | -0,087* | -0,045 | 0,197** | 0,139** | -0,010 | -0,004 | -0,028 | -0,017 | 0,055 | -0,043 |
| 19 | -0,084* | 0,101* | 0,022 | 0,101* | 0,032 | 0,103* | -0,011 | -0,051 | 0,076 | 0,004 |
| 20 | 0,131** | -0,082 | 0,022 | -0,125** | 0,118** | -0,009 | 0,009 | 0,268** | -0,052 | -0,040 |
| Ö | | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
| 12 | | 0,022 | | | | | | | | |
| 13 | | 0,115* | -0,071 | | | | | | | |
| 14 | | 0,042 | -0,090 | 0,211** | | | | | | |
| 15 | | -0,042 | 0,091 | 0,000 | 0,018 | | | | | |
| 16 | | -0,005 | 0,074 | 0,017 | 0,047 | -0,043 | | | | |
| 17 | | -0,034 | 0,076 | -0,019 | -0,177** | 0,126** | 0,019 | | | |
| 18 | | 0,075 | -0,006 | -0,020 | -0,024 | 0,050 | -0,013 | -0,012 | | |
| 19 | | 0,076 | -0,041 | -0,053 | -0,156** | 0,032 | -0,048 | 0,068 | 0,111** | |
| 20 | | -0,005 | -0,041 | 0,310** | 0,150** | 0,149** | -0,035 | -0,034 | -0,040 | 0,032 |

Ö: Özellikler; 1: Yumurta Ağırlığı; 2: Şekil indeksi; 3: Kırık Yumurta; 4: Ca Birikmesi; 5: Boğumlu yumurta; 6: Çatlak Yumurta; 7: Gizli Çatlak Yumurta; 8: Opaklık; 9: Döllü Yumurta; 10: Toplam Embriyo Ölümleri; 11: Canlı Civciv Sayısı; 12: Civciv Ağırlığı; 13: Tona Skoru; 14: Canlı Ağırlık; 15: FPD; 16: Dirsek Yanıklığı; 17: Ayak Bacak Problemleri; 18: Parmak Çarpıklığı; 19: Toplam Yaralanma; 20: Toplam Tüy Skoru; *: P=0,05; **: P=0,01

Yumurta ağırlığı kuluçka süresini, çıkış gücünü, civcivin ağırlığını ve erken dönem embriyo ölümlerini etkileyebilmektedir (Altan ve ark., 1998). Bu bilgilere ek olarak civciv kalitesinin de yumurta kalite özelliklerinden etkilendiği bildirilmektedir (Özsoy, 2019). Bu çalışmada da bildirilen bulguları destekleyici olarak yumurta ağırlığı ile civciv ağırlığı arasında güçlü ve pozitif bir ilişki belirlenmiştir. Bu duruma benzer olarak birçok araştırmada da yumurta ağırlığı ile civciv ağırlığı arasında yüksek ve önemli ilişkiler tespit edilmiştir (Dere ve ark., 2005; Saatçi ve ark., 2006; Yılmaz & Çağlayan, 2008).

Yumurtanın iç ve dış kalite özelliklerini damızlıklara ait genetik faktörler, üretim koşulları, hastalıklar ve yaralanmalar, yaş, besleme, sıcaklık ve stres durumları da etkileyebilmektedir (Durmuş & Kutlu, 2019). Faktörlere bağlı olarak yumurta kalite özellikleri iyi olan damızlıklardan da iyi kalitede civcivler elde edilebilmektedir (Decuypere ve ark., 2001; Tona ve ark., 2007). Bu çalışmada da birçok vücut kusuru özelliği yumurta verim dönemine bağlı olarak yumurta dış kalitesinde olduğu gibi değişim göstermiştir. Benzer olarak kuluçka özelliklerinde de bu farklılık belirlenmiştir. Özellikle yaşın ilerlemesi ile birlikte ortaya çıkan bazı fizyolojik değişimler ve kafeste yetiştirme koşullarının verdiği stres öncelikle yumurta kalitesini ve bununla ilişkili olarak da civciv kalitesini etkilemiş olabilir.

Yumurta kabuğu, gelişen embriyoya fiziksel koruma ve besin sağladığı ve yumurta içeriğinin mikrobiyal kontaminasyonunu önlediği için yumurtaların en önemli komponentlerinden biridir (Fathi ve ark., 2007; Nys ve ark., 2011). Yumurta kabuğunda kırık-çatlak olması, kirlilik, kabuksuz, benekli (koyu benekli yumurtalar) ve deforme yumurtalar hem sofralık yumurta kalitesini hem de döllü yumurtalarda kuluçka performansını önemli düzeyde etkilemektedir. Yumurta kabuğu sorunlarına ise birçok faktör (hastalık, stres, yaş, mikotoksinler, yetersiz beslenme, yaralanmalar vb.) etki etmektedir. Tavuklarda yapılan çalışmalarda, hayvanların sağlık durumunun iyileştirilmesine yönelik stratejilerin yumurta kabuğu kalitesini de iyileştirdiği bildirilmektedir (Gautron ve ark., 2021; Zhao ve ark., 2021).

Yumurtaların şekil indeksi, yumurtanın genişliğinin uzunluğuna oranı olarak ifade edilmekte ve kalitenin belirlenmesi açısından önemlidir. Yuvarlak yumurtaların viyollere alınması ve depolanması esnasında kırılma ihtimalleri veya zarar görme ihtimalleri daha yüksektir. Küçük yumurtalarda aynı sorunlara neden olabilmektedir. Kuluçkalık yumurta kalitesi açısından sorunlu olan yumurtaların depolanması, taşınması ve kuluçkalama işlemi sorunlara yol açabilmektedir (Türkoğlu & Sarıca, 2014; Alkan ve ark., 2016). Çalışmamızda şekil indeksi pik öncesi döneme göre (35-46. hafta) ilerleyen dönemlerde azalmıştır.

Yumurta kabuğunun opaklığı, yumurta dış kalitesini ciddi şekilde etkiler ve yumurta kabuğu veya zarındaki değişiklikler, özelliğin yapısal temeli olarak kabul edilmektedir (Ren ve ark., 2023). Yumurta kabuklarındaki opaklık (saydamlık), yumurta içerisindeki nemin kabuk zarı ile birlikte dışa aktarılmasının ve bunun yumurta kabuğunda birikmesinin bir sonucu olarak ortaya çıkar. Bu durum da ışık iletiminin artmasına neden olur (Solomon, 1991; Wang ve ark., 2017). Yarı saydam olan, yani opaklığı düşük olan yumurta kabuğuna da Salmonella vb. kolayca nüfuz edebilir (Chousalkar ve ark., 2010). Çalışmamızda da yumurta verim dönemlerine bağlı olarak opaklığın değiştiği görülmektedir. Yumurtlama dönemi ilerledikçe opaklık azalmış saydam görünüm artmıştır. Bu durumun yumurta kabuk kalitesinin yaş ilerledikçe kötüleşmesi ile ilişkili olduğu düşünülmektedir. Fakat artan yaş ile birlikte yumurta kabuğu opaklığının fenotipik değişimini inceleyen sınırlı çalışma mevcuttur (Ren ve ark., 2023). Yumurta kabuğu opaklığı kısmen genetik faktörlerden etkilenmekte olup (Zhang ve ark., 2021), ortam sıcaklığı, nemi ve rasyondaki fosfor düzeyi vb. diğer faktörlerden de kısmen etkilenmektedir (Nie, 2013). Çalışma sonuçlarımızla benzer olarak, yaşlı tavuklarda (60 hafta) yumurta kabuğu opaklığının genç tavuklara (24 ve 42 hafta) göre daha kötü olduğu tespit edilmiştir (Solomon, 1991). Bu duruma yaşın daha etkili olduğu düşünülmektedir. Çünkü araştırmacılar çalışmalarında yaşın artması ile birlikte kabuk kalınlığı ve kırılma direnci özelliklerinin de kötüleştiğini, aynı zamanda kabuk oranının da azalma eğiliminde olduğunu bildirmişlerdir (Sarıca ve ark., 2010; Onbaşılar & Avcılar, 2011). Bu bildirişten farklı olarak Yılmaz ve ark. (2020) kabuk kalınlığının yaş ile değişmediğini bildirmiştir.

Çalışmamızda kekliklerde yumurta verim dönemleri baz alınarak sonuçlar irdelenmiştir. Verim dönemi aslında yaşı da ifade etmektedir. Bu nedenle yaş ile sonuçları değerlendirme gerekliliği ortaya çıkmaktadır. Çünkü genel bir değerlendirme yapıldığında kuluçkalık yumurtaların sağlandığı ebeveyn sürülerin yaşı kuluçka sonuçlarını, civciv kalitesini büyük oranda etkilemiştir. Bu çalışmadaki yumurta verim dönemleri dikkate alındığında verim durumu ile yaşın yumurta kalitesi, kuluçka sonuçları ve civciv kalitesi üzerine etkili olduğu düşünülmektedir. Vücut kusuru özellikleri üzerine kafes şartları ve kekliklerin davranış özelliklerinin etkili olduğu düşünülmektedir. Özellikle çiftleşme davranışı sırasında hırçın olmaları, erkeklerin çiftleşme amacıyla dişilerin sırtına çıktığında ayakları ile sırt bölgesinde yaralanmalara, gagasıyla da baş ve boyun bölgesinde yaralanmalara neden olduğu gözlenmiştir. Ayrıca bu durum tüy skorunun da kötüleşmesine neden olmuştur. İlerleyen yaş ile birlikte çiftleşme isteğinin azalması, vücut yaralanmalarının da azalmasında etkili olduğu düşünülmektedir.

Tona skoruna göre, skorlama puanı 100'e yaklaştıkça civciv kalitesi artmakta, 100'den uzaklaştıkça civciv kalitesi düşmektedir (Kamanlı & Durmuş, 2014). Civcivlerin tona skorunun yüksek olması canlı kalabilme gücünü ortaya koymaktadır. Çalışmamızda yumurta verim dönemlerine bağlı olarak civciv tona skoru değeri önemli farklılıklar göstermiştir. Yumurta veriminin başladığı ilk dönemde (pik verim öncesi dönem) elde edilen civcivlerin kalitesi ilerleyen dönemlerde düşmüştür. Civciv ağırlıkları ise dönemlere bağlı olarak değişmemiştir. Durmuş (2018), yüksek canlı ağırlığa sahip civcivlerin daha kaliteli olduğunu bildirmektedir. Çalışmamızın sonuçları bu bilgiyi desteklememektedir. Çalışmamızda kötüleşen civciv kalitesi ile yumurta kabuk opaklığının da ilişkili olduğu düşünülmektedir. Çünkü opaklık ve tona skoru arasında zayıf değerde de olsa önemli ilişki tespit edilmiştir.

## Sonuç

Çalışma sonuçları kekliklerde yumurta üretimi ve kuluçka faaliyetlerinde yaş dönemlerine bağlı olarak iyileştirme faaliyetleri yapılması gerektiğini göstermektedir. Pik öncesi yumurta verim döneminde (erken yaşlarda) daha kötü olan sağlık ve refah ile ilgili vücut kusuru özelliklerinin kafes ortamında ilk çiftleşmelerden kaynaklı hırçınlığın bir sonucu olarak meydana gelebileceği düşünülmektedir. Bu nedenle ebeveyn kekliklerde çiftleşme dönemi öncesinde gaga kesimi ve tırnak bakımı çok önemli bir uygulama olarak ön plana çıkmaktadır. Özellikle yumurta kabuk opaklığının ilerleyen yaşa bağlı olarak azalması (saydamlığın artması), üzerinde durulması gereken diğer önemli bir konudur. Yumurtalarda opaklık ve çıkış gücü arasındaki ilişkiler değerlendirilerek yeni çalışmalar yapılması gereklidir. Kabuk kalitesini iyileştirici uygulamaların opaklık üzerine de olumlu etkide bulunacağı düşünülmektedir.

Kekliklerde erken yumurta verim döneminde vücut yaralanmaları ile ilerleyen yaşla birlikte kötüleşen tüy kondisyonu, kabuk opaklığında azalma, kuluçka performansı ve civciv kalite özelliklerinde kötüleşmeyi önleyici uygulamalar hem üretim performansının hem de hayvan refahının artırılması bakımından faydalı olacaktır.

## Beyanlar

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Bu çalışma 1. yazarın yüksek lisans tez çalışmasından özetlenmiştir.

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# *In silico* analyses of miRNAs that Target Odorant Binding and Chemosensory Proteins in *Bemisia tabaci*

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Tomato
Linen

ABSTRACT

The whitefly, *Bemisia tabaci*, damages various crops by releasing honeydew and spreading. Although farmers and pest control experts primarily rely on insecticides to manage whiteflies, the notable issue is their tendency to develop resistance to major insecticide categories, posing a significant challenge. This result has led to the improvement of new drugs or insecticide mixtures. In addition, some plant-based studies have been conducted to control whiteflies, and RNA interference (RNAi) technology has been used in recent years. This study aimed to identify the relationships between tobacco, cotton, tomato, and linen miRNAs and odorant-binding protein (*OBP*) and chemosensory protein (*CSP*) genes in whiteflies by using *in silico* approaches. We determined that 115 miRNAs belonging to these plants targeted 13 *CSP* and 8 *OBP* genes of *B. tabaci*. Obtaining findings are important to reduce dependency on chemicals and pesticides in pest management.

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## Introduction

Various pests and pathogens cause a reduction in plant growth and development. These pathogens, including viruses, bacteria, fungi and oomycetes, can be commensal, symbiotic, and pathogenic characteristics (Wang et al., 2022; Karanfil et al., 2023; Randa-Zelyut et al., 2023). Certain pathogens can directly penetrate plant tissues, while others enter through wounds or natural openings. Vector-borne pathogens can be introduced directly into vascular tissues. Each tissue invasion method differs in different cells and tissue types (Faulkner et al., 2012; Huang et al., 2020; Kashyap et al., 2020).

Besides plant pathogen microorganisms, plant pests are also responsible for 18-26% of worldwide crop loss annually (Culiney et al., 2014). These pests include insects, mites, nematodes, birds, and others. They can cause damage to crops by feeding on plant tissues, transmitting diseases, competing for nutrients, or inhibiting plant growth and development. Effective management of plant pests is essential for maintaining agricultural productivity and ensuring food security (Lucas et al., 2011).

The whitefly, *B. tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is one of the most important agricultural pests (Stansly et al., 2010; Inoue-Nagata et al., 2016; Czosnek et al., 2017). It affects crops through direct feeding or transmission of viruses (Brown et al., 1995; Navas-Castillo et al., 2011). More than 350 viral species, including Begomovirus, Carlavirus, Crinivirus, Ipomovirus, and Torradovirus, have been documented to infect over 1000 plants (Jones et al., 2003; Abd-Rabou et al., 2010; Götz et al., 2016; Rodríguez et al., 2019; Lu et al., 2019). Yellowing, leaf folding, reduced growth, and malformed fruit can be seen in affected plants (Khan et al. 2018). *B. tabaci* Middle East-Asia Minor1 (MEAM1) and *B. tabaci* Mediterranean (MED) species are highly invasive and have inflicted significant economic losses (Luo et al., 2002; Chu et al., 2006).

It has been proved that controlling whiteflies is difficult. Insecticides are used to manage this pest because of their convenience and efficacy. However, targeting whiteflies with insecticides is problematic due to their feeding habits on the underside of the leaves (Simmons et al., 2000; Simmons et al.,2005; Simmons et al., 2011; Horowitz et al., 2020). Therefore, whitefly management can be accomplished through a combination of physical and mechanical methods, biological control, plant-based products and/or biotechnical strategies (Perring et al., 2018; Razze et al., 2016; Ibrahim et al., 2017; Shejulpatil et al., 2019). Furthermore, biotechnological strategies for *B. tabaci* include transgenesis and RNA interference (RNAi) (Pan et al., 2012; Hunter et al., 2021).

The ability to perceive chemicals and detect odors is vital for the normal life cycle of insects, playing a crucial role in numerous physiological behaviors, including mating, host-seeking, and distinguishing food sources (Leal et al., 2013; French et al., 2015). Proteins associated with insect olfaction primarily comprise odorant binding proteins (*OBP*s), chemosensory proteins (*CSP*s), odorant receptors (ORs), and sensory neuron membrane proteins (Liu et al., 2012; Liu et al., 2015). *OBPs* and *CSPs* within this group are small soluble proteins found in high concentrations in the sensillum lymph of insects, particularly in the head region (Honson et al., 2005; Pelosi et al., 2006; Zhou et al., 2010). These proteins play vital roles in aiding insects to detect chemical signals, thereby significantly influencing their behaviors (Tunstall et al., 2012). Consequently, they represent important molecular targets for designing and developing new pest management systems (Qiao et al., 2009; Sun et al., 2012). Our aim was to determine the targets of whitefly chemical and odor genes in specific plants and to target *B. tabaci* by using *in silico* methods.

## Materials and Methods

### *Retrieval of Tobacco, Cotton, Tomato and Linen*

Mature miRNAs belonging to tobacco, cotton, tomato and linen miRNAs were retrieved from miRBase (https://www.mirbase.org). miRBase is a public repository and standard online reference source for all documented miRNA sequences, offering textual annotations and gene nomenclature (Kozomara et al. 2019). We obtained 60 tobacco, 50 cotton, 101 tomato and 16 linen mature miRNAs from miRBase and examined them in this study.

*Obtaining from CSP and OBP genes*

The sequence of 13 *CSP* and 8 *OBP* genes of *B. tabaci* were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/). These genes and accession numbers were shown in Table 1.

Table 1. *CSP* and *OBP* sequences

| Gene Name | Accession Number |
|---|---|
| *CSP1* | KT694344 |
| *CSP2* | KT694345 |
| *CSP3* | KT694346 |
| *CSP4* | KT694347 |
| *CSP5* | KT694348 |
| *CSP6* | KY305449 |
| *CSP7* | KY305450 |
| *CSP8* | KY305451 |
| *CSP9* | KY305452 |
| *CSP10* | KY305453 |
| *CSP11* | KY305454 |
| *CSP12* | KY305455 |
| *CSP13* | KY305456 |
| *OBP1* | KY305457 |
| *OBP2* | KY305458 |
| *OBP3* | KY305459 |
| *OBP4* | KY305460 |
| *OBP5* | KY305461 |
| *OBP6* | KY305462 |
| *OBP7* | KY305463 |
| *OBP8* | KT358500 |

### *Target Prediction*

RNAhybrid tool was used (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid) to target predictions. For this purpose, a total of 227 miRNAs belonging to tobacco, cotton, tomato and linen were investigated whether they target *OBP* and *CSP* genes or not. The results were evaluated according to Marakli (2020). The positions and MFE values of miRNAs were recorded and the graph was drawn using MATLAB (MATLAB , R2023b MathWorks, Natick, MA, USA).

### *Phylogenetic Tree*

After the detection of miRNA that targets *CSP* and *OBP* genes, a phylogenetic tree was constructed to examine evolutionary relationships. For this purpose, the MEGA X (Kumar et al., 2018) program was used with the following criteria: Neighbor-Joining method (Saitou et al., 1987), genetic distances computed using the p-distance model (Nei and Kumar,2000) and even bootstrap resampling using 1000 replicates (Felsenstein, 1985). After the phylogenetic tree was created in MEGA X, Interactive Tree of Life (iTOL) (https://itol.embl.de) was used to manage and display the phylogenetic tree (Letunic and Bork, 2024).

## Results

According to the rules for target prediction, we identified 23 and 21 tobacco miRNAs that target *CSP* and *OBP* genes, respectively. For cotton, the numbers of miRNAs were 10 for *CSP* and 12 for *OBP*. Moreover, tomato miRNAs were 34 and 21 for *CSP* and *OBP* genes, respectively while 7 and 9 linen miRNAs were detected. We detected that some miRNAs target both *CSP* and *OBP* genes. The table containing plant genes, their interacting miRNAs, the corresponding interaction positions, and the MFE values were shown in Table 2a,b,c. The positions and MFE values of miRNAs were indicated in Figure 1.

The evolutionary relationships among a total of 100 miRNAs belonging to tobacco, tomato, linen and cotton which target *CSP* and *OBP* genes were evaluated. We observed that all sequences were divided into 3 groups in the phylogenetic tree. Furthermore, miRNAs found in 4 different plants were detected in each clade (Figure 2). Our analysis revealed that some of the miRNAs are classified as sister taxa. For example, sly-miR10535a and sly-miR10535b were found in sister clade that were predicted to interact with *CSP* 11, while nta-miR479a was a sister group to nta-miR479b which targeted *OBP* 8. Furthermore, sly-miR171d shared high sequence homology with sly-miR171e, targeting *CSP* 7. These miRNAs are predicted to interact with the same genes, supporting the notion that similar sequences may target similar genes (Friedman et al., 2009).

Nevertheless, the sequences appear to target the same gene even if they are not similar. For instance, nta-miR1919 and sly-miR1919a were found in sister clades and both of them were predicted to target *CSP* 5. Similarly, nta-miR399a and ghr-miR399a form another pair of sister clades, targeting *CSP* 6. Furthermore, there was another sister clade including sly-miR397-5p and lus-miR397a which targeted *CSP* 10.

Table 2a. Detail information for miRNA and their targets

| Gene | Plant | miRNA | Position | MFE (kcal/mol) |
|---|---|---|---|---|
| *CSP1* | Tobacco | nta-miR168a | 197 | -34.2 |
| | Cotton | ghr-miR3476-5p | 55 | -33.2 |
| | | ghr-miR7496a | 1 | -22.4 |
| | Tomato | sly-miR394-3p | 18 | -24.2 |
| | | sly-miR477-5p | 140 | -24.3 |
| | Linen | lus-miR395e | 268 | -23.8 |
| *CSP2* | Tobacco | nta-miR477b | 307 | -21.6 |
| | Cotton | ghr-miR482b | 318 | -22.2 |
| | Tomato | sly-miR9470-3p | 318 | -21.4 |
| | | sly-miR166c-5p | 99 | -23.7 |
| | | sly-miR9476-5p | 248 | -22.6 |
| | | sly-miR9479-5p | 265 | -21.8 |
| | | sly-miR399b | 316 | -21.5 |
| *CSP3* | Tomato | sly-miR482e-5p | 221 | -25 |
| | | sly-miR164a-3p | 326 | -24.2 |
| | | sly-miR403-5p | 175 | -21.2 |
| | | sly-miR482d-5p | 40 | -24.3 |
| | | sly-miR10537 | 326 | -20.7 |
| | | sly-miR7981f | 172 | -23.6 |
| *CSP4* | Tobacco | nta-miR5303c | 5 | -24.4 |
| | Cotton | ghr-miR7497 | 3 | -24.1 |
| | | ghr-miR7504a | 9 | -22.6 |
| | Tomato | sly-miR156e-3p | 23 | -24.6 |
| | | sly-miR477-3p | 108 | -26.3 |
| | | sly-miR10531 | 139 | -28.2 |
| | Linen | lus-miR167a | 7 | -25.7 |
| *CSP5* | Tobacco | nta-miR1919 | 46 | -24.3 |
| | | nta-miR6148b | 337 | -21.3 |
| | Tomato | sly-miR1919a | 46 | -24.9 |
| | | sly-miR1919c-5p | 254 | -23.3 |
| | | sly-miR9471a-3p | 58 | -24 |
| *CSP6* | Tobacco | nta-miR399a | 205 | -25.4 |
| | | nta-miR477b | 183 | -26.7 |
| | | nta-miR1919 | 29 | -26.7 |
| | | nta-miR6025c | 179 | -21.7 |
| | | nta-miR6150 | 457 | -23.5 |
| | | nta-miR5303c | 180 | -27.9 |
| | | nta-miR6161d | 168 | -22.9 |
| | Cotton | ghr-miR399a | 554 | -23.7 |
| | | ghr-miR3476-5p | 420 | -26.7 |
| | | ghr-miR7489 | 222 | -28 |
| | Tomato | sly-miR6023 | 552 | -20.9 |
| | | sly-miR482c | 218 | -21.7 |
| | | sly-miR6026 | 323 | -22.4 |
| | | sly-miR319c-5p | 546 | -27.3 |
| | | sly-miR9469-3p | 599 | -27.5 |
| | | sly-miR167b-3p | 215 | -22.7 |
| | | sly-miR10536 | 161 | -21.9 |
| | Linen | lus-miR398f | 686 | -28.5 |
| *CSP7* | Cotton | ghr-miR7489 | 151 | -22.2 |
| | Tomato | sly-miR171d | 172 | -27.1 |
| | | sly-miR395a | 50 | -25.5 |
| | | sly-miR171e | 164 | -25.8 |
| | Linen | lus-miR398f | 164 | -25.5 |
| *CSP8* | Tobacco | nta-miR6148a | 279 | -22.2 |
| | | nta-miR6150 | 288 | -20.2 |
| | Cotton | ghr-miR2948-5p | 18 | -20.9 |
| | Tomato | sly-miR1918 | 114 | -22.6 |
| | | sly-miR394-3p | 1 | -21.7 |
| | Linen | lus-miR398f | 149 | -20.5 |

Table 2b. Detail information for miRNA and their targets

| Gene | Plant | miRNA | Position | MFE (kcal/mol) |
|---|---|---|---|---|
| *CSP9* | Tobacco | nta-miR6020b | 251 | -20.9 |
| | | nta-miR6144 | 235 | -22.1 |
| | | nta-miR5303b | 44 | -23 |
| | | nta-miR6161c | 161 | -22.8 |
| | Cotton | ghr-miR2950 | 20 | -26 |
| | Tomato | sly-miR1918 | 177 | -26.4 |
| | | sly-miR9469-3p | 234 | -21.4 |
| *CSP10* | Tobacco | nta-miR6154b | 77 | -22.7 |
| | Cotton | ghr-miR3476-5p | 54 | -23.7 |
| | Tomato | sly-miR397-5p | 151 | -23.3 |
| | | sly-miR9471a-3p | 119 | -23.9 |
| | Linen | lus-miR397a | 151 | -24.1 |
| *CSP11* | Tobacco | nta-miR477b | 176 | -24.9 |
| | | nta-miR6145a | 163 | -23 |
| | Cotton | nta-miR6163 | 22 | -20.4 |
| | | ghr-miR7506 | 279 | -33.3 |
| | Tomato | sly-miR1918 | 75 | -21.8 |
| | | sly-miR6022 | 323 | -22.8 |
| | | sly-miR403-5p | 271 | -21 |
| | | sly-miR10531 | 141 | -25.5 |
| | | sly-miR10535a | 99 | -22.5 |
| | | sly-miR10535b | 99 | -21.6 |
| | Linen | lus-miR395e | 156 | -22.1 |
| | | lus-miR398c | 199 | -25.7 |
| | | lus-miR398e | 198 | -28 |
| | | lus-miR398f | 199 | -24.6 |
| *CSP12* | Tobacco | nta-miR397 | 66 | -23.8 |
| | | nta-miR479a | 38 | -20.1 |
| | | nta-miR6149b | 285 | -25.5 |
| | Linen | lus-miR169d | 1 | -23 |
| *CSP13* | Tobacco | nta-miR1919 | 281 | -21.8 |
| | | nta-miR6150 | 262 | -7.2 |
| | | nta-miR6155 | 159 | -23.8 |
| | Cotton | nta-miR6157 | 95 | -21.3 |
| | | ghr-miR482b | 40 | -23.6 |
| | | ghr-miR2948-5p | 18 | -26.8 |
| | Tomato | sly-miR6022 | 101 | -20.4 |
| | | sly-miR482c | 60 | -25.4 |
| *OBP1* | Tobacco | nta-miR479b | 182 | -21.5 |
| | Cotton | ghr-miR169b | 37 | -23.5 |
| | Tomato | sly-miR6027-5p | 54 | -21.4 |
| | | sly-miR9471b-3p | 144 | -24.1 |
| | | sly-miR9471a-3p | 247 | -22.6 |
| | | sly-miR156e-5p | 22 | -26.6 |
| | | sly-miR156e-3p | 135 | -24.2 |
| | | sly-miR399b | 138 | -27.8 |
| | | sly-miR10536 | 185 | -22.3 |
| | Linen | lus-miR159c | 149 | -24.1 |
| | | lus-miR396d | 130 | -21.5 |
| | | lus-miR398e | 249 | -20.7 |
| *OBP2* | Tobacco | nta-miR168a | 637 | -27.9 |
| | | nta-miR6158a | 39 | -25.3 |
| | Cotton | ghr-miR160 | 6 | -25.3 |
| | | ghr-miR7503 | 108 | -20 |
| | Tomato | sly-miR1919a | 585 | -20.3 |
| | | sly-miR9476-5p | 95 | -20.8 |

Table 2c. Detail information for miRNA and their targets

| Gene | Plant | miRNA | Position | MFE (kcal/mol) |
|---|---|---|---|---|
| *OBP3* | Tobacco | nta-miR171a | 623 | -21.9 |
| | | nta-miR6154b | 266 | -23.5 |
| | Cotton | ghr-miR160 | 20 | -26.6 |
| | | ghr-miR7510a | 90 | -30.8 |
| | Tomato | sly-miR1918 | 706 | -23.8 |
| | | sly-miR6023 | 84 | -21.4 |
| | | sly-miR397-3p | 29 | -22.4 |
| | Linen | lus-miR167a | 211 | -23.4 |
| | | lus-miR159b | 87 | -25.2 |
| | | lus-miR172j | 117 | -26.6 |
| | | lus-miR159c | 88 | -25.5 |
| | | lus-miR398f | 67 | -29 |
| *OBP4* | Tobacco | nta-miR6144 | 250 | -24.6 |
| | | nta-miR6154b | 42 | -26.9 |
| | | nta-miR6155 | 392 | -26.3 |
| | Tomato | sly-miR395a | 353 | -24.3 |
| | | sly-miR10531 | 234 | -21.3 |
| *OBP5* | Tobacco | nta-miR6021 | 307 | -24.6 |
| | | nta-miR6024 | 485 | -20 |
| | | nta-miR6145e | 340 | -20.5 |
| | Cotton | ghr-miR172 | 415 | -23 |
| | Tomato | sly-miR9478-3p | 145 | -23.4 |
| | Linen | lus-miR166b | 386 | -28.4 |
| | | lus-miR172j | 214 | -20.9 |
| *OBP6* | Tobacco | nta-miR482b-5p | 13 | -22.5 |
| | | nta-miR169t | 9 | -21 |
| | | nta-miR477a | 152 | -22.8 |
| | | nta-miR477b | 152 | -21.2 |
| | | nta-miR6144 | 141 | -22 |
| | | nta-miR6145e | 318 | -25.2 |
| | Cotton | ghr-miR2949a-5p | 388 | 25.9 |
| | | ghr-miR2949c | 388 | -23.7 |
| | Tomato | sly-miR482e-5p | 14 | -23.5 |
| | | sly-miR6026 | 127 | -23 |
| | | sly-miR169e-5p | 8 | -21.7 |
| | | sly-miR398a | 352 | -30.4 |
| | | sly-miR10533 | 152 | -20.3 |
| | Linen | lus-miR398c | 353 | -27.3 |
| | | lus-miR172j | 6 | -22.8 |
| | | lus-miR398e | 353 | -27.4 |
| | | lus-miR398f | 352 | 31.6 |
| *OBP7* | Tobacco | nta-miR477a | 690 | -25.4 |
| | | nta-miR477b | 690 | -25.3 |
| | | nta-miR6145a | 227 | 22.3 |
| | Cotton | nta-miR6163 | 267 | -25.6 |
| | | ghr-miR7485 | 586 | -28.7 |
| | Tomato | sly-miR9469-3p | 123 | -24.4 |
| | | sly-miR9476-5p | 621 | -24.2 |
| | | sly-miR10533 | 563 | -20.1 |
| | Linen | lus-miR398c | 9 | -30.6 |
| | | lus-miR398e | 7 | -31 |
| *OBP8* | Tobacco | nta-miR168a | 28 | -25.3 |
| | | nta-miR479a | 285 | -22 |
| | | nta-miR479b | 285 | -21.3 |
| | | nta-miR6025e | 14 | -22 |
| | | nta-miR6156 | 162 | -21.1 |
| | | nta-miR6145d | 354 | -21.3 |
| | | nta-miR6145e | 354 | -21.6 |
| | | nta-miR5303c | 5 | -22.3 |
| | Cotton | ghr-miR399c | 120 | -20.4 |
| | | ghr-miR7497 | 325 | -22.4 |
| | | ghr-miR7505 | 410 | -21.4 |
| | | ghr-miR7512 | 398 | -23.7 |
| | Linen | lus-miR398f | 218 | -25.5 |

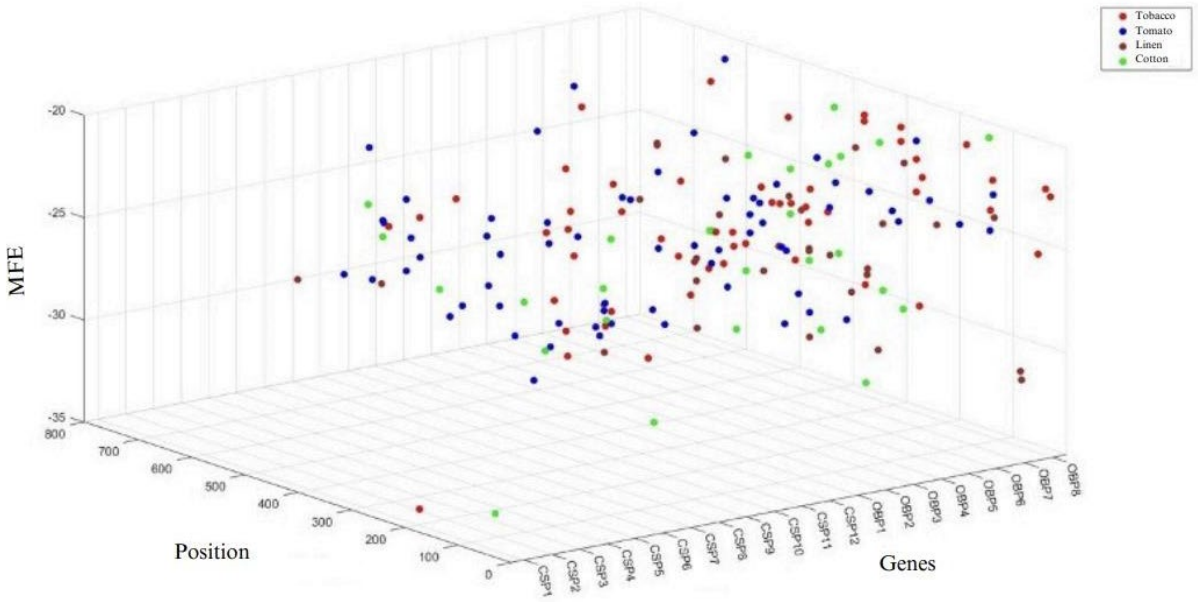


Figure 1. The 3D graphics of miRNAs. Red, blue, brown and green dots indicated tobacco, tomato, linen and cotton, respectively.

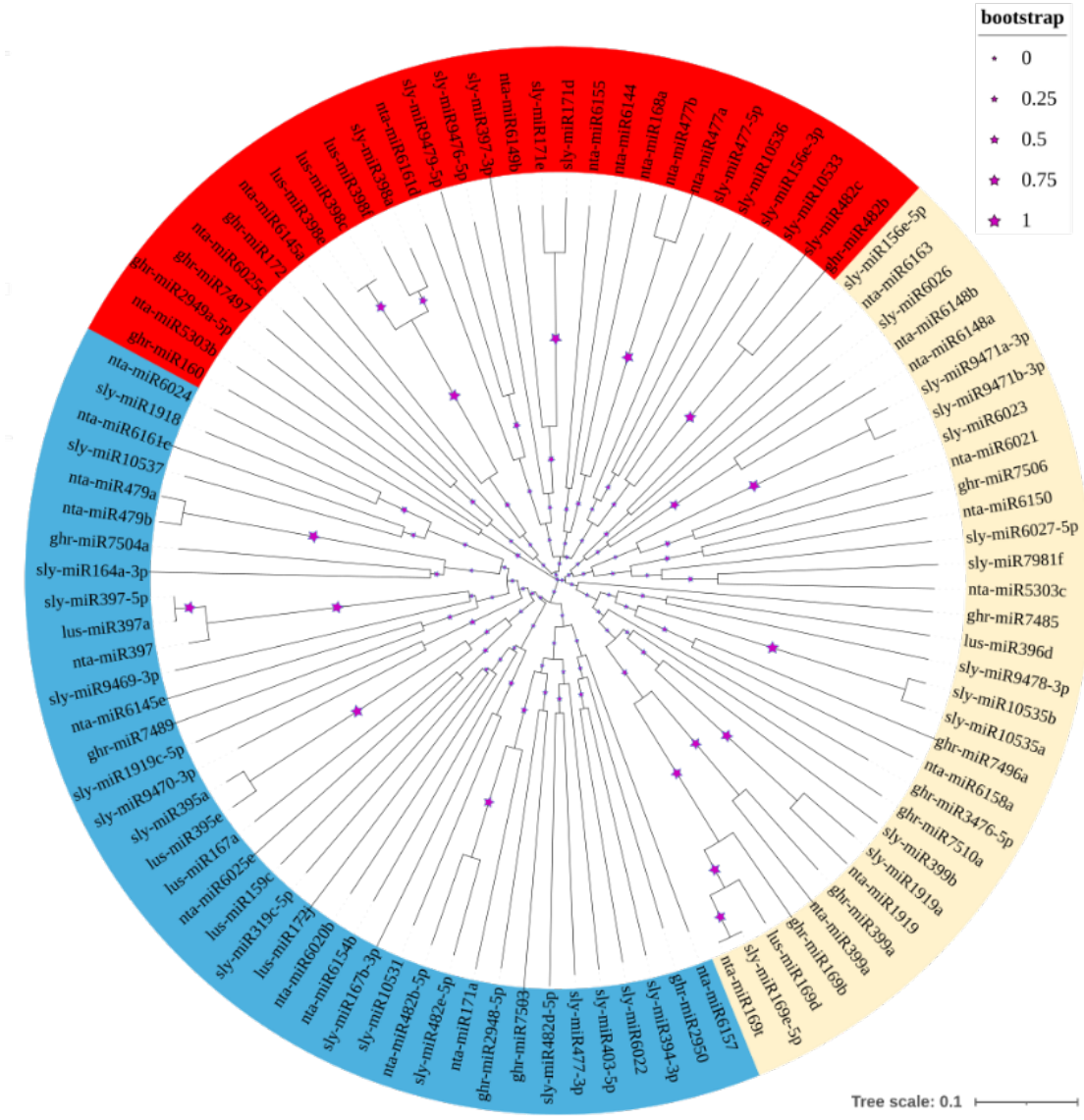


Figure 2. Phylogenetic tree generated using miRNAs belonging to tobacco, tomato, linen and cotton.

On the other hand, nta-miR482b-5p and sly-miR482e-5p were sister groups to nta-miR169t and sly-miR169e-5p, and targeted *OBP* 6. Similarly, lus-miR172j and ghr-miR172 were found as a sister group, targeting *OBP* 5. This observation suggests that while sequence similarity may often correlate with similar gene targets, this relationship is not universally applicable (Affonso-Grunz and Müller, 2015).

## Discussion

The whitefly is an important pest that causes serious damage to various plants, and it is crucial to improve new management practices. The use of RNAi technology among applications has been gaining increasing interest in recent years (Animasaun et al., 2023). In these studies, RNAi-mediated gene silencing has been successfully applied to different genes. In this study, we analyzed the relationships between miRNAs - *CSP*s and miRNAs – *OBP*s to determine the potential of candidate miRNAs to be used in the management of this pest. *CSP*s and *OBP*s are responsible for capturing external odorants and transporting them to olfactory receptors which are crucial for the development of the insect olfactory system (Leal et al., 2013; Li et al., 2014; Pelosi et al., 2014). In insects, the number of *CSP* genes varies widely, with nearly 70 in *Locusta migratoria* (Zhou et al., 2013). Similarly, the number of *OBP*-coding genes is different across insect species ranging from 13 in some ants (McKenzie et al., 2014) to over 100 in certain mosquitoes (Manoharan et al., 2013).

In previous studies, *CSP*s and *OBP*s were studied in RNAi studies. Waris et al. (2018) showed that the silencing of a chemosensory protein of *Nilaparvata lugens* changed the behavior of ligand-binding specificity and decreased the behavioral responses. Rebijith et al. (2016) reported that partially silenced *OBP* from *Aphis gossypi* affected antennal response to compounds. Moreover, five sensory-related genes were screened in another pest, *Agrilus planipennis*, for RNAi and they focused on three *OBP*s (Fan et al., 2022). These genes showed significant expression differences between newly emerged and mature emerald ash borers. Following dsRNA injection, gene expression was notably down-regulated. Gong et al. (2012) revealed that silencing *SexiCSP3* in *Spodoptera exigua* females reduced survival, oviposition, and egg hatching, highlighting RNAi's role in studying reproductive genes. These results showed that RNAi technology can be used for insecticides. On the other hand, there is no study about the relationship between plant miRNAs, *CSP*s and *OBP*s genes in whiteflies. We evaluate the interactions between 4 different plants (tobacco, cotton, tomato and linen) that are affected by this pest and 13 *CSP* and 8 *OBP* genes in *B. tabaci*. Recent studies have increasingly focused on understanding the molecular mechanisms underlying the interactions between *B. tabaci* and miRNAs. These small RNAs play a crucial role in regulating gene expression, impacting various physiological processes (Hasegawa et al., 2020; Wang et al., 2020). Researchers are particularly interested in how miRNAs influence the insect's development, reproduction, and adaptability to environmental stressors, as well as how they might contribute to its resistance to insecticides. A plant-mediated strategy using artificial miRNA (amiRNA) targeting three important whitefly genes (*Sxl, AChE,* and *Orc*) was transferred to tobacco plants. They reported decreased whitefly populations with fewer eggs hatching and slower development (Zubair et al., 2020). Furthermore, Gong et al. (2023) provided basic data on whitefly miRNA patterns, highlighting novel_miR-1517 as a key miRNA that regulates *CYP6CM1* gene. This gene is involved in the response to imidacloprid, a pesticide, suggesting that miRNAs could be potential targets for managing imidacloprid resistance in whiteflies. In another study, Shen et al. (2024) identified Bta-miR-998 and Bta-miR-129 which were associated with temperature tolerance.

## Conclusion

Previous studies have demonstrated that silencing certain *CSP* (Waris et al., 2018) and *OBP* (Rebijith et al., 2016) genes can alter insect behavior. Considering this, we aimed to identify miRNAs in tomato, tobacco, linen, and cotton, which are commercially significant crops commonly affected by *B. tabaci*. Our analysis revealed a total of 115 miRNAs and 21 genes, which were subsequently matched *in silico*. The results indicated that these genes were compatible with the identified miRNAs. Based on these findings, future studies can focus on targeting these genes using the identified miRNAs to protect these plants against whiteflies. This approach offers a sustainable and environmentally friendly alternative to pest control, reducing reliance on pesticides and chemicals.

## Declarations

### *Conflict of Interest*

The authors declare that there are no conflicts of interest regarding the research.

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# Foliar Application of Folic Acid on Cabbage Seedlings Grown under Restricted Irrigation Conditions Can Alleviate the Negative Effects

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ABSTRACT

Drought, a critical abiotic stress worsened by climate change, poses a substantial threat to crop production and global food security. White cabbage is classified as a moderately drought-sensitive crop. The function of folic acid, otherwise known as folate, in the plant's response to drought conditions is not yet fully understood. The aim of the study was to evaluate the potential of folic acid in enhancing certain growth parameters and physiological traits of cabbage seedlings under limited irrigation conditions. In this investigation, the effects of FA as a foliar application at 0, 100, and 200 µM (FA0, FA1, and FA2, respectively) were examined on white cabbage seedlings grown under full-irrigation (I0) and restricted irrigation (I1), set to 50% of full capacity irrigation scheme (I0). Drought stress adversely affected the plant growth properties of cabbage seedlings, whereas FA treatments mitigated the adverse effects of drought stress on the plant growth properties of cabbage seedlings. Under restricted irrigation, plants treated with 100 µM FA (FA1) had higher plant height, stem diameter and leaf area, while plants treated with 200 µM FA (FA2) had higher plant dry weight and plant dry matter content. Plant fresh weight increased with FA treatments under restricted irrigation, but no significant difference was observed between doses. On the other hand, leaf relative water content (LRWC), which decreased under restricted irrigation conditions, increased with FA applications regardless of the dose, while electrical conductivity (EC), which increased under the same conditions, decreased with 100 µM FA (FA1) application. Leaf number, root fresh weight, root fresh weight, root dry weight, root dry matter content and chlorophyll value (SPAD) were not affected by FA treatments under both restricted and full irrigation conditions. In conclusion, foliar spray of folic acid in cabbage can be recommended as a potential application to alleviate drought stress.

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## Introduction

Increasing environmental stresses caused by of climate change trigger various responses in plants, including alterations in growth rate, productivity, cellular metabolism, and gene expression (Muhammad et al., 2024). One of the adverse effects of climate change is soil water deficit, which leads to drought stress (Shinde et al., 2024). Rosa et al (2022) in their review of global agricultural landscapes for 130 primary crops; they estimated that exposure to water scarcity is highly dependent on geographical location and month of the year, with 76% of global agricultural land facing water scarcity for at least one month per year and another 42% of these lands facing five months per year.

Drought conditions trigger a large number of important events at the morphological, physiological and biochemical levels that can cause serious dysfunctions in plant metabolism (Anjum et al., 2011; Sanchez-Reinoso et al., 2018). As Yildirim et al. (2021b) posited, insufficient hydration in plants has been demonstrated to engender a reduction in photosynthesis, which consequently results in a decrease in vegetative growth. Moreover, the stem and leaves of plants exhibit greater sensitivity to water stress compared to the roots. In addition to alterations in metabolism, such as cell division and cell elongation, drought causes loss of turgor, deterioration in enzyme activities, and decreased photosynthesis efficiency (Bhargava and Sawant, 2013; Osakabe et al., 2014). The effect of drought is known to vary according to the severity and duration of the stress experienced, as well as the response of the plants (Farooq et al., 2009).

Cabbage (*Brassica oleracea* var. *capitata* L.), belonging to the Brassicaceae family, is predominantly distributed in the northern extratropical region of the world and originated from wild species growing on the European coasts of the Atlantic and Mediterranean (Leike, 1989). Brassica vegetables, including cabbage, comprise many economically important species cultivated worldwide (Pavlović et al., 2018). The development of Brassica crops,

which are commercially cultivated especially in Mediterranean, semi-arid and arid regions, and consequently the yield and quality of crops can be greatly affected by drought (Zhang et al., 2014). As drought is projected to increase gradually but steadily in much of the Mediterranean region, future projections indicate that the situation will worsen (Daliakopoulos et al., 2014). In fact, environmental conditions significantly affect cabbage production. Studies have shown that the growth and yield of white cabbage are negatively affected by drought and that this crop is moderately sensitive to drought (Maggio et al., 2005; Seidel et al., 2017). Despite this sensitivity, white cabbage has been shown to tolerate limited water availability to a certain extent (Pavlović et al., 2018), although the exact resilience mechanisms are not yet fully understood.

Vitamin B9 (folic acid; FA) and its derivatives (folates) are significant contributors to the metabolism of both carbohydrates and nitrogen (Stakhova et al., 2000). Folic acid serves as a crucial cofactor in plant one-carbon metabolism, aiding in the production of nucleic acids and amino acids (Alsamadany et al., 2022). Numerous studies have shown that foliar application of folic acid can boost vegetative growth in many plant species, including wheat (Mohamed, 2013), potato (Ibrahim et al., 2015), and broad bean (Dawood and El-Metwally, 2018). Research has also indicated that folic acid can mitigate oxidative stress caused by environmental factors through its antioxidant properties in plants (Cui et al., 2018; Gliszczyńska-Świgło, 2007). The external application of folic acid has been demonstrated to regulate plant responses to various environmental stressors, including drought, salt, and metal exposure. In this regard, exogenous folic acid plays a significant role in reducing the negative impacts of abiotic stresses such as drought (Poudineh et al., 2015; Ibrahim et al., 2021; Alsamadany et al., 2022; Khan et al., 2022), salinity (Kilic et al., 2016; Alsamadany et al., 2022; Al-Elwany et al., 2022), and metal toxicity (Sahito et la., 2024). A review of the literature on foliar applications of folic acid revealed a lack of research on its use to enhance drought tolerance in cabbage. To fill this knowledge gap, the current study investigated the effects of foliar applied folic acid on specific developmental and physiological characteristics of cabbage seedlings grown under water-limited conditions. The results of this research are expected to provide valuable insights for future investigations in this area.

## Materials and Methods

The study was was conducted in the greenhouses of Atatürk University, Plant Production Application and Research Centre, and in the laboratories of the Department of Horticulture of the Faculty of Agriculture. Seeds of the commercial standard white head cabbage variety "Yalova 1" treated with thiram to prevent possible fungal diseases, were used as plant material in this study.

The study was conducted as a pot experiment under controlled greenhouse conditions (average temperature 25°C and humidity 60%). Cabbage seeds were sown in multi-well trays containing a mixture of peat and perlite (1:1, v/v) and seedlings were transplanted after 30 days into 1.5 L pots with a 2:1 (v:v) soil:peat mixture. The study was designed as a completely randomized factorial design with two factors; two irrigation level treatments (I0 and I1) and three doses of folic acid (FA0, FA1 and FA2). There were three replications and 5 pots per replication.

Seedlings of homogeneous size and morphology, aged two weeks, were segregated into two primary groups. One group received irrigation equivalent to field capacity for the control treatment (fully irrigated; I0), while the other group was subjected to restricted irrigation conditions, receiving 50% of the I0 treatment (I1). The volumetric determination of irrigation water application and soil moisture content in the pots was conducted utilizing a portable moisture meter. Within each major group, pots were subdivided into three subgroups to administer folic acid ($C_{19}H_{19}N_7O_6$) at concentrations of 0 (FA0), 100 (FA1), and 200 µM (FA2) (Alsamadany et al., 2022). Seedlings were subjected to foliar application of FA five times at 15, 20, 25, 30, and 35 days post-transplantation. The plants in the control group were treated with foliar rainwater. The volume of applied solution was 10 mL per plant per application. Tween-20, a non-ionic surfactant, was incorporated at 0.05% (v/v) in all foliar treatments.

To evaluate the impacts of the irrigation levels and FA applications on cabbage seedlings, some measurements, weighing and observations were carried out. In the study, plant height, leaf number, stem diameter, leaf area, plant fresh and dry weight, root fresh and dry weight, plant and root dry matter content, leaf relative water content (LRWC), SPAD values, and electrical conductivity (EC) were determined. SPAD value is measured with portable SPAD-502 chlorophyll meters, which express the relative chlorophyll content. The total leaf area of each plant was determined by the leaf area meter (CID Bio-Science, USA).

The determination of electrical conductivity (EC) was carried out as described by Kaya et al. (2003). Discs (1 cm diameter) were taken from fully opened true leaves of two randomly selected plants and transferred to glass bottles containing 30 ml of distilled water. The samples were shaken in a shaker for 24 hours. The electrical conductivity of the shaken water was then measured and the permeability (degree of damage) of the cell membranes determined (EC1). The samples were then autoclaved at 121°C for 20 min and the second electrical conductivity measurement was carried out in the sample water at room temperature (EC2) and the final EC value was obtained by calculating the percentage of EC1/EC2.

For the measurement of leaf relative water content (LRWC), discs (1 cm diameter) were taken from fully opened true leaves of two randomly selected plants and their fresh weight (FW) was determined by rapid weighing on a high precision balance. The discs were then placed in a glass beaker containing pure water, so that the underside of the leaf was in contact with the water, and kept in the dark for 24 hours. The excess water on the discs was then gently removed with blotting paper and the turgor weights (TW) of the discs were again weighed on a precision balance and recorded. The discs were then dried in an oven at 72ºC for 48 hours and weighed by same balance again to determine the dry weight (DW). LRWC values were calculated based on the following formula (equation 1) (Shams et al., 2019):

$$LRWC = [(FW - DW) / (TW - DW)] \times 100 \quad (1)$$

The data were analyzed by using the SPSS 27 package program, and the difference between the means was determined by Duncan multiple range tests.

## Results and Discussion

Figure 1, Table 1, and Table 2 illustrate the effects of folic acid on the growth and physiological characteristics of cabbage seedlings subjected to water deficit.

The effects of folic acid doses and irrigation levels on plant height, stem diameter, number of leaves and leaf area were found to be significant at the $p<0.05$ level, except for FA treatments for stem diameter. Cabbage seedlings exposed to water deficit showed a significant decrease in plant height, stem diameter, number of leaves and leaf area by 6.19%, 19.49%, 21.17% and 31.56% compared to the control plants under well-irrigated conditions. As reported by Fathi and Tari (2016), drought stress during the vegetative period has multifaceted effects on plant development, including leaf and stem development, photosynthesis and accumulation of plant components. The study findings were consistent with the results of Jang et al. (2024), who reported drought stress-induced decrease in leaf area, fresh weight, and leaf water content of Chinese cabbage seedlings. Samancıoğlu et al. (2016) also confirmed that low irrigation levels reduce the growth parameters of cabbage seedlings. However, foliar application of folic acid has been shown to mitigate the adverse effects of deficient irrigation on the growth characteristics of cabbage seedlings (Figure 2). In plants experiencing water stress and treated with folic acid, FA1 and FA2, significant increases in plant height (11.02 and 5.43%), stem diameter (12.79 and 1.83%), and leaf area (21.02 and 11.74%) were observed compared to the control (F0). Folic acid treatments had no statistically significant impact on cabbage seedlings' leaf number, regardless of watering conditions (Figure 1). Gorelova et al. (2017) documented the significant regulatory role of folates in plant growth and development processes. In the study by Aljuaid et al. (2022), the application of folic acid was found to significantly improve the shoot fresh-weight of plants subjected to stress, particularly at concentrations of 0.1 and 0.2 mM, in comparison to untreated plants. A similar outcome was reported in a study conducted in coriander, where foliar FA application was found to enhance plant growth under conditions of deficient irrigation (Khan et al., 2022).

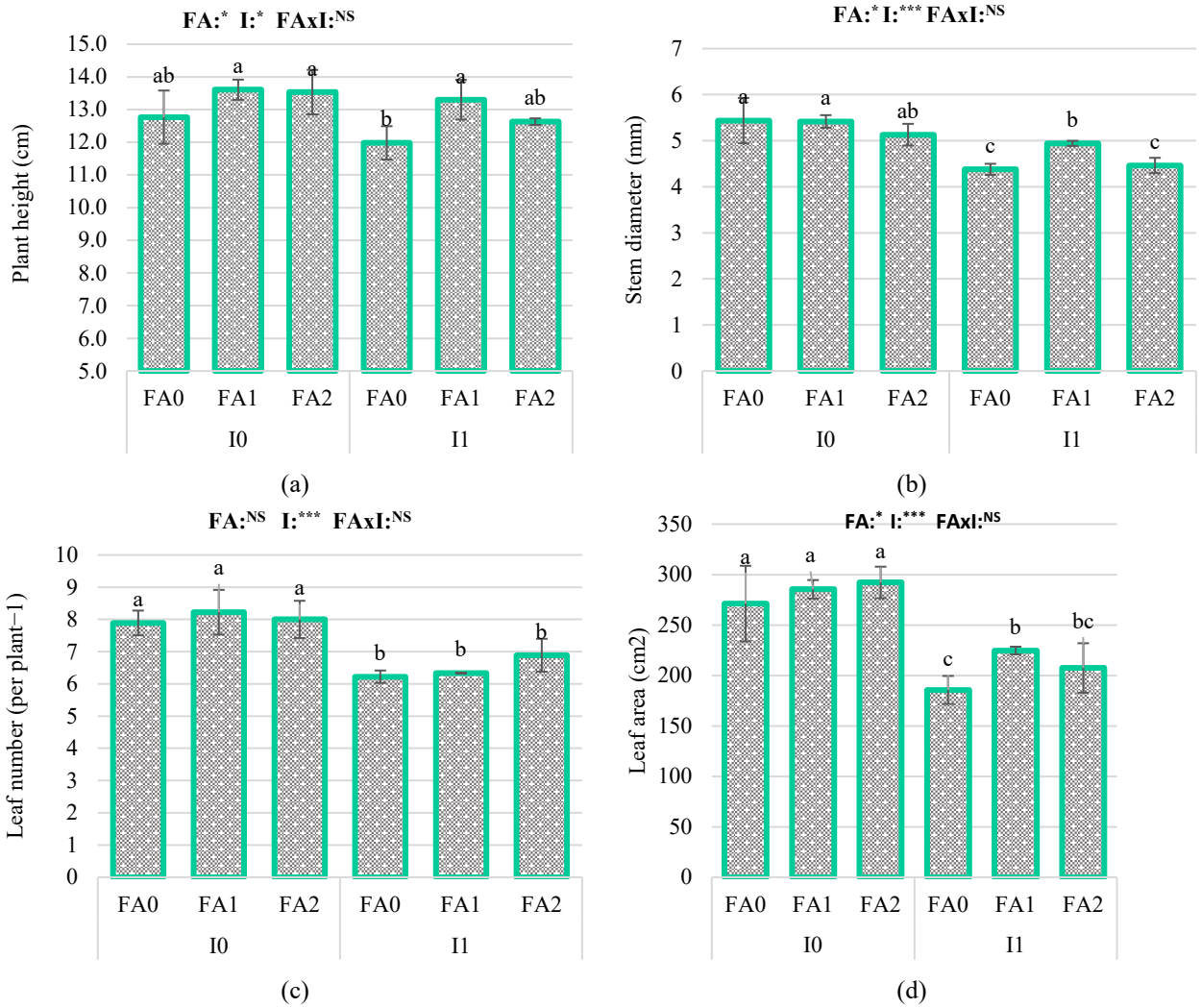


Figure 1. Effect of foliar application of 0 (FA0), 100 (FA1), and 200 µM (FA2) on (a) plant height (cm), (b) stem diameter, (c) leaf number, (d) leaf area in I0 and I1.

Graph values are the mean ± SE of three replicates. Bars exhibited with different letters indicated a significant difference between means ($p \leq 0.05$).

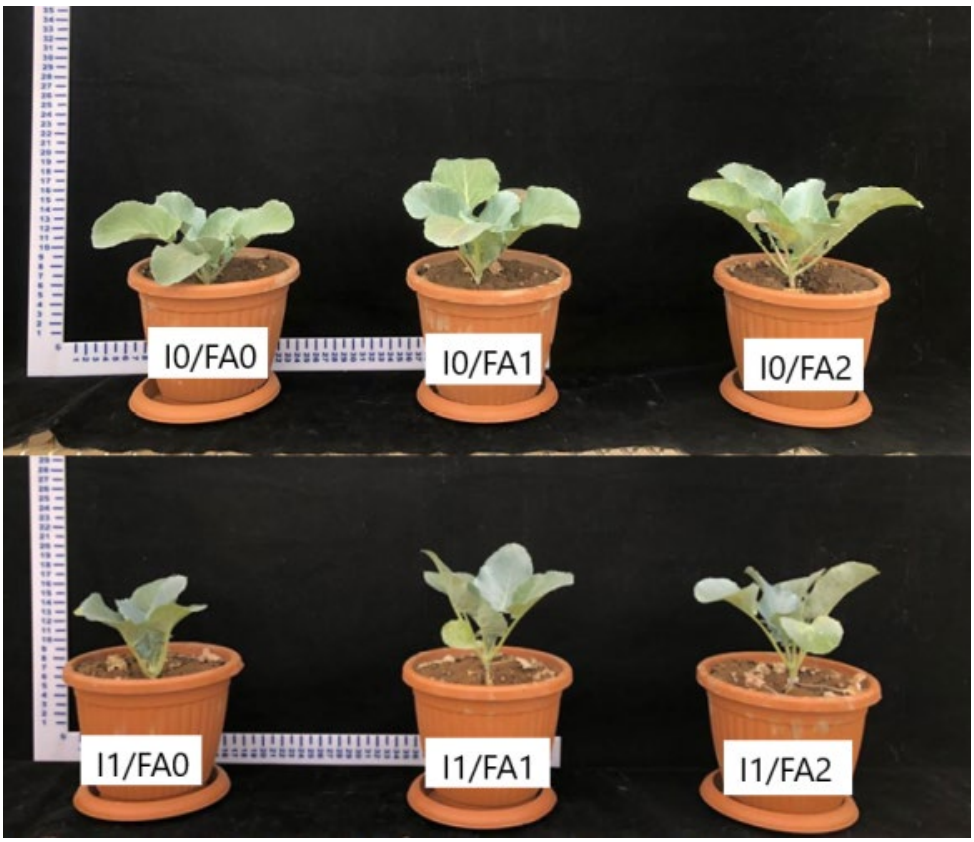


Figure 2. Response of cabbage seedlings to folic acid applications under varying irrigation conditions.
I0: 100% irrigation; I1, 50% irrigation; FA0, 0 µM FA; FA1, 100 µM FA; FA2, 200 µM FA.

Table 1. Effect of 0 (FA0), 100 (FA1) and 200 µM (FA2) foliar applications on plant fresh and dry weight, plant dry matter content, root fresh and dry weight and root dry matter content at I0 and I1.

| Irrigation | Folic acid | Plant fresh weighth (g) | Plant dry weighth (g) | Plant dry matter content (%) | Root fresh weight (g plant$^{-1}$) | Root dry weight (g plant$^{-1}$) | Root dry matter content (%) |
|---|---|---|---|---|---|---|---|
| I0 | FA0 | 15.37±0.56 a | 3.40±0.18 a | 22.18±1.13 a | 4.44±0.94 a | 3.45±0.09 a | 14.05±1.38 b |
| | FA1 | 15.54±0.44 a | 3.59±0.16 a | 23.13±0.95 a | 4.20±0.25 a | 3.42±0.03 a | 14.46±0.09 b |
| | FA2 | 15.39±1.39 a | 3.41±0.20 a | 22.14±1.42 a | 4.81±0.40 a | 2.94±0.07 a | 13.08±0.64 b |
| I1 | FA0 | 8.91±0.13 c | 1.80±0.07 c | 20.20±0.58 b | 2.26±0.13 b | 1.85±0.04 b | 17.02±1.84 a |
| | FA1 | 9.16±0.68 b | 1.97±0.08 bc | 21.54±2.14 ab | 2.36±0.38 b | 2.05±0.05 b | 17.92±1.76 a |
| | FA2 | 9.43±0.41 b | 2.12±0.07 b | 22.5±0.59 a | 2.02±0.13 b | 1.52±0.04 b | 18.90±1.09 a |
| Falic Acid (FA) | | * | * | * | NS | NS | NS |
| Irrigation (I) | | *** | *** | ** | *** | *** | *** |
| FA× I | | NS | NS | NS | NS | NS | NS |

Table values are the mean ± SE of three replicates. Means with different letters in the same column indicate a significant difference ($p \leq 0.05$).

On the other hand, a marked decline in plant fresh weight, plant dry weight and plant dry matter content was observed, amounting to 42.03%, 47.06% and 8.93%, respectively, as a consequence of the restricted irrigation. Concurrently, the fresh and dry weights of the cabbage seedlings' roots decreased by 49.10% and 46.38%, respectively, while root dry matter content exhibited an increase of 21.14%. Our results are consistent with those of the study by Şahin et al. (2018), which found that severe drought stress caused a 46.11-48.29% decrease in the relative fresh and dry weights of plant shoots. On the other hand, as a result of restricted irrigation, plant fresh weight, plant dry weight and plant dry matter content of cabbage seedling decreased significantly by 42.03%, 47.06% and 8.93%, respectively. Meanwhile, the restriction of irrigation led to a decline in the fresh and dry weights of cabbage seedlings' roots by 49.10% and 46.38%, respectively, while root dry matter content exhibited an increase of 21.14%. However, folic acid foliar application significantly increased plant fresh weight under restricted irrigation conditions, though no differences were observed between doses. The most favourable outcomes were observed for plant dry weight and plant dry matter content of cabbage seedlings, with 17.78% and 11.39%, respectively, attained in the 200 µM FA (FA2) treatment under restricted irrigation conditions. Conversely, the impact of folic acid on root fresh weight and root dry weight of seedlings under restricted irrigation conditions was found to be insignificant. (Table 1). Khan et al. (2022) reported that foliar application of folic acid to coriander plants under IR75 and IR50 irrigation regimes resulted in higher plant fresh biomass (28% and 131%) and dry biomass (63% and 66%) in comparison to plants not treated with folic acid.

Table 2. Effect of 0 (FA0), 100 (FA1) and 200 µM (FA2) foliar applications on plant fresh weight, plant dry weight, plant dry matter content, root fresh weight, root dry weight and root dry matter content at I0 and I1.

| Irrigation | Folic Acid | SPAD | LRWC (%) | EC (%) |
|---|---|---|---|---|
| I0 | FA0 | 50.07±0.88 c | 89.27±0.65 b | 43.63±1.11 d |
| | FA1 | 47.69±1.07 d | 90.31±3.45 b | 39.26±0.86 e |
| | FA2 | 47.43±1.09 d | 94.87±2.98 a | 41.31±1.4 e |
| I1 | FA0 | 59.04±0.15 a | 67.35±1.95 d | 64.02±1.24 a |
| | FA1 | 54.62±0.59 b | 74.78±1.43 c | 55.26±1.19 c |
| | FA2 | 55.16±0.88 b | 72.52±0.95 c | 57.79±1.06 b |
| Falic Acid (FA) | | *** | *** | *** |
| Irrigation (I) | | *** | *** | *** |
| FA× I | | NS | ** | ** |

Table values are the mean ± SE of three replicates. Means with different letters in the same column indicate a significant difference ($p \leq 0.05$)

Finally, it was shown that SPAD and EC values of plants increased by 17.92% and 46.73%, respectively, under restricted irrigation conditions. In contrast, leaf relative water content (LRWC) decreased by 24.56% compared to control plants. In a study examining the effects of drought stress on cabbage seedlings, it was found that plants maintained more LWRC (69.12%) under well-watered conditions, while lower LWRC (56.06%) was recorded under stress conditions (Yildirim et al., 2021a). Xu and Leskovar (2014)'s study also reported a significant decrease in the relative water content (RWC) value under conditions of water stress. Also in the current study, regardless of dosage, FA application resulted in an increase (11.03% for FA1 and 7.68% for FA2) in LRWC, which decreased due to deficit irrigation. Furthermore, under the same conditions, a 13.68% decrease in the increased EC value was observed with the application of 100 µM FA (FA1). Ibrahim et al. (2021) reported that foliar FA applications at restricted irrigation levels significantly improved RWC in green bean leaves compared to untreated plants in a two-season study (Ibrahim et al., 2021). As a last point, foliar FA1 and FA2 applications reduced the increased SPAD value due to similarly restricted irrigation by 7.49% and 6.57%, respectively, regardless of the dose (Table 2).

## Conclusion

The study's findings indicated that a restricted irrigation treatment resulted in a decline in several pivotal parameters of cabbage seedlings' growth. The parameters that demonstrated a decline included plant height, stem diameter, leaf number, leaf area, plant fresh and dry weight, plant dry matter content and root fresh and dry weight. In addition, restricted irrigation increased root dry matter content, SPAD value and EC, while decreasing LRWC. However, folic acid application increased plant height, stem diameter, leaf area, plant fresh and dry weight and plant dry substance content in cabbage seedlings under restricted irrigation conditions. The best results in terms of plant height, stem diameter and leaf area were observed at 100 µM FA (FA1) folic acid dose in the restricted irrigation group. A folic acid dose of 200 µM (FA2) was found to be most effective in increasing plant dry weight and dry matter content in the drought-affected group. Furthermore, it was observed that folic acid sprays, irrespective of the dose, enhanced plant fresh weight, which had been diminished by the effects of drought. The study concluded that foliar application of folic acid in cabbage can be recommended as a possible intervention to reduce the effects of drought stress.

## Declarations

This paper was presented in 6th International Conference on Food, Agriculture and Animal Sciences.

***Author Contribution Statement***

R.K. carried out all stages such as designing, conducting, analyzing the research, preparing pictures and tables, and writing the article.

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# Determination of Water Quality in Dairy Cattle Enterprises: A Case of Niğde Province

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ABSTRACT

In dairy farms, water is crucial for the health, productivity, and welfare of animals. Water is a fundamental component in all biological processes, and insufficient water intake can negatively impact milk production, reproductive health, and overall animal welfare. The water requirement for dairy cattle depends on various factors such as age, weight, milk yield, environmental temperature, and nutritional status. An adult dairy cow can consume approximately 80-150 liters of water per day. This requirement increases in high-yielding cows. Adequate water intake directly affects milk production, as approximately 87% of milk is composed of water. The quality of water is as crucial as its quantity. This study investigates the quality of drinking water in dairy farms within Niğde Province, Turkey, focusing on its implications for livestock health and productivity. Water samples were collected from 11 livestock enterprises, encompassing water tanks and troughs, and analyzed for electrical conductivity (EC), pH, nitrate ($NO_3$), nitrite ($NO_2$), and phosphate phosphorus ($PO_4$) concentrations. Results showed EC values averaging 0.803 $dSm^{-1}$, within acceptable standards for livestock, although high concentrations in certain tanks raised concerns regarding mineral content and potential health impacts. The pH ranged from 7.27 to 8.20, remaining suitable for all livestock classes. $NO_3$ concentrations averaged 21.834 $mgL^{-1}$, with no samples below the 10 $mgL^{-1}$ threshold, highlighting risks from prolonged exposure. In contrast, $NO_2$ concentrations averaged 0.251 $mgL^{-1}$, remaining within safe limits. $PO_4$ concentrations were minimal, averaging 0.056 $mgL^{-1}$, and posed no significant risks. The findings underscore the importance of periodic water quality monitoring in livestock farms to mitigate risks of contamination and ensure optimal health and productivity. These findings highlight the necessity for customized water management techniques to fit particular farm settings and advance our understanding of the complex effects of water quality on livestock performance.

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## Introduction

Water is an essential nutrient for the vital functions of living beings. For this reason, we must always ensure that clean and continuous water is available to animals on the farm. However, sometimes caretaker errors, differences in the animal's needs in climate conditions (winter and summer), differences in feeding, physiological differences in the animal, the nutrient content of the feed, and the level of minerals in the feed can cause the animal's water needs to increase or reduce. In such cases, insufficient or poor quality water given to dairy cattle can limit milk production, limit the animal's growth, and cause health problems. For this reason, always having enough clean water in front of the animal supports normal rumen function, high feed intake, digestion, and nutrient absorption. Water also maintains blood volume, meets tissue needs, and makes up approximately 87% of the milk secreted by the cow (Paul & Kim, 2017).

The need for a steady supply of high-quality water is sometimes overlooked by managers of animal production systems, who instead focus on changing feed to improve performance (Beede, 2012).

Water's pH indicates how acidic or alkaline it is, and it greatly affects both microbial activity and water palatability. A livestock's feed intake and general health may be impacted by gastrointestinal problems brought on by too acidic or alkaline water. Extreme pH levels can also have an impact on the toxicity and solubility of other dissolved materials, including minerals and metals, which can further alter the quality of water. Generally, the ideal pH range for animal water is between 6.5 and 8.5; any variations could have negative health implications.

However, water is necessary for many vital processes in the animal body, including the delivery of nutrients and hormones, the removal of waste products, the control of

osmotic blood pressure, and the regulation of secretions such as milk, saliva, and body temperature (Yoshihara et al., 2016). In livestock production, it's just as crucial to guarantee that ruminant animals always have access to enough water as it is to have feed on hand (Utley et al., 1970). There are three ways to receive water: drinking water, feed water, and metabolic water (produced during the breakdown of nutrients). Most livestock production systems do not measure the latter two types of water (Romanzini et al., 2024).

When assessing the suitability of water for livestock, various criteria must be considered, with local conditions and the availability of alternative sources playing significant roles (FAO, 1994). Water sources such as small, shallow wells and streams are more prone to contamination and poor water quality compared to larger wells or running streams. Groundwater, in particular, is often more chemically imbalanced than surface water. Seasonal changes also influence water quality; during hot and dry periods, factors such as extreme evaporation from stock watering ponds or tanks can lead to higher salt concentrations, increased water consumption due to heat, and elevated water temperatures. These conditions can render marginally suitable water sources unusable. The age and condition of animals further affect their vulnerability, with young, frail, and lactating animals being particularly susceptible. Additionally, the composition of feed plays a critical role; dry pastures and protein-rich supplementary feeds, which have lower moisture content and higher salt levels, can harm livestock and reduce their salt tolerance. Some feed additives are specifically controlled by adding salt to slow down consumption. Lastly, different animal species have varying abilities to tolerate salt levels in their water, necessitating tailored water quality management for each species (FAO, 1994).

Regardless of the size of the operation, livestock farming requires a constant availability of clean, safe water to support animal development, reproduction and milk production. Water quality and agricultural ecosystems interact in a dynamic and complex manner. Farms often use groundwater sources such as wells or surface water such as rivers and lakes to provide drinking water for their livestock. Because of their close proximity to these water sources, farms are vulnerable to contamination from agricultural runoff, which can include pathogens from manure as well as pollutants such as phosphates, nitrates and pesticides. The health of animals can be seriously compromised by contamination of drinking water, leading to illness, reduced weight gain and, in severe situations, even death. Livestock water quality is directly impacted by farming activities. Water quality problems can be caused by improper water management, fertilizer and pesticide contamination, and inadequate water supplies. According to farm management guidelines, the water supply must be protected from pollutants and checked regularly to ensure excellent quality. Additionally, it is important to integrate water management with other agricultural techniques, including crop management and soil health (USEPA, 2005, USEPA, 2015).

The quality of drinking water varies significantly depending on the source and environmental conditions, leading to variations in water quality parameters. These include electrical conductivity (EC), pH, nitrate ($NO_3$), nitrite ($NO_2$), and phosphate ($PO_4$), which all play critical roles in determining water suitability for animal consumption (NRC, 2001). The amount of dissolved minerals including calcium, magnesium, and salt is directly correlated with EC, a measurement of water salinity. Especially when exposed for an extended period of time, animals may get dehydrated or consume less feed as a result of high EC values that decrease water palatability (McDowell, 2003). To avoid possible health hazards for cattle, it is crucial to identify water sources with high dissolved salts (FAO, 1985). Nitrogen and Phosphorus are the two nutrients that are most important for animal production. While other forms of nitrogen (mostly $NO_2$) are thought to be potentially extremely toxic and disease-causing, high amounts of nitrogen in drinking water in the $NO_3$ form induce methemoglobinemia, or "blue baby disease." Because it eutrophicates surface water bodies, phosphorus in the $PO_4$ form is of concern (Hubbard et al., 2004).

Different types of livestock have different water needs. Factors that influence water intake include the animal's size, diet, production level (e.g., milk production in dairy cows), environmental conditions, and water quality. Clean, fresh water should always be available on the farm, with particular attention during times of heat stress or high physical activity. Monitoring water intake can serve as an early indicator of health problems, as reduced water consumption often precedes illness. The water requirements of different livestock species, taking into account factors such as environmental conditions, age and physiological condition, are shown in Table 1 and 2.

Table 1. Daily total water intake of cattle and cows

| Animal Type and Daily Total Water Intake (DTWI) | | | | | (lt/animal) | References |
|---|---|---|---|---|---|---|
| Cattle DTWI | 360 kg | 408 kg | 500 kg | 544 kg | 816 kg | Parker & Brown, 2003 |
| | 15-22 | 25-37 | 23-33 | 28-66 | 33-78 | Cemek et al., 2011 |

| | 4.4°C | 10°C | 26°C | 32.2°C | |
|---|---|---|---|---|---|
| Temperatures | | | | | NRC,1974, Olkowski, 2009 |
| Growing Cattle 182-364 kg DTWI | 15.1-23 | 16.3-25.7 | 25.4-40.1 | 36-56.8 | |
| Finishing Cattle 273-454 kg DTWI | 22.7-32.9 | 24.6-35.6 | 37.9-54.9 | 54.1-78 | |
| Wintering Pregnant Cows 409-500 kg DTWI | 25.4-28.7 | 27.3-24.6 | - | - | |
| Lactating Cows 409 kg DTWI | 43.1 | 47.7 | 67.8 | 81 | |

| | | |
|---|---|---|
| Lactating Cows 600 kg | 15 kg milk yield daily 10°C | 59 |
| | 15 kg milk yield daily 32°C | 89 |
| | 30 kg milk yield daily 10°C | 92 |
| | 30 kg milk yield daily 32°C | 146 |
| | 45 kg milk yield daily 10°C | 124 |
| | 45 kg milk yield daily 32°C | 203 |

| Temperature | Beef Cows 500 kg | Beef Cows 590 kg | Beef Cows 680 kg | |
|---|---|---|---|---|
| 4°C | 31-48 | 35-55 | 39-58 | Spencer et al., 2016 |
| 18°C | 41-58 | 45-65 | 48-71 | |
| 32°C | 51-68 | 54-75 | 58-81 | |

Table 2. Water consumption of other livestock and poultry animals

| Animal Type | Situation | Water consumption (lt/animal) | References |
|---|---|---|---|
| Mature Bulls 636-727 kg | 4.4°C<br>10°C<br>26°C<br>32.2°C | 30.3-32.9<br>32.6-35.6<br>50.7-54.9<br>71.9-78 | NRC,1974; Olkowski, 2009 |
| Calf | 1 month<br>4 month | 5-8<br>11-13 | Parker & Brown, 2003; Cemek et al., 2011 |
| Calf 227 kg (6 kg DMI) | 5°C<br>16°C<br>27°C<br>32°C | 16<br>20<br>28<br>40 | Winchester & Morris, 1956; Henning et al., 2000; Higgins & Carmen, 2008 |
| Calf 340 kg (8 kg DMI) | 5°C<br>16°C<br>27°C<br>32°C | 23<br>29<br>39<br>55 | |
| Heifer | 5 months<br>18-24 months | 14-17<br>28-36 | Parker & Brown, 2003<br>Cemek et al., 2011 |
| Dry Cow | Jersey<br>Guernsey | 49-59<br>52-61 | |
| Dry Cow 500 kg | 5°C<br>16°C<br>27°C<br>32°C | 28<br>35<br>47<br>67 | Winchester & Morris, 1956<br>Henning et al., 2000; Higgins & Carmen, 2008 |
| Lactating Cow 500 kg | 5°C<br>16°C<br>27°C<br>32°C | 31<br>38<br>51<br>73 | |
| Rams<br>Sheep<br>Ewe<br>Lambs<br>Goats (Per a body kg)<br>Meat goats | | 7.6<br>7.6<br>11.3<br>0.4-5.7<br>1.43-3.5<br>0.7 | Parker & Brown, 2003<br>Cemek et al., 2011 |
| | | lt/bird/week | |
| Broilers 1-8 week<br>Broilers 1-8 week<br>White Leghorn Hens 1-20 week | | 0.10-0.48<br>0.22-2<br>0.2-1.6 | Parker & Brown, 2003<br>Cemek et al., 2011; NRC,1994; Olkowski, 2009 |
| Chicken 1-18 week with white eggs or brown eggs<br>Brown Egg Laying Hens 1-20 week | | 0.20-1.30<br>0.2-1.5 | |

Cows consuming dry feed like hay require more water to support digestion, as dry feeds increase the demand for fluid intake compared to fresh forages. Environmental factors such as temperature and humidity significantly impact livestock water consumption. In hot weather, animals lose more water, so this leads to increasing their daily water requirements. Conversely, in cold climates, water intake may decrease, but it is critical to the health of livestock that they continue to have access to clean, unfrozen water. Several rules and conditions should be considered to guarantee the purity of the water given to animals. Some of the most important indicators of drinking water quality parameters are heavy metals, nitrates, nitrites, dissolved salts, pH, total dissolved solids (TDS), and microbial contamination. It is very important for livestock to have a safe source of water. If the water quality is below standards, health problems can occur in the animals, or their feed intake can decrease. Mineral or organic contaminants in water lead to a reduction in productivity and can lead to various diseases. When assessing the quality of water to be used for animal husbandry, consideration should be given to whether animal productivity declines, whether the water has disease-spreading properties, and whether the animal product affects human health in a manner that is potentially harmful to human health (Cemek et al., 2011). Although water quality for livestock animals varies widely, it will cause harmful effects if there are high levels of some substances in it. Poultry is known to be the most sensitive group to water among farm animals. Although providing animals with poor-quality water does not cause widespread specific production problems in animals, it can cause low productivity and possible problems. Selected water quality criteria for farm animals are given in Table 3.

The novelty of this study lies in its comprehensive approach to analyzing these parameters across multiple livestock enterprises. By evaluating a range of water sources, including water troughs and storage tanks, the study provides a detailed understanding of how these parameters interact and influence the overall quality of drinking water. By providing useful insights for enhancing water safety and animal welfare on farms, the findings add to the expanding body of information on the significance of routine water quality monitoring and management. Contaminated or dirty water can lead to the spread of diseases and reduce water intake by animals. It is necessary to regularly test and clean water sources. High mineral and microbial contamination levels can cause digestive disorders and metabolic diseases.

Table 3. Recommended livestock drinking water quality guide

| | Limits | Explanations | References |
|---|---|---|---|
| Water Salinity (EC) | <1000 mg/l<br><1.5 dS/m | Excellent for all classes of livestock and poultry | FAO, 1985<br>Guyer, 1996, FAO, 2002,<br>NAS, 1972<br>NAS, 1974 |
| | 1000-3000 mg/l<br>1.5-5 dS/m | Satisfactory for all classes of livestock. May cause temporary mild diarrhea in livestock not used to it. If the water reaches the upper limits, poultry may experience watery droppings. | |
| | 3000-5000 mg/l<br>5-8 dS/m | Sufficient for farm animals, but may be rejected by animals that are not used to it. If sulfate salts predominate, the animals may experience temporary diarrhea. Poor water for poultry, often resulting in watery droppings, increased mortality, and reduced growth, particularly in turkeys. | |
| | 5000-7000 mg/l<br>8-11 dS/m | All farm animals, with the exception of lactating or pregnant animals, may use this water. Animals may refuse it until they get used to it, and it can have a laxative effect. It is not suitable for chickens. | |
| | 7000-10000 mg/l<br>11-16 dS/m | Significant risk to sheep, horses, and pregnant or lactating cows, as well as to the young of these animals. It can be used on older horses or ruminants. Unsuitable for pigs and most likely poultry. | |
| | > 10000 mg/l<br>> 16 dS/m | This water is insufficient for all livestock and poultry categories. | |
| pH | 6.5-8.5 | Only EPA info available; no cow studies have been done. Low pH (<6) causes corrosiveness and gives water a metallic taste. High pH gives the water a slippery feel, soda taste, and leaf deposits. | NRC,2001 |
| $NO_3$ | 0-44 | Generally considered safe | Freedman & Fleming, 2003 |
| | 45-132 | Sure – if other sources (Such feed etc) are lower in N2. | |
| | 133-220 | Harmful- if used for an extended length of time | |
| | 221-659 | Risky- potential death risks | |
| | 660-799 | Not safe- high risk of death | |
| | >800 | Not safe- high risk of death-Should not be used | |
| $NO_3$-N | <10 mg/l | Livestock guidelines | NRC, 2001 |
| | 0-10 | Generally considered safe | Freedman & Fleming, 2003 |
| | 10--19 | Sure – if other sources (Such feed etc) are lower in $N_2$. | |
| | 20-39 | Harmful- if used for an extended length of time | |
| | 40-99 | Risky- potential death risks | |
| | 100-199 | Not safe- high risk of death | |
| | >200 | Not safe- high risk of death-Should not be used | |
| | 10 | Livestock guidelines | USEPA, 2003 Shaw et al., 2006 |
| | 22.7 | Upper level | USEPA, 2003 |
| | 20 | Upper level | Socha et al., 2002*<br>Shaw et al.,2006 |
| | 100 | Risky- Maximum upper level | NAS, 1974; Shaw et al., 2006 |
| | 23 | Livestock guidelines | CCME, 2005 |
| $NO_2$-N | 10 mg/l | Livestock guidelines | NAS, 1972 |
| Nitrate + Nitrite ($NO_3$-N + $No_2$-N) | 100 | Livestock guidelines | NAS, 1972<br>CCME, 2005 |
| Phosphorus ($PO_4$) | 0.7 ppm | Upper level and also maximum upper level | USEPA, 2003<br>Shaw et al., 2006 |

* Socha et al., (2002) reported that these guidelines are acomposite of several sources; (NRC, 1974; NRC, 1980; Bergsrud & Linn, 1990; Puis, 1994; Hutcheson, 1996;).

For the aforementioned reasons, this article aims to discuss the importance of water for dairy cattle, the effects of water quality and quantity, and the issues to be considered in water supply. In this context, drinking water samples were taken from dairy cattle enterprises in Niğde province and analyzed, and the results were compared with the relevant standards to determine the current situation and aimed to contribute to similar research, relevant stakeholders and farm owners by emphasizing the importance of the subject.

## Materials and Methods

### *Materials*

Drinking water samples taken from dairy farms were used as material in the study. Livestock enterprises from which animal drinking water samples were taken are established in Niğde province and its districts (Table4). Animal drinking water samples were randomly selected sampling from 11 livestock farms, from points likely to be affected by pollution.

Table 4. Identifiers information of enterprises

| N | Code | Location | NDC | Race | NS |
|---|---|---|---|---|---|
| 1 | E1 (E1K$^a$-E1Y$^b$) | Niğde/Kayı | 100 | Holstein crossbred | 1* 2** |
| 2 | E2 (E2K$^a$-E2Y$^b$) | Niğde/Bor | 35 | Holstein crossbred | 1* 2** |
| 3 | E3 (E3K$^a$-E3Y$^b$) | Niğde/Edikli | 120 | Holstein crossbred, Montofon crossbred, Simmental crossbred | 1* 2** |
| 4 | E4 (E4K$^a$-E4Y$^b$) | Niğde/Edikli | 90 | Holstein crossbred, Montofon crossbred, Simmental crossbred | 1* 2** |
| 5 | E5 (E5K$^a$-E5Y$^b$) | Niğde/Edikli | 20 | Holstein crossbred, Montofon crossbred, Simmental crossbred | 1* 2** |
| 6 | E6 (E6K$^a$-E6Y$^b$) | Niğde/Kayı | 90 | Holstein crossbred | 1* 2** |
| 7 | E7 (E7K$^a$-E7Y$^b$) | Niğde/Edikli | 60 | Holstein crossbred | 1* 2** |
| 8 | E8 (E8K$^a$-E8Y$^b$) | Niğde/Badak | 50 | Holstein crossbred | 2* 2** |
| 9 | E9 (E9K$^a$-E9Y$^b$) | Niğde/Ovacık | 200 | Holstein crossbred, Simmental crossbred | 1* 2** |
| 10 | E10 (E10K$^a$-E10Y$^b$) | Niğde/Edikli | 45 | Holstein crossbred, Montofon crossbred, Simmental crossbred | 1* 2** |
| 11 | E11 (E11K$^a$-E11Y$^b$) | Niğde/Ovacık | 15 | Holstein crossbred, Montofon crossbred, Simmental crossbred | 1* 2** |

N: Number; NDC: Number of Dairy Cattle; NS: Number of Samples; a Water samples were taken from water storage tank,*From water storage tank b Water samples were taken from troughs ** From different points of troughs

The main criteria when taking animal drinking water samples was the volunteering of the enterprises owner. Since enterprises owners did not want detailed information to describe their farms, location information and other detailed information about the enterprises were not provided. Therefor only enterprises information that can identify the type of enterprises and animal water systems are included. Identifiers ınformation of enterprises are given in Table 4. In addition, all enterprises are integrated, closed systems. The barn types in the enterprises were recorded as semi-open. Enterprises use city mains water as a drinking water source, but due to water distribution in farm, all of them have water tanks. In enterprises, animal drinking water is first stored in water tanks and then delivered to troughs. It can be said that there is no routine in farms' drinking water distribution practices. When the animal's drinking water runs out in the troughs, drinking water is transferred from the tank to the troughs.

***Methods***

While the collection of animal drinking water samples, strict adherence to standard drinking water sample collection rules was ensured. During the water collection process, the selection of enterprises from which water samples were taken was determined based on transportation conditions and the voluntary participation of the operators. Some operators did not permit access to their enterprises or the collection of water samples. Consequently, the number of enterprises that could be reached and sampled was determined according to this limitation. For each enterprise, three different drinking water samples were collected: one from the tank and two from different points of the troughs. The samples were coded based on their collection locations. Samples taken from the water tank were labeled with the code "E-K," while those taken from the troughs were coded "E-Y." In the case of enterprise no. 8, two separate samples were collected from the drinking water tank due to its large size.

Pre-sterilized laboratory analysis bottles were used to collect the drinking water samples. Once collected, the samples were transported on the same day to the Çukurova University, Agricultural Structures and Irrigation Department Laboratory via a cold chain system using a refrigerated vehicle. Upon arrival, the samples were logged in the laboratory registration sheet. The water samples were then filtered using blue band filter paper and transferred to plastic bottles that had been cleaned with a chromic acid solution. These bottles were labeled according to proper technical procedures. Depending on the availability of time and labor, the water samples were either analyzed immediately or stored in a refrigerator at +4°C until the analysis was performed.

In the animal drinking water samples obtained;

Electrical Conductivity (EC) analyzes were completed with an EC meter and pH analyzes were completed with a pH meter (Electrometric Method). Nitrite ($NO_2$) analyzes (SM 4500-$NO_2$- B / Spectrometric Method) and Nitrate ($NO_3$) analyzes (SM 4500-$NO_3$- B / Spectrometric Method) in waters; It was performed on a Shimadzu brand spectrophotometer device according to Standard Methods, 2017. In the phosphate phosphorus ($PO_4$) analysis, the SM 4500-P-E Ascorbic Acid Method was applied using the same spectrophotometer. MERCK brand chemicals were used in the analyses.

## Results and Discussion

The results obtained from the analyses carried out on animal drinking water samples taken from enterprises are given in this section with tables and graphs.

***EC and pH Concentrations in Animal Drinking Water Samples***

EC concentrations of samples taken from animal drinking water are given in Figure 1. Among the EC values obtained as a result of the analyses, the highest values were recorded in the animal drinking water tanks of enterprise no. 8, with 4 $dSm^{-1}$ and 3.88 $dSm^{-1}$, respectively. The

lowest value, 0.20 $dSm^{-1}$, was measured in the animal drinking water tank of enterprise no. 4. The average of all EC analyzes performed is 0.803 $dSm^{-1}$. While the average of measurements taken only from water tanks was 0.98 $dSm^{-1}$, the average of samples taken only from troughs was 0.70 $dSm^{-1}$.

The EC analysis results of animal drinking water samples obtained from enterprises were found to be compatible with the standards. For the wellbeing and production of animals, drinking water's EC is an essential factor. The concentration of dissolved salts and minerals in the water is correlated with the water's EC. Elevated concentrations of salts, as indicated by high EC levels, can cause dehydration, digestive problems, and decreased feed intake in animals. On the other hand, low EC values usually indicate high-quality water with minimal dissolved contaminants. Animals receive water that supports optimal health and performance when EC levels in drinking water are regularly monitored, reducing potential health issues and increasing overall productivity.

The pH level appears to influence various factors such as palatability, efficacy of chlorination, corrosive characteristics, and numerous additional attributes of drinking water (Hersom & Crawford, 2008). A slight degree of alkalinity is considered more acceptable than acidity in herbivorous species. A pH value lower than 5.5 induces acidosis, which presents a potential risk for weight reduction and diminished production. Deviations from these specified pH ranges may result in decreased water and feed consumption, alterations in digestive processes, diarrhea, and suboptimal feed conversion ratios (Looper, 2012).

The results of the pH analysis are given in Figure 2. As seen from Figure 2, pH values are within the desirable range (Table 3) since the highest value is 8.20 and the lowest value is 7.20. According to the guidelines given in Table 3, these waters can be used safely for all classes of livestock and poultry in terms of pH. High water pH is the result of many interacting chemical and biological processes. Animals may experience burning or irritation in their eyes, oral cavity irritation, and thirst refusal if pH levels are higher than acceptable levels. Dairy cows that drink water with a pH higher than 9.0 may experience health issues linked to mild or chronic alkalosis. Water pH extremes have the potential to dissolve things from ditches, pipes, and other materials. Some of these may be harmful or give the water a disagreeable flavor; in particular, high-pH materials can give off a metallic taste that cattle seem to dislike. The negative effects of animals consuming extremely basic or alkaline water are not well documented.

**$NO_3$, $NO_2$ and $PO_4$ Concentrations in Animal Drinking Water Samples**

Both nitrate and nitrite have the potential to cause toxicity in animal populations, with nitrite exhibiting significantly higher toxicity compared to nitrate. Manifestations of acute poisoning include increased urine output, cyanosis, and restlessness, which may result in vomiting, seizures, and mortality. There may be some confusion regarding the limit values for nitrate and nitrite, as the concentrations are sometimes presented in relation to their respective nitrogen components (N), in particular as nitrate-N and nitrite-N. While both nitrate and nitrite can be toxic to animals, nitrite is 10 to 15 times more lethal than nitrate (Case, 1963).

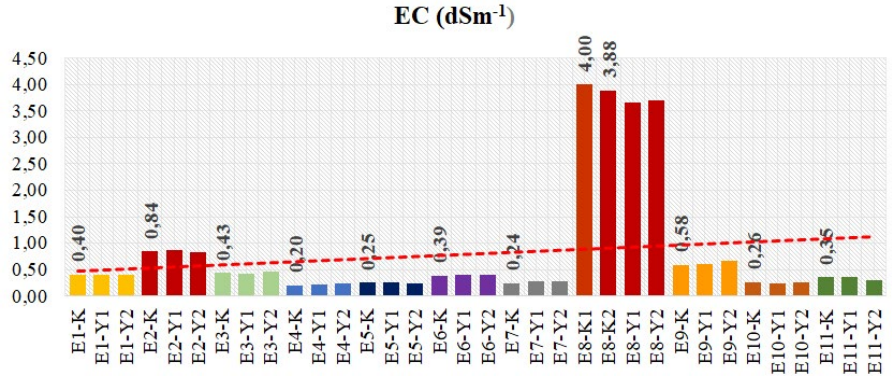


Figure 1. EC concentrations in animal drinking water samples

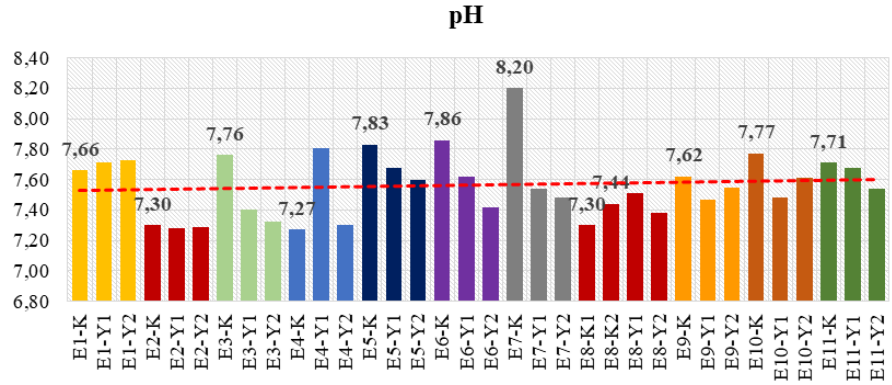


Figure 2. pH concentrations in animal drinking water samples

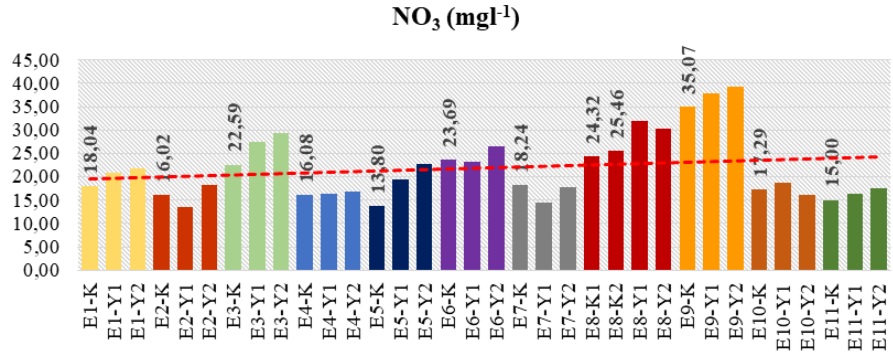


Figure 3. $NO_3$ concentrations in animal drinking water samples

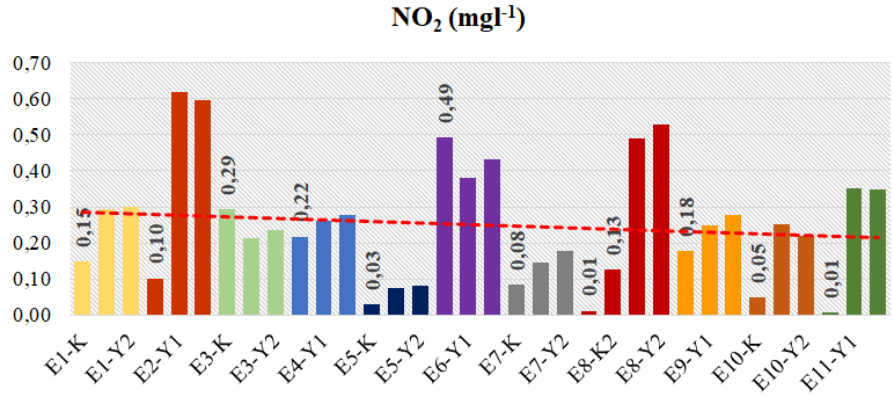


Figure 4. $NO_2$ concentrations in animal drinking water samples

The results of the $NO_3$ analysis are given in Figure 3. As seen from Figure 3, $NO_3$ values are not within the desirable range (Table 3). Because the results are mostly in the ranges which are described as harmful if used for an extended length of time. According to the obtained $NO_3$ concentrations, the lowest value was calculated as 13.606 $mgl^{-1}$, the highest value was 39.313 $mgl^{-1}$and the average was 21.834 $mgl^{-1}$. None of the samples have $NO_3$ concentrations lower than 10 $mgl^{-1}$. In all results, 52.95% of the samples contain 13-19.5 $mgl^{-1}$ $NO_3$, 32.35% of the samples contain 20-30 $mgl^{-1}$ $NO_3$, and 14.70% of the samples contain 31-39.2 $mgl^{-1}$ $NO_3$. Even if these results are within the recommended limit values, water samples in livestock enterprises should be analyzed periodically against concentration risks accumulating over time.

The results of the $NO_2$ analysis are given in Figure 4. As seen from Figure 4, $NO_2$ values are within the desirable range according to the literature given in Table 3. When the obtained $NO_2$ concentrations were examined, the highest value was found to be 0.618 $mgl^{-1}$, the lowest value was 0.008 $mgl^{-1}$, and the average was 0.251 $mgl^{-1}$.

$NO_3$ are infrequently found in potable water and exhibit lower toxicity; conversely, $NO_2$ are significantly toxic and carcinogenic in nature, with nitrogenous fertilizers and livestock farming potentially increasing their concentration (Schütz, 2012, Wright, 2007). In the context of ruminants, nitrates are assimilated via the oral route into the rumen, where they undergo conversion into $NO_2$. The $NO_2$ are then assimilated into the circulatory system, thereby impairing the oxygen transport capacity of erythrocytes (RBCs), leading to fatal outcomes primarily due to asphyxiation resulting from inadequate oxygenation (Hersom & Crawford, 2008; Schütz, 2012; Wright, 2007; Hubbard et al., 2004).

The results of the $PO_4$ analysis are given in Figure 5. As a result of the study, the highest value among the phosphorus concentrations in the waters obtained was calculated as 0.131 $mgl^{-1}$, the lowest value as 0.017 $mgl^{-1}$, and the average value as 0.056 $mgl^{-1}$. When the obtained data are compared with the literature values (Table 3), it can be said that the results are below the desired limits.

The proposed limits for humans and/or cattle correspond to the upper acceptable limits for most chemicals. However, it should be noted that the values listed as the highest acceptable values may vary. Nitrate and sulfate, two TDS elements, also have different desirable upper limits. As seen in Table 3. The U.S. Environmental Protection Agency (EPA) sets the safe limit for cattle at 22.7 ppm, but the National Academy of Sciences lists 100 ppm as the safe limit for nitrate nitrogen. The desired upper limits for livestock may vary depending on a number of factors, including the age of the cattle, the amount of water they consume per unit of body weight (lactating cows drink more water than growing animals), and whether or not they are cattle adapted to the water and the amount of nitrates that the livestock feed contributes (Socha et al., 2002).

Williams et al. (2002) reported a 23% increase in weight gain among heifers provided with access to cleaner water, in contrast to those obtaining water directly from a pond. Williams et al., (1994), indicated a 20% reduction in weight among 18-month-old steers consuming water from dugouts during the summer over a duration of 71 days (Umar et al., 2014).

Temperature exerts a significant influence on the assessment of water quality for livestock animals, which in turn impacts their hydration, nutritional intake, respiratory rates, various physiological functions, milk production, weight gain, and overall performance (Wilks et al., 1990; Brod et al., 1982).

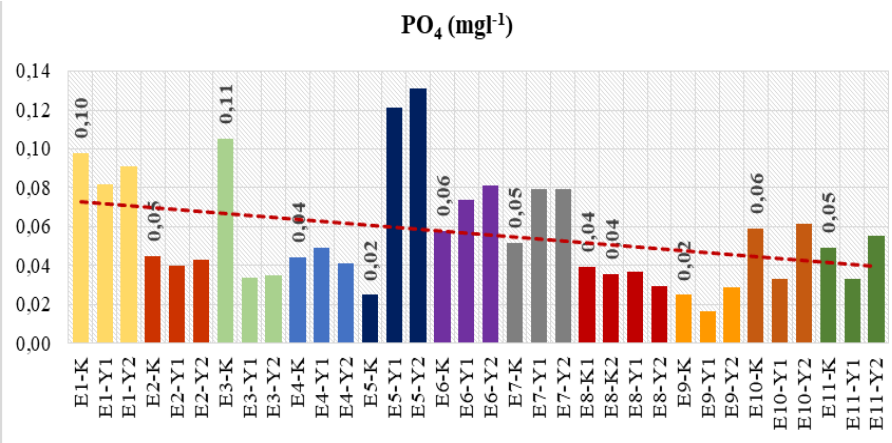


Figure 5. $PO_4$ concentrations in animal drinking water samples

Table 5. Descriptive statistics

| | EC ($dSm^{-1}$) | pH | $NO_3$ ($mgl^{-1}$) | $NO_2$ ($mgl^{-1}$) | $PO_4$ ($mgl^{-1}$) |
|---|---|---|---|---|---|
| Mean | 0.803 | 7.56 | 21.834 | 0.251 | 0.056 |
| Standard Error | 0.194 | 0.04 | 1.187 | 0.028 | 0.005 |
| Median | 0.398 | 7.55 | 19.024 | 0.241 | 0.047 |
| Mode | 0.403 | 7.30 | none | 0.294 | 0.025 |
| Standard Deviation | 1.129 | 0.21 | 6.921 | 0.164 | 0.029 |
| Sample Variance | 1.274 | 0.04 | 47.895 | 0.027 | 0.001 |
| Kurtosis | 4.139 | 0.95 | 0.376 | -0.258 | 0.396 |
| Skewness | 2.380 | 0.69 | 1.059 | 0.568 | 1.027 |
| Range | 3.800 | 0.93 | 25.707 | 0.610 | 0.115 |
| Minimum | 0.200 | 7.27 | 13.606 | 0.008 | 0.017 |
| Maximum | 4.000 | 8.20 | 39.313 | 0.618 | 0.131 |
| Count | 34.000 | 34.00 | 34.000 | 34.000 | 34.000 |

EC: Electrical conductivity, $NO_3$: Nitrate, $NO_2$: Nitrite, $PO_4$: Phosphate Phosphorus

The influence of temperature on quality occurs through alterations in palatability and acceptability by the animals or by perturbing the microflora within the gastrointestinal tract. Typically, livestock exhibit a preference for cool water, particularly under elevated thermal conditions (Arias & Mader, 2011). Besides, the quality of drinking water for animals varies depending on where the water is supplied. In the summer months, water contained in shallow ponds and small troughs experiences elevated temperatures, which can impede an animal's ability to satisfy its hydration needs, thereby leading to a reduction in feed consumption, ultimately resulting in diminished production and growth rates. Conversely, deeper aquatic systems such as tanks, ponds, and groundwater stored in larger troughs typically do not reach temperatures sufficient to influence consumption behaviors. Similarly, animals have been observed to prefer consuming warmed water during winter periods when ambient temperatures approach the freezing point (Umar et al., 2014).

Livestock productivity is impacted when upper contamination limits are exceeded, yet there is disagreement over how this affects animal performance. The precise reason for the influence on livestock output is unknown until more investigation is conducted. In certain circumstances, high concentrations may not directly affect production but instead make water less palatable. As a result, less water is used, which lowers performance without endangering animals due to toxicity issues. In other instances, high concentrations might cause toxicity issues, especially with regard to bacteria and trace elements, which can impair function and, in the case of prolonged exposure, result in death (Schlink et al., 2010).

Descriptive statistics for the analysis performed on animal drinking water samples are given in Table 5.

## Conclusion

This study investigated the quality of drinking water in livestock enterprises, analyzing key parameters such as EC, pH, $NO_3$, $NO_2$, and $PO_4$ concentrations. The results revealed that while the EC values were generally within acceptable ranges, with a mean of 0.803 $dSm^{-1}$, the highest EC levels observed in some water samples (up to 4.0 $dSm^{-1}$) highlight the need for regular monitoring to ensure water quality does not negatively impact animal health. Most of the samples, particularly from water troughs, exhibited lower EC values, which indicate relatively high-quality water with minimal dissolved salts and minerals. These findings suggest that, in general, the water quality regarding salinity is within acceptable limits for livestock consumption, supporting animal hydration and overall health. Elevated EC values observed in some water tanks (up to 4.00 $dSm^{-1}$) may, however, warrant further investigation, especially for those animals that are not accustomed to such levels of dissolved minerals. Prolonged exposure to high salinity levels could lead to dehydration, reduced feed intake, and digestive issues, particularly in sensitive livestock groups. Therefore, regular monitoring of EC levels, particularly in water storage tanks, is recommended.

The pH levels, ranging from 7.27 to 8.20, were also within the safe zone, indicating that the water in the studied enterprises is unlikely to cause issues related to palatability or digestion and indicating that the water pH was within the desirable range for livestock health. Water with a pH within this range is generally acceptable for both ruminants and non-ruminants, as it supports palatability and prevents issues such as eye irritation or oral discomfort that can occur with more alkaline or acidic water. Given the variation in pH observed across different enterprises, it is crucial to monitor pH levels periodically to ensure they remain within safe limits for all livestock, especially during periods of water system adjustments or when using new water sources. However, $NO_3$ concentrations exceeded the desirable limits in many samples, with an average concentration of 21.83 $mgL^{-1}$. Nitrate concentrations in the water samples showed significant variability, with values ranging from 13.61 to 39.31 $mgL^{-1}$ and a mean concentration of 21.83 $mgL^{-1}$. These concentrations, although generally within the recommended safety limits, are concerning because prolonged exposure to nitrate levels above 20 $mgL^{-1}$ can cause toxicity, particularly in young or pregnant animals, and lead to health issues like methemoglobinemia. Given that 32.35% of the samples exceeded the 20-30 $mgL^{-1}$ range, it is advisable to monitor nitrate concentrations closely and implement mitigation strategies (such as dilution or treatment) if nitrate levels approach harmful thresholds. Regular testing of water sources is essential to mitigate long-term risks associated with elevated nitrate concentrations.

Nitrite levels, on the other hand, were found to be within the desirable range, with concentrations ranging from 0.008 $mgL^{-1}$ to 0.618 $mgL^{-1}$ and an average of 0.251 $mgL^{-1}$ .Nitrite is considerably more toxic than nitrate and can cause severe health issues in animals, particularly when consumed over extended periods. The low nitrite concentrations observed in this study suggest that the water quality in terms of nitrite contamination is largely safe for livestock, but continued vigilance is necessary to prevent any potential accumulation over time, particularly in water sources impacted by agricultural runoff or livestock waste. $PO_4$ levels, although low, remain an important consideration for long-term water quality management. $PO_4$ concentrations were relatively low, ranging from 0.017 $mgL^{-1}$ to 0.131 $mgL^{-1}$, with a mean of 0.056 $mgL^{-1}$. These values are well below the upper acceptable limits, suggesting that $PO_4$ contamination in the drinking water is not a significant concern. However, elevated phosphate levels, if detected in future assessments, could indicate pollution from agricultural runoff, which may require treatment to prevent eutrophication in nearby water bodies.

Birds, animal feces, animal carcasses, runoff from exposed paddocks, extensive livestock operations, and sewage waste can contaminate livestock enterprises' water. Low productivity, illness, or animal mortality may arise from this. Local authorities should take necessary precautions to prevent consumption of these water bodies by livestock. A vital component of farm production and livestock health is the quality of the drinking water quality. Animals exposed to contaminated water may experience severe health issues that affect their general performance, growth rates, and reproduction ability. Farms may safeguard both animal health and financial sustainability by implementing sustainable water management techniques, monitoring water sources often, and addressing contamination threats. Farm owners should put in place routine testing procedures to keep an eye on the quality of the water and take appropriate measures when needed.

Maintaining the health and production of livestock depends on providing them with clean drinking water. Livestock farmers may increase the productivity and sustainability of their operations by providing for the unique requirements of various livestock species and by comprehending the intricate relationships between farm management techniques and water quality. Maintaining water quality and making sure cattle are healthy requires routine inspections, appropriate waste disposal, and the application of best practices.

Numerous elements, such as species, nutrition, temperature, and physiological state, affect how much water cattle consume. In order to meet the needs of each species, livestock farm managers must make sure that their animals have access to clean, sufficient water supplies. Maintaining the health, productivity, and well-being of livestock requires routine monitoring of the quality and quantity of water, especially during times of heightened demand like lactation, hot weather, or growth. Farmers may ensure animal welfare and farm efficiency by optimizing management procedures by knowing the unique water consumption requirements of their animals.

In conclusion, the majority of the water samples from the examined businesses meet acceptable water quality criteria; nevertheless, special attention should be paid to the nitrate concentrations, especially in water sources that are used for lengthy periods of time. Regularly checking for EC, pH, and other contaminants and putting remedial measures like water treatment or source diversification into place will assist guarantee the best possible water quality for the productivity and health of cattle. Animal welfare can be preserved and possible production losses brought on by low water quality can be reduced by following these procedures. Overall, the findings of this study emphasize the need for continuous monitoring of drinking water quality in livestock enterprises, particularly concerning nitrate and nitrite levels. Ensuring optimal water quality not only supports animal health but also enhances productivity. This results contributes to the growing understanding of the relationship between water quality and livestock performance, highlighting the importance of maintaining safe drinking water standards in agricultural settings to promote animal welfare and farm productivity.

***Declarations***

Ethical Approval Certificate is not required.

***Author Contribution Statement***

MEC: Investigation, conceptualization, formal analysis, writing the original draft, review and editing

MB: Data collection, investigation, conceptualization, review and editing

All authors have read and agreed to the published version of the manuscript.

***Conflict of Interest***

The authors declare no conflict of interest.

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# The Effects of Pre-Slaughter Fasting Periods on Tonic Immobility, Slaughter, Carcass and Meat Quality Traits in Medium- and Fast-Growing Broiler Chickens

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ABSTRACT

In this study, the effects of pre-slaughter fasting periods (8, 12, and 16 h) on stress levels, body weight loss, slaughter, carcass, and meat quality characteristics were investigated in broiler chickens with different growth rates (medium and fast-growing). In the experiment, 15 male and 15 female broilers each from the ANADOLU-T A1 (medium growing) dam line and ROSS-308 hybrid (fast growing) at 42 days of age were used. Body weight losses did not differ significantly among fasting periods, but medium-growing broilers exhibited higher body weight losses across all fasting groups (P<0.05). The carcass yield was higher in fast-growing broilers (P<0.05), while abdominal fat ratios were higher in medium-growing broilers (P<0.05). The amount of residual feed in the gizzard, digestive system, and gizzard ratios decreased with increasing fasting duration (P<0.05). Digestive system ratios also differed between genotypes (P<0.05). An increase in breast ratio was observed with longer fasting periods (P<0.05). On the other hand, the fast-growing genotype had higher breast ratios, lower wing ratios, lower back ratios, and lower neck ratios (P<0.05). Fasting periods did not significantly affect the *L**, *a**, and *b** color values of breast and thigh meat, but differences between genotypes were significant, with higher values observed in the fast-growing genotype (P<0.05). Breast pH values did not differ among fasting periods but were higher in the fast-growing genotype (P<0.05). Thigh pH values, however, varied significantly between fasting periods and genotypes (P<0.05). The medium-growing genotype had higher thigh pH values, and thigh pH increased with longer fasting periods (P<0.05). White striping and woody breast scores in breast muscles were higher in the fast-growing genotype, with partial increases observed as fasting duration increased. In conclusion, the results indicate that a 12-h pre-slaughter fasting period is sufficient for digestive tract clearance and carcass quality. However, meat quality defects in fast-growing genotypes should be monitored.

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# Orta ve Hızlı Büyüyen Etlik Piliçlerde Kesim Öncesi Açlık Sürelerinin Tonik İmmobilite, Kesim, Karkas ve Et Kalite Özellikleri Üzerine Etkileri

MAKALE BİLGİSİ

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Tonik immobilite
Göğüs pH
Göğüs rengi

ÖZ

Bu çalışmada, farklı gelişme düzeylerine sahip etlik piliçlerde kesim öncesi 8, 12 ve 16 saatlik açlık sürelerinin, piliçlerin stres düzeyi, canlı ağılık kaybı ile kesim, karkas ve et kalite özelliklerine etkileri ortaya koyulmuştur. Denemede, 42 günlük yaşta ANADOLU-T A1 ana hattı (orta gelişen) ve ROSS-308 hibritinin (hızlı gelişen) her birinden 15 erkek-15 dişi etlik piliç kullanılmıştır. Canlı ağırlık kayıpları açlık süreleri arasında farklı bulunmamış, tüm açlık gruplarında orta gelişen etlik piliçlerde daha yüksek canlı ağırlık kaybı oranları belirlenmiştir (P<0,05). Karkas randımanı hızlı gelişen etlik piliçlerde daha yüksek, abdominal yağ oranı ise orta gelişen piliçlerde daha yüksek bulunmuştur (P<0,05). Taşlıkta kalan yem miktarı, sindirim sistemi ve taşlık oranları açlık süresinin artması ile azalmıştır (P<0,05). Sindirim sistemi oranı genotipler arasında da farklılık göstermiştir (P<0,05). Açlık süresinin artması ile göğüs oranında artış belirlenmiştir (P<0,05). Diğer yandan hızlı gelişen genotip daha yüksek göğüs, daha düşük kanat, daha düşük sırt ve daha düşük boyun oranlarına sahip olmuştur (P<0,05). Açlık süreleri göğüs ve but etlerinde *L**, *a** ve *b** renk değerleri üzerinde farklı etkiler yapmamış, ancak genotipler arasında farklılıklar önemli bulunmuş, hızlı gelişen genotipte daha yüksek değerler belirlenmiştir (P<0,05). Göğüs pH değeri açlık süreleri arasında farklılık göstermemiş, hızlı gelişen genotipte daha yüksek değerler bulunmuştur (P<0,05). But pH değeri ise açlık süreleri ve genotipler arasında farklılık göstermiştir (P<0,05). Orta gelişen genotip daha yüksek but pH değerlerine sahip olmuş, açlık süresinin artması ile but pH değeri yükselmiştir (P<0,05). Göğüs kaslarında görülen beyaz çizgi ve odunsu göğüs skorları hızlı gelişen genotipte daha yüksek iken, açlık sürelerinin artması ile kısmi artışlar belirlenmiştir. Bu sonuçlar, kesim öncesi 12 saatlik açlık uygulamasının sindirim kanalı temizliği ve karkas kalitesi için yeterli olduğunu, ancak hızlı gelişen genotiplerde et kalite kusurlarının izlenmesi gerektiğini ortaya koymaktadır.

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## Giriş

Etlik piliç üretiminde verimlilik ve et kalitesi ile üretimin devamlılığını sağlayacak uygulamalar ön plana çıkmaktadır. Üretim döneminde verimlilik üzerine genotip, bakım-besleme koşulları, yerleşim sıklığı, hayvan refahı ve sağlığı ile kümes içi koşulları en etkili faktörlerdir. Tüm bu aşamalar en optimum şartlarda gerçekleşse bile, kesim öncesi ve kesim esnasında uygulanan işlemler hayvan refahı, kesim ve karkas özellikleri üzerine etkili olabilmektedir (Sarıca ve ark., 1995; Bilgili, 2002; Saraiva ve ark., 2020). Kesim öncesi piliçlere uygulanan işlemler, açlık ve susuzluk süresi, yakalama uygulamaları, kesimhanelere taşıma sistemi ve mesafesi, kesimhanelerde bekletme süresi gibi etkenler piliç eti kalitesinde önemli farklılıklar oluşturabilmektedir (Bilgili, 1995; Sarıca ve ark., 2024a; Wu ve ark., 2024).

Etlik piliçler kesim öncesinde, nakliye ve kesimhanelerde bekletme süresi de dikkate alınarak kesim öncesinde yaygın olarak 9-12 saatlik aç bırakma; taşıma ve bekletme alanında ise aç ve susuz bırakma uygulamasına maruz kalmaktadır (Pereira ve ark., 2013). Kümeste uygulanmaya başlayan yem çekme uygulamasının amacı kesim esnasında sindirim kanalının boşalması sağlanarak iç çıkarma anında karkasa dışkı veya barsak içeriğinin bulaşmasının önlenmesidir (Bilgili, 1988; Bilgili, 2002). Diğer yandan kesim anına kadar verilen yemin sindirimi tamamlanmadığı için gereksiz yem verilmesi önlenmekte, taşlık temizliği daha kolay yapılabilmektedir (Bilgili, 2002). Kontrollü çevre koşullarında açlık periyodu genellikle 10-12 saat olarak uygulanmaktadır. Sekiz saatten daha az açlık süreleri sindirim kanalında yem ve su kaynaklı doluluk nedeniyle iç çıkarma esnasında karkasa dışkı bulaşmasına neden olabilmektedir. 13-14 saati aşan açlık süresinde ise bağırsak mukozasındaki hasarlar nedeniyle kolayca parçalanma kaynaklı dışkı bulaşma riski ortaya çıkmaktadır (Bilgili & Hess, 1997; Duke ve ark., 1997; Komiyama ve ark., 2008; Xue ve ark., 2021). Ayrıca artan açlık periyodu karaciğerden safra bulaşmasını arttırabilmektedir (Northcutt ve ark., 1997; Bilgili, 2002).

Kesim öncesi açlık uygulamasının en önemli etkisi canlı ağırlık kaybı ile ortaya çıkmakta, bu durum kesim randımanını olumsuz etkileyebilmektedir (Ali ve ark., 1999; Beraquent, 1999; Berri, 2000; Karaçay ve ark., 2008; Gewehr ve ark., 2023). Üretimde zorunlu olarak uygulanan yem çekme işlemi ve ilave bazı işlemler (yakalama, taşıma, kesim alanında bekletme, çevre koşulları vb.) refah parametreleri üzerine olumsuz etkilerde bulunmaktadır (Mitchell & Kettlewel, 2009; Jacobs ve ark., 2017; Saraiva ve ark., 2020). Piliçlerin yakalanması ve taşınmasında kullanılan yöntemler yanında açlık süresi ve çevre sıcaklığının etkisi ile yaralanmalar, ayak ve kanat kırılmaları, göğüs renk değişiklikleri veya ölümlere kadar kayıplar ortaya çıkabilmektedir (Langkabel ve ark., 2015; Siegel & Honaker, 2014; Sarıca ve ark., 2024a). Bu uygulamalar sonucunda oluşan stres düzeyine bağlı olarak piliçlerde refah düzeyi ciddi oranda azalmaktadır (Mitchell & Kettlewell, 1998; 2009). Piliçlerin kesimhanelere taşınma süresinin uzaması, taşınmadan sonra bekletme alanında kalma süresi, aç ve susuz kalma süresini arttırarak refahı kötüleştirmektedir (Mitchell & Kettlewel, 2009; Saraiva ve ark., 2016; Grandin, 2017; Saraiva ve ark., 2020).

Kesim öncesi açlık süresinin etkileri genellikle hızlı gelişen etlik piliçlerde ele alınmış, farklı gelişme düzeyleri arasında karşılaştırmalar literatürde çok fazla yer almamıştır. Bu çalışmada, orta düzeyde gelişen (ANADOLU-T etlik piliç ana ebeveyn hattından üretilen) piliçler ile hızlı gelişen etlik piliçlerde (ROSS-308) aynı uygulamaya tepkiler bakımından karşılaştırmalar yapılmıştır. Çalışmada, kesimhanelerde geleneksel uygulama olan 12 saatlik açlık süresinin altında (8 saat) ve üstünde (16 saat) açlık sürelerinin farklı gelişme özelliğine sahip etlik piliçlerde stres düzeyi (tonik immobilite), kesim, karkas ve et kalite özelliklerine etkileri değerlendirilmiştir.

## Materyal ve Yöntem

### *Hayvan Materyali ve Deneme Planı*

Bu çalışma, Ondokuz Mayıs Üniversitesi Ziraat Fakültesi Hayvancılık Araştırma ve Uygulama Çiftliği Kanatlı Üretim ve Araştırma biriminde bulunan etlik piliç deneme kümesi ve kesim ünitesinde yürütülmüştür. Hayvan materyali olarak orta gelişme düzeyine (61.5 g/gün canlı ağırlık artışı) sahip olan 42 günlük yaşta ANADOLU-T A1 ana hattına ait 15 erkek ve 15 dişi ile hızlı gelişen (74.1 g/gün canlı ağırlık artışı) ROSS-308 hibritine ait 15 erkek-15 dişi etlik piliç kullanılmıştır. Her iki genotip içerisinde 8, 12 ve 16 saatlik aç bırakma süreleri uygulanarak 3 farklı muamele grubu oluşturulmuştur. Her bir genotip için her muamele grubunda ortalama canlı ağırlığa yakın 5 dişi-5 erkek etlik piliç kullanılmıştır.

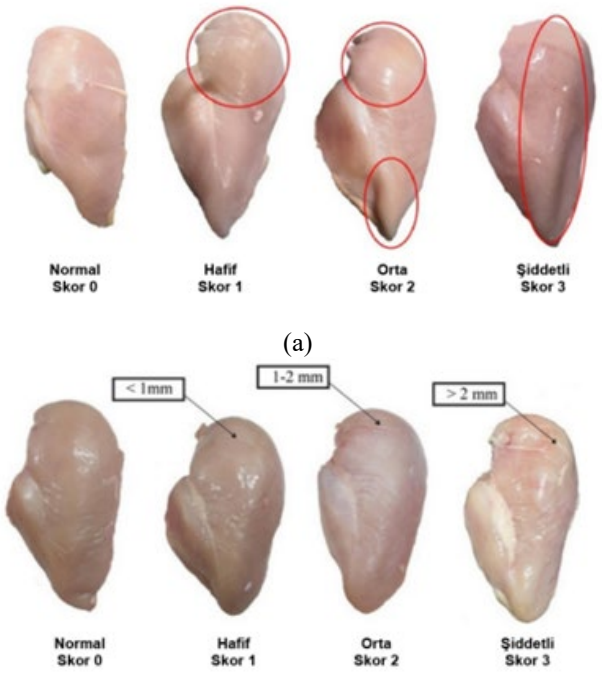
Çalışmada 6 adet deneme bölmesi kullanılmış ve her bölmede de 5 dişi-5 erkek piliç yer almıştır. Her bölme 4 $m^2$ (1,80 × 2,20cm) taban alanına sahip tel örgülü bölmelerden oluşmuştur. Bölmelerde altlık olarak 10 cm kalınlığında kaba rende talaşı kullanılmıştır. Her bölmede 5 kg kapasiteli bir yemlik ve 2 nipel suluk yer almıştır. Çalışmadaki etlik piliçler 42 günlük yaşa kadar standart yetiştirme koşullarına uygun olarak barındırılmış ve 42 günlük yaşta kümes içi sıcaklık 21-22ºC ve nispi nem %50-%70 arasında tutulmuş ve 24 saatlik aydınlık süre uygulanmıştır. Tüm piliçlere 42. güne kadar yeme ve suya serbest ulaşım imkânı sağlanmıştır. Yemler ticari bir yem fabrikasından temin edilmiş ve açlık periyodunu da kapsayan 35-42 günler arasında %18 HP ile 3100 kcal/kg ME içeriğine sahip yemle besleme yapılmıştır. Etlik piliçlere kesim öncesi açlık uygulaması 42. günde yapılmış ve kesim işlemleri 43 günlük yaşta gerçekleştirilmiştir.

### *Çalışmada Ele Alınan Özellikler*

Kesim öncesi 42 günlük yaşta tüm piliçlere bireysel takip amacıyla kanat numarası takılmıştır. Piliçlerin aynı anda kesimi için aç bırakma sürelerinin ayarlaması kesimden 8, 12 ve 16 saat önce gerçekleştirilmiştir ve bu gruplar çalışmanın muamelelerini oluşturmuştur. Aç bırakma uygulaması başlamadan ve kesimden önce piliçler bireysel olarak 1 g hassasiyetli terazi kullanılarak tartılmıştır. Böylelikle hem kesim öncesi canlı ağırlığı (g) hem de aç bırakma süresine bağlı canlı ağırlık kaybı (g ve % olarak) hesaplanmıştır.

Aç bırakma süresinin piliçlerin stres ve korku düzeylerine etkilerini belirleyebilmek için kesimden hemen önce tonik immobilite testi (TI) uygulanmıştır. Uygulama her bir açlık grubu için her genotip grubundan 3 dişi-3 erkek piliçte gerçekleştirilmiştir. Seçilen piliçler TI testi için ayrı bir odaya alınmış, bireysel olarak teste tabi tutulmuştur. Yumuşak ve U seklindeki beşiğe sırtüstü ya da sağ tarafına doğru yatırılarak baş kısmı aşağıda kalacak şekilde göğüs kısmına hafif bastırılarak bu şekilde 15 saniye tutulmuş ve piliç serbest bırakıldıktan sonra 10 saniye içinde hareketsiz kalan piliçlerde TI'nın sağlandığı varsayılmıştır. Piliç kalkana kadar geçen süre 1 m uzaklıkta bulunan gözlemci tarafından kronometre ile ölçülüp kaydedilmiş ve bu süre TI süresi olarak kaydedilmiştir. Tekrarlanan 5 müdahalenin ardından TI gerçekleşmemişse o piliç uyumamış olarak kaydedilmiştir. Test periyodu maksimum 5 dakika ile sınırlandırılmış ve bu süre sonunda uyanmayan piliçlerde TI süresi 300 saniye olarak kabul edilmiştir. Ayrıca her piliç için kaçıncı yatışta TI sağlandığı da saptanmış ve uyarılma sayısı olarak kaydedilmiştir (Marx ve ark., 2008; Prieto & Campo, 2010).

TI testi tamamlandıktan sonra tüm piliçler kesim ünitesine nakledilmiştir. Kesim öncesi bireysel canlı ağırlığı alınan piliçlerin kesimi gerçekleştirilmiştir. Kesim, karkas ve et kalite özellikleri tüm piliçlerde (30 dişi-30 erkek) bireysel olarak belirlenmiştir. Kesimi yapılan piliçler 55-60°C'lik suda 15-20 sn bekletilmiş ve tüy yolma makinesi ile tüyleri yolunmuştur. Ardından sıcak karkas ağırlığı (g), yenilebilir iç organ ağırlıkları (kalp, karaciğer, taşlık), abdominal yağ miktarı (g), sindirim sistemi ağırlığı (g) ve eğer içerisinde sindirilmeden kalan içerik varsa ağırlığı (g) belirlenmiştir. Elde edilen değerlerden, karkas randımanı (%), yenilebilir iç organların oranı (%), abdominal yağ oranı (%) ve sindirim sistemi oranı (%) kesimdeki canlı ağırlığın yüzdesi olarak hesaplanmıştır. Kesimden hemen sonra karaciğerde 3 farklı noktadan renk ölçümü ($L^*$: parlaklık, $a^*$: kırmızılık, $b^*$: sarılık) yapılmıştır (Konica Minolta CR-400). Karkasların soğuk hava deposunda (+4°C) 24 saat bekletilmesinden sonra soğuk karkas, göğüs, but, sırt, kanat ve boyun ağırlıkları (g) alınmıştır. Bu değerlerden soğuk karkas randımanı (%) kesimdeki canlı ağırlığın yüzdesi ve karkas parça oranları (%) da soğuk karkas ağırlığının yüzdesi olarak hesaplanmıştır. Kesimden 24 saat sonra but ve göğüs etinin 3 farklı bölgesinde derisiz et rengi ($L^*$: parlaklık, $a^*$: kırmızılık, $b^*$: sarılık) Konica Minolta CR-400 renk cihazı ile belirlenmiştir. Göğüs ve but etinin pH'sı (Testo205 pH meter) aynı bölgelerden yapılan ölçümlerle belirlenmiştir (Fanatico ve ark., 2007; Sarıca ve ark., 2014). Göğüs etinde görsel kalitenin belirlenmesi için *Pectoralis major* kasında odunsu göğüs ve beyaz çizgi oluşumu skorlanmıştır (Şekil 1a ve Şekil 1b). Odunsu göğüs oluşumu el muayenesi yardımıyla göğüsteki sertleşmelerin derecesine göre belirlenmiştir. *Pectoralis major* kasının yumuşak ve normal yapıda olmasına 0 skoru, kasın baş (kraniyal) bölgesinin sert diğer bölgelerin yumuşak ve normal olmasına 1 skoru, kasın baş ve alt bölgesinin sert olmasına 2 skoru ve kasın boydan boya sert olduğu durumlarda ise 3 skoru verilmiştir (Tijare ve ark., 2016; Khalil ve ark., 2021; Şekil 1a). Beyaz çizgi oluşumları ise normal ve çizgi olmayan göğüs için 0 skoru, genellikle 1 mm' den daha az kalınlıkta hafif çizgilenme için 1 skoru, çok belirgin olan 1-2 mm kalınlıkta büyük beyaz çizgiler için 2 skoru ve göğüs kasının neredeyse tamamını kaplayan 2 mm' den daha kalın çizgi oluşumları için 3 skoru verilmiştir (Kuttapan ve ark., 2016; Khalil ve ark., 2021, Şekil 1b).




Şekil 1. Odunsu göğüs (a) ve beyaz çizgili göğüs için görsel puanlama ölçeği (b)
Figure 1. Visual scoring scale for woody breast (a) and white-striped breast (b)

Et kalite özelliklerinden sızdırma kaybını belirlemek için göğüs ve but etinden alınan örnekler (~5 g) kilitli poşetler içerisine konularak 2-4°C'de buzdolabında birbirleriyle temas etmeyecek şekilde 48 saat depolanmıştır. Bu sürenin sonunda et örnekleri kâğıt havlu ile kurutularak son ağırlıkları tespit edilmiştir. Sızdırma kaybı ilk örnek ağırlığının yüzdesi olarak hesaplanmıştır. Pişirme kaybını belirlemek amacıyla göğüs ve but etinden alınan örnekler (~20 g) 200 °C'de ön ısıtılmış elektrikli fırında açık alüminyum tavada 15 dakika pişirilmiştir. Ardından bu örnekler 15°C'de 30 dakika soğutulup kâğıt havlu ile kurutularak son ağırlıkları tespit edilmiştir. Pişirme kaybı, pişirmeden önceki ağırlığın yüzdesi olarak hesaplanmıştır (Bianchi ve ark., 2007).

***İstatistik Analizler***

Bu çalışma, 2 farklı genotip (orta gelişen A1 saf ana ebeveyn hattı ve hızlı gelişen ROSS-308) ve 3 farklı açlık süresi (8, 12 ve 16 saat) ile 2 x 3 faktöriyel deneme deseninde gerçekleştirilmiştir. Tüm veri analizlerinde SPSS 21.0 paket programından yararlanılmıştır. Her bir özellik için verilerin uygunluğu Shapiro-Wilk testi kullanılarak teyit edilmiştir. Analizler faktöriyel ANOVA kullanılarak doğrusal modele göre yapılmıştır. Modele genotip ve açlık süresi sabit faktörler olarak dahil edilmiş ve ayrıca ikili interaksiyon da modele eklenmiştir. Anlamlılık 0,05 düzeyinde test edilmiş ve çoklu karşılaştırmalarda Tukey HSD testi kullanılmıştır. Et kalite özelliklerinden göğüs etinde odunsu ve beyaz çizgi oluşumu için açlık süresi ve genotip ana etkileri modele ayrı ayrı dahil edilmiş ve Kruskal-Wallis-H testi ile analiz edilmiştir.

**Bulgular ve Tartışma**

Çalışmada farklı açlık süreleri uygulanan piliçlerde yapılan TI testlerinde açlık grupları ve genotipler bakımından farklılıklar önemli bulunmamıştır (Çizelge 1). Buna karşın orta-ağır gelişen genotip (A1), hızlı gelişen genotipe (ROSS-308) göre sayısal olarak daha düşük TI süresine sahip olmuş; yine uygulamanın tekrar sayısı orta ağır genotipte daha düşük bulunmuştur. Açlık süreleri sonunda ölçülen TI süresi, sayısal olarak 8 ve 12 saatlik açlık sürelerinde benzer iken, 16 saatlik açlık süresinde daha düşük bulunmuştur.

Kesim öncesi stres faktörleri, yetiştirme sistemleri, mevsim, bakım-besleme koşulları işletmeden işletmeye değişebilmektedir. Kesim öncesi açlık periyodunun 6, 9, 12 ve 15 saat uygulanması ile solunum sıklığı, sıkıntılı görünüm ve tüy çekme gibi davranışlarda özellikle 12-15 saatlik açlık sürelerinde önemli artışlar olduğu belirtilmiştir (Pereira ve ark., 2013). Açlık süresinin artması ile kan glikojen düzeyindeki azalmaya bağlı olarak piliçlerde stres düzeyi yükselmekte (Warriss ve ark., 1988), bu durum kesim ve karkas özelliklerini etkilemektedir (Wu ve ark., 2024). Ancak çalışmamızda TI süresi ve uyarılma sayıları ne genotiplerin gelişme düzeyinden ne de açlık sürelerinden etkilenmemiştir. Pereira ve ark. (2013), etlik piliçlerde kesim öncesi 15 saatlik aç bırakmanın stres düzeyini artırdığını bildirmiştir, ancak çalışmamızda gelişme düzeyinden bağımsız olarak 16 saate kadar aç bırakmanın strese neden olmadığı belirlenmiştir.

Başlangıç canlı ağırlıkları orta ve hızlı gelişen genotiplerde farklı bulunmuş (2581,8 g ve 3110,1 g; P<0,05; Çizelge 2); açlık periyodu sonunda canlı ağırlık kaybı oranları ise farklılık göstermemiştir (%2,41 ve %2,23). 8, 12 ve 16 saatlik açlık periyodlarında canlı ağırlık kayıpları % 2,19, 2,39 ve 2,45 olarak gerçekleşmiş ve farklılıklar önemli bulunmamıştır. Buna karşılık açlık süresinin artmasıyla canlı ağırlık kayıpları da yükselmiştir. Sarıca ve ark. (1995), 8, 10 ve 12 saatlik açlık uygulamaları arasında canlı ağırlık kayıpları bakımından farklılık belirlememişler, ancak açlık süresinin azalmasıyla daha düşük canlı ağırlık kayıpları olduğunu bildirmişlerdir. Canlı ağırlık kayıpları, piliçlerin başlangıç ağırlıkları, kesim esnasına kadarki su tüketimi ve taşıma koşullarına göre değişebilmektedir (Bilgili, 2002; Warriss ve ark., 2004). Uzun süreli açlık uygulamalarında (24 saat) canlı ağırlık kayıpları %7,16'ya kadar çıkabilmekte (Veerkamp, 1986), bu kayıpların azaltılması amacıyla 10 saat kesim öncesi açlık periyodu uygulanan piliçlerin içme suyuna 1-2 g/L sakkaroz verilmesi ile canlı ağırlık kayıpları %3,76, %3,49 ve %3,0 olarak kısmen düşürülebilmiştir (Karaçay ve ark., 2008). Diğer taraftan 4, 8, 12, 16 ve 20 saatlik kesim öncesi açlık periyodları uygulanan piliçlerde belirlenen canlı ağırlık kayıpları (sırasıyla %1,59, %2,70, %3,41, %3,92, %4,34) çalışmamızda bulunan değerlerden yüksektir (Wu ve ark., 2024). Schneider & Gewehr (2023), 4, 8, 12 ve 16 saatlik kesim öncesi açlık periyodlarının 25 günlük yaştaki etlik piliçlerde canlı ağırlık kayıplarını %2,0-4,92 arasında (P<0,05); 42 günlük piliçlerde ise %1,60-4,68 arasında (P<0,05) belirlemişlerdir. Canlı ağırlık kayıplarında en önemli etkenler bağırsak içeriğinin azalması, kaslardaki su düzeyindeki düşüş ve enerji metabolizmasındaki bozulmadır (Sterten ve ark., 2010; Faucitano, 2018; Xue ve ark., 2021). Çalışmada orta gelişme düzeyine sahip genotip (A1) hızlı gelişen genotipe (ROSS-308) göre her üç açlık süresinde de daha yüksek canlı ağırlık kaybı ortaya koymuştur (P<0,05; A1: %2,41, ROSS-308: %2,23). Bu durum orta gelişen ve aynı yaşta kesilen piliçlerin açlık periyodundan daha fazla etkilendiğini ifade etmektedir.

Karkas randımanı genotipler arasında önemli bulunurken (P<0,05), açlık periyodları bakımından farklılıklar önemli bulunmamıştır. Abdominal yağ düzeyi orta gelişen piliçlerde daha yüksek (A1: %2,19 ve ROSS-308: %1,60; P<0,05) bulunmuştur (Çizelge 2).

Her iki genotipte de açlık sürelerinin artması karkas randımanının yükselmesine neden olmakla birlikte hızlı gelişen etlik piliçler daha yüksek değerlere sahip olmuştur. Hızlı gelişen piliçlerde daha fazla karkas ağırlık kaybı belirlenmiş, ancak farklılıklar önemli bulunmamıştır.

Çizelge 1. Tonik immobilite (TI) süresi (sn) ve uyarılma sayısı (n)
*Table 1. Tonic immobility (TI) duration (sec) and induction attempts (n)*

| Açlık süresi (saat) | Genotip | TI süresi (sn) | TI uyarılma sayısı (n) |
|---|---|---|---|
| 8 | A1 | 181,50 | 1,25 |
| | ROSS-308 | 174,75 | 1,50 |
| 12 | A1 | 122,50 | 1,00 |
| | ROSS-308 | 236,25 | 1,25 |
| 16 | A1 | 64,50 | 1,25 |
| | ROSS-308 | 185,00 | 2,25 |
| OSH | | 22,718 | 0,180 |
| Ana etkiler | | | |
| Açlık süresi | | 0,545 | 0,381 |
| 8 | | 178,13 | 1,38 |
| 12 | | 179,38 | 1,13 |
| 16 | | 124,75 | 1,75 |
| Genotip | | 0,112 | 0,182 |
| A1 | | 122,83 | 1,17 |
| ROSS-308 | | 198,67 | 1,67 |
| İnteraksiyon | | 0,453 | 0,625 |

OSH: Ortalama standart hata.

Çizelge 2. Kesim ve karkas özellikleri
*Table 2. Slaughter and carcass traits*

| Açlık süresi | Genotip | İlk canlı ağırlık (g)[1] | Kesim ağırlığı (g) | Canlı ağırlık kaybı (%)[2] | Karkas verimi (%) | Karkas ağırlık kaybı (%) | Abdominal yağ (%) |
|---|---|---|---|---|---|---|---|
| 8 | A1 | 2593,2 | 2529,0 | 2,51 | 75,7 | 1,29 | 2,21 |
| | ROSS-308 | 3114,9 | 3047,0 | 2,17 | 79,3 | 1,38 | 1,69 |
| 12 | A1 | 2591,5 | 2528,8 | 2,39 | 76,5 | 1,17 | 2,12 |
| | ROSS-308 | 3166,0 | 3104,8 | 1,95 | 79,8 | 3,40 | 1,57 |
| 16 | A1 | 2560,7 | 2494,9 | 2,63 | 76,3 | 1,07 | 2,25 |
| | ROSS-308 | 3049,4 | 2984,1 | 2,16 | 79,8 | 2,44 | 1,52 |
| OSH | | 41,686 | 41,296 | 0,109 | 0,156 | 0,382 | 0,063 |
| Ana etkiler | | | | | | | |
| Açlık süresi | | 0,764 | 0,743 | 0,681 | 0,224 | 0,600 | 0,786 |
| 8 | | 2854,1 | 2788,0 | 2,34 | 77,5 | 1,34 | 1,95 |
| 12 | | 2878,8 | 2816,8 | 2,17 | 78,1 | 2,29 | 1,84 |
| 16 | | 2805,1 | 2739,5 | 2,39 | 78,0 | 1,76 | 1,89 |
| Genotip | | <0,001 | <0,001 | 0,049 | <0,001 | 0,113 | <0,001 |
| A1 | | 2581,8 | 2517,6 | 2,51 | 76,2 | 1,18 | 2,19 |
| ROSS-308 | | 3110,1 | 3045,3 | 2,09 | 79,6 | 2,41 | 1,60 |
| İnteraksiyon | | 0,914 | 0,909 | 0,970 | 0,961 | 0,522 | 0,748 |

A1: Orta gelişen genotip, ROSS-308: Hızlı gelişen genotip, OSH: Ortalama standart hata. [1]: Açlık uygulaması öncesi canlı ağırlığı ifade etmektedir. [2]: Açlık uygulaması öncesi ve sonrası arasındaki yüzde canlı ağırlık kaybını ifade etmektedir.

Çizelge 3. Yenilebilir iç organlar ve sindirim sistemi özellikleri[1]
*Table 3. Edible inner organs and digestive system traits*

| Açlık süresi | Genotip | Taşlıkta kalan yem (%)[1] | Sindirim sistemi oranı (%)[1] | Taşlık oranı (%)[1] | Karaciğer | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Oran (%)[1] | *L** | *a** | *b** |
| 8 | A1 | 26,42 | 4,66 | 1,01 | 1,83 | 33,55 | 18,70 | 11,03 |
| | ROSS-308 | 27,24 | 3,79 | 0,95 | 1,64 | 35,56 | 18,43 | 11,58 |
| 12 | A1 | 21,70 | 4,18 | 0,90 | 1,68 | 32,14 | 18,07 | 9,70 |
| | ROSS-308 | 27,54 | 3,43 | 0,84 | 1,62 | 34,68 | 18,19 | 10,23 |
| 16 | A1 | 21,94 | 4,02 | 0,80 | 1,74 | 34,63 | 16,95 | 10,83 |
| | ROSS-308 | 20,93 | 3,55 | 0,76 | 1,72 | 32,20 | 18,18 | 9,97 |
| OSH | | 0,726 | 0,050 | 0,016 | 0,021 | 0,356 | 0,227 | 0,322 |
| Ana etkiler | | | | | | | | |
| Açlık süresi | | 0,014 | 0,005 | 0,018 | 0,178 | 0,322 | 0,206 | 0,232 |
| 8 | | 26,83$^{b}$ | 4,22$^{a}$ | 0,98$^{a}$ | 1,73 | 34,55 | 18,57 | 11,30 |
| 12 | | 24,62$^{ab}$ | 3,81$^{b}$ | 0,87$^{b}$ | 1,65 | 33,48 | 18,13 | 9,98 |
| 16 | | 21,46$^{a}$ | 3,78$^{b}$ | 0,78$^{b}$ | 1,73 | 33,35 | 17,56 | 10,40 |
| Genotip | | 0,200 | <0,001 | 0,828 | 0,039 | 0,325 | 0,429 | 0,916 |
| A1 | | 23,35 | 4,43 | 0,95 | 1,75 | 33,44 | 17,90 | 10,55 |
| ROSS-308 | | 25,39 | 3,59 | 0,94 | 1,66 | 34,15 | 18,27 | 10,59 |
| İnteraksiyon | | 0,147 | 0,817 | 0,079 | 0,254 | 0,012 | 0,381 | 0,593 |

[1]: Kesim ağırlığının yüzdesi olarak ifade edilmiştir. A1: Orta gelişen genotip, ROSS-308: Hızlı gelişen genotip, OSH: Ortalama standart hata. Aynı sütunda farklı harflerle gösterilen ortalamalar arasındaki farklılıklar istatistiki olarak önemlidir (P<0,05).

Kesim öncesi açlık süreleri arasında, karkas randımanı, karkas ağırlık kaybı ve abdominal yağ düzeyleri bakımından farklılık bulunmamıştır. Sarıca ve ark. (1995), tüm açlık uygulamalarında sürekli yem verilen gruba göre karkas randımanının yüksek olduğunu, ancak açlık uygulamaları arasında farklılık bulunmadığını belirtmişlerdir. Benzer sonuçlar Karaçay ve ark. (2008) tarafından da bildirilmiş, serbest yemleme grubu açlık gruplarından daha düşük kesim randımanı değerleri göstermiştir. Wu ve ark. (2024), 4-20 saatlik açlık sürelerinde, sürenin artması ile karkas randımanının arttığını, abdominal yağ düzeylerinin ise farklılık göstermediğini belirtmişlerdir. Abdominal yağ düzeyi etlik piliç ebeveynlerinde yapılan seleksiyon çalışmaları ile önemli düzeyde azaltılmasına karşın (Arthur & Albers, 2003; Sarıca ve ark., 2024b), uygulanan yetiştirme sistemleri ve büyütme dönemi yemleme uygulamalarından daha fazla etkilenmektedir (Sarıca ve ark., 2024a). Oral Toplu ve ark. (2021), büyütme döneminde yem kısıtlaması uygulamalarında hızlı gelişen etlik piliçlerde serbest beslenenlerde %76,6 olan karkas randımanının, kısıtlı yemlenenlerde %73,5'e kadar düştüğünü (P<0,05), bununla birlikte abdominal yağ düzeylerinde de %2,04'ten %1,66'ya düşme şeklinde sonuçlar bildirmişlerdir.

Açlık süresi uygulamalarının en önemli göstergeleri olan sindirim sistemi içeriği ve karaciğerde ortaya çıkan renk değişiklikleri Çizelge 3'te verilmiştir. Taşlıkta kalan yem bakımından açlık sürelerinin etkileri önemli bulunmuş, en düşük değer 16 saat açlık uygulamasında (%21,46) belirlenmiş, 12 saat ve 8 saat açlık uygulamaları arasında benzer sonuçlar elde edilmiştir (%24,62 ve %26,83). Genotipler arasında taşlıkta kalan yem bakımından farklılıklar önemli bulunmamıştır. Aynı şekilde sindirim sistemi oranı hem genotipler hem de açlık süreleri arasında farklı

bulunmuştur. Hızlı gelişen genotip daha düşük sindirim sistemi oranına sahip iken (P<0,05); 12 ve 16 saatlik açlık periyodlarında benzer sonuçlar elde edilmiştir. Taşlık oranları açlık periyodları arasında farklılık göstermiş, ancak genotipler arasındaki farklılıklar önemli bulunmamıştır. Karaciğer oranı ise genotipler arasında farklılık gösterirken (P<0,05), açlık uygulamaları arasındaki farklılıklar önemli bulunmamıştır. Karaciğer renk değişimleri (*L**, *a**, *b**) genotip ve açlık periyodlarındaki farklılıklar önemli bulunmazken, *L** değerinde interaksiyon etkisi önemli bulunmuştur. 16 saatlik açlık uygulamasındaki A1 genotipinde daha yüksek *L** değeri görülmesi interaksiyon etkisini ortaya çıkarmıştır (Çizelge 3).

Etlik piliçlerde kesim öncesi açlık periyodu uygulamaları (6-7 saatten az) sindirim kanalında, özellikle kursak ve taşlıkta yem ve su ile doluluğa neden olmakta, bu durum ise kesim esnasında bulaşmaların temelini oluşturmaktadır (Northcutt, 2001; Bilgili, 2002). Çalışmamızda 8 ve 12 saatlik açlık uygulamaları arasında taşlıkta kalan yem miktarı bakımından önemli farklılıklar bulunmamakla birlikte, açlık süresinin artmasıyla kalan yem miktarında azalma görülmüştür (P<0,05). Buna bağlı olarak toplam sindirim sistemi ve taşlık oranlarında önemli düzeyde azalma olmuştur. Benzer bulgular Warriss ve ark. (2004) sindirim kanalı ağırlığındaki değişimlerin 8-12 saatlik açlık periyodlarında önemli azalmalar göstermesine karşılık, 24 saatlik açlık süresinde etkinin daha yüksek olduğu; susuz kalma süresinin artmasına bağlı olarak hem ağırlıkta hem de bağırsak hasarlarında artış olduğunu belirtmişlerdir. Petrolli ve ark. (2016) zamana bağlı sindirim kanalı ağırlığında azalma olmasına karşın, açlık süresince içme suyuna glikoz, sodyum bikarbonat ve vitamin-E verilmesinin bu özelliklerde bir değişime neden olmadığını belirtmişlerdir. Xue ve ark. (2021), kesim öncesi açlık süresinin 0-10 saatler arasında olduğu çalışmalarında proventrikulus içeriği, taşlık içeriği ve tüm sindirim kanalı içeriğinin açlık sürelerine bağlı olarak önemli düzeyde azaldığını belirtmişlerdir. Kesim öncesi açlık süresinin uzaması karaciğer renginin parlaklığında (*L**) olumsuz etkiler ortaya çıkarmaktadır (Trampel ve ark., 2005; Karaçay ve ark., 2008).

Açlık süresinin artması ile daha yüksek göğüs oranları belirlenmiş (P<0,05); but, kanat, sırt ve boyun oranlarındaki farklılıklar önemli olmamıştır. 12 ve 16 saatlik açlık periyodlarında kısmen daha yüksek göğüs oranları bulunmuştur (P<0,05). Hızlı gelişen genotip (ROSS-308) daha yüksek göğüs (P<0,05), kısmen daha yüksek but, daha düşük kanat (P<0,05), daha düşük sırt (P<0,05) ve daha düşük boyun (P<0,05) oranlarına sahip olmuştur (Çizelge 4). Özellikle hızlı, orta ve yavaş gelişen etlik piliçlerde önemli karkas parçalarından göğüs ve but oranlarında farklılıklar görülmesi (Sarıca ve ark., 2019) kesim esnasındaki uygulamalardan daha önemlidir. Buna karşın, Wu ve ark. (2024) açlık süresinin artması ile göğüs ve but oranlarında artışlar olduğunu belirtmişlerdir.

Kesimden 24 saat sonra belirlenen göğüs ve but etlerine ait pH ile *L**, *a** ve *b** renk değerleri Çizelge 5'te verilmiştir. Göğüs pH değeri açlık sürelerinden etkilenmemiş, hızlı gelişen genotipte daha yüksek göğüs pH değeri belirlenmiştir (P<0,05). But etinde ise açlık süresinin artması ile pH yükselmiş (P<0,05), yavaş gelişen genotip daha yüksek but pH değerlerine sahip olmuştur (P<0,05). Göğüs *L**, *a** ve *b** değerleri genotipler arasında farklılık göstermiş (P<0,05), hızlı gelişen genotip daha yüksek değerlere sahip olmuştur. But eti *L**, a ve *b** değerlerinde de genotipler arasında farklılıklar önemli bulunmuş (P<0,05), *L** değeri hızlı gelişen genotipte, *a** ve *b** değerleri orta gelişen genotipte daha yüksek bulunmuştur. But ve göğüs eti rengi açlık sürelerinden etkilenmemiştir. Yapılan çalışmaların önemli bir kısmında kesim öncesi açlık sürelerinin but ve göğüs etindeki renk değişimi üzerine etkili olmadığı (Barbut, 1998; Boschini, 2011; Pereira ve ark., 2013; Petrolli ve ark., 2016; Gewehr ve ark., 2023) belirtilirken; Karaçay ve ark. (2008) ile Wu ve ark. (2024), göğüs eti *L** değerindeki değişimi önemli bulmuşlardır. Komiyema ve ark. (2008), 4, 8, 12 ve 16 saat kesim öncesi açlık uygulamalarında göğüs eti *L** ve *a** değerleri bakımından farklılıkların önemli olduğunu; *L** değerinde açlık uygulama süresinin artması ile düşüş olurken, *a** değerinde yükselme olduğunu, *b** değerinde ise farklılık görülmediğini bildirmişlerdir.

Çizelge 4. Karkas parça oranları (%)[1]
*Table 4. Carcass part ratios (%)[1]*

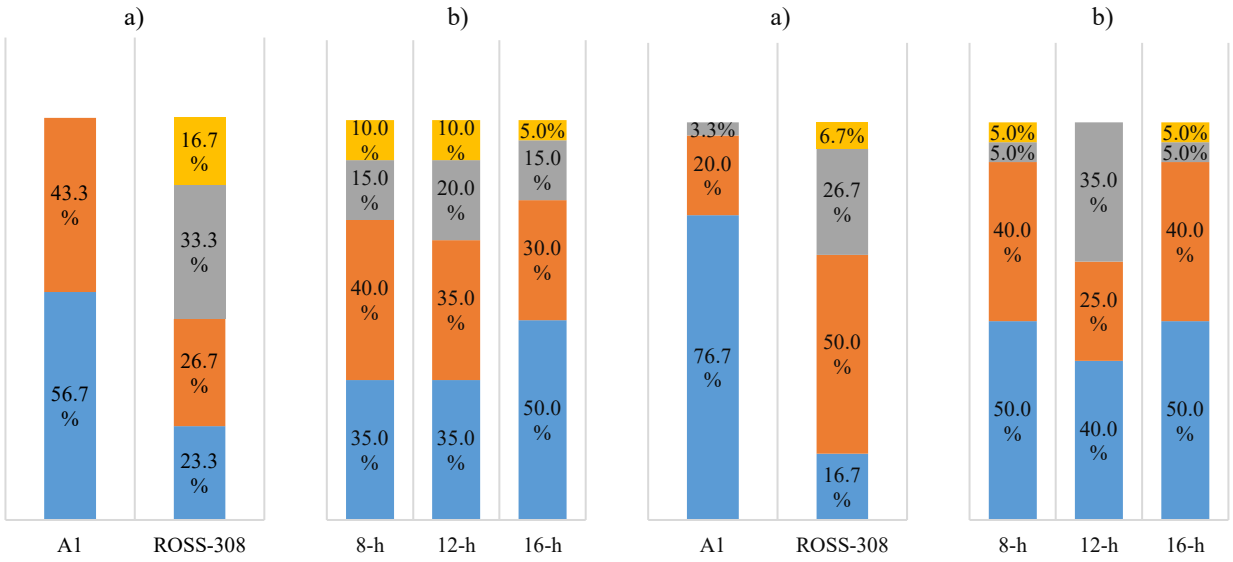
| Açlık süresi | Genotip | Göğüs | But | Kanat | Sırt | Boyun |
|---|---|---|---|---|---|---|
| 8 | A1 | 35,50 | 29,66 | 10,48 | 18,47 | 6,28 |
| | ROSS-308 | 37,77 | 30,43 | 9,53 | 16,60 | 5,78 |
| 12 | A1 | 35,81 | 29,55 | 10,14 | 17,73 | 6,79 |
| | ROSS-308 | 40,90 | 29,68 | 9,27 | 16,90 | 5,93 |
| 16 | A1 | 36,01 | 29,27 | 9,89 | 18,91 | 6,31 |
| | ROSS-308 | 40,07 | 29,62 | 8,93 | 16,71 | 5,93 |
| OSH | | 0,246 | 0,251 | 0,131 | 0,235 | 0,069 |
| Ana etkiler | | | | | | |
| Açlık süresi | | 0,019 | 0,607 | 0,191 | 0,691 | 0,137 |
| 8 | | 36,64[b] | 30,05 | 10,01 | 17,54 | 6,03 |
| 12 | | 38,36[a] | 29,62 | 9,71 | 17,32 | 6,36 |
| 16 | | 39,04[a] | 29,45 | 9,41 | 17,81 | 6,12 |
| Genotip | | <0,001 | 0,411 | 0,001 | 0,001 | <0,001 |
| A1 | | 35,53 | 29,49 | 10,17 | 18,37 | 6,46 |
| ROSS-308 | | 39,41 | 29,91 | 9,24 | 16,74 | 5,88 |
| İnteraksiyon | | 0,058 | 0,870 | 0,988 | 0,467 | 0,339 |

[1]: Karkas parça oranları soğuk karkas ağırlığının yüzdesi olarak ifade edilmiştir. A1: Orta gelişen genotip, ROSS-308: Hızlı gelişen genotip, OSH: Ortalama standart hata. Aynı sütunda farklı harflerle gösterilen ortalamalar arasındaki farklılıklar istatistiki olarak önemlidir (P<0,05).

Çizelge 5. Et kalite özellikleri
*Table 5. Meat quality traits*

| Açlık süresi | Genotip | Göğüs eti | | | | | | But eti | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | pH | *L** | *a** | *b** | SK | PK | pH | *L** | *a** | *b** | SK | PK |
| 8 | A1 | 5,84 | 59,00 | 1,39 | 7,41$^{b}$ | 3,93 | 15,23 | 6,21 | 56,17 | 4,79 | 6,05 | 2,15 | 16,56 |
| | ROSS-308 | 5,88 | 61,22 | 1,50 | 7,84$^{b}$ | 4,59 | 18,82 | 6,13 | 59,18 | 3,41 | 7,06 | 2,16 | 17,61 |
| 12 | A1 | 5,84 | 59,24 | 1,07 | 7,93$^{b}$ | 4,24 | 14,85 | 6,25 | 56,25 | 4,38 | 6,53 | 1,38 | 13,24 |
| | ROSS-308 | 6,00 | 63,14 | 1,69 | 8,30$^{ab}$ | 4,31 | 16,81 | 6,24 | 60,14 | 3,30 | 7,60 | 1,96 | 16,06 |
| 16 | A1 | 5,83 | 57,44 | 0,94 | 6,46$^{c}$ | 3,04 | 16,40 | 6,39 | 55,70 | 4,02 | 5,72 | 1,81 | 15,23 |
| | ROSS-308 | 6,05 | 61,61 | 1,28 | 8,91$^{a}$ | 4,36 | 14,09 | 6,23 | 57,98 | 3,53 | 7,33 | 2,21 | 13,29 |
| OSH | | 0,019 | 0,293 | 0,089 | 0,162 | 0,247 | 0,572 | 0,015 | 0,350 | 0,179 | 0,197 | 0,126 | 0,635 |
| Ana etkiler | | | | | | | | | | | | | |
| Açlık süresi | | 0,241 | 0,065 | 0,272 | 0,406 | 0,562 | 0,438 | 0,001 | 0,284 | 0,733 | 0,459 | 0,272 | 0,153 |
| 8 | | 5,86 | 60,11 | 1,44 | 7,63 | 4,26 | 17,03 | 6,17$^{b}$ | 57,67 | 4,10 | 6,55 | 2,16 | 17,09 |
| 12 | | 5,92 | 61,19 | 1,38 | 8,12 | 4,27 | 15,83 | 6,25$^{ab}$ | 58,19 | 3,84 | 7,06 | 1,67 | 14,65 |
| 16 | | 5,94 | 59,52 | 1,11 | 7,68 | 3,70 | 15,25 | 6,31$^{a}$ | 56,86 | 3,77 | 6,54 | 2,00 | 14,26 |
| Genotip | | <0,001 | <0,001 | 0,048 | 0,001 | 0,174 | 0,350 | 0,007 | <0,001 | 0,007 | 0,002 | 0,196 | 0,612 |
| A1 | | 5,84 | 58,56 | 1,13 | 7,27 | 3,74 | 15,50 | 6,28 | 56,04 | 4,40 | 6,10 | 1,78 | 15,01 |
| ROSS-308 | | 5,98 | 61,99 | 1,49 | 8,35 | 4,42 | 16,58 | 6,20 | 59,10 | 3,42 | 7,33 | 2,11 | 15,66 |
| İnteraksiyon | | 0,116 | 0,339 | 0,510 | 0,013 | 0,592 | 0,104 | 0,151 | 0,646 | 0,583 | 0,792 | 0,628 | 0,310 |

A1: Orta gelişen genotip, ROSS-308: Hızlı gelişen genotip, OSH: Ortalama standart hata. *L**: Parlaklık, *a**: Kırmızılık, *b**: Sarılık, pH: Kesimden sonra 24. saattaki pH değeri, SK: Sızdırma kaybı, PK: Pişirme kaybı. Aynı sütunda farklı harflerle gösterilen ortalamalar arasındaki farklılıklar istatistiki olarak önemlidir (P<0,05).




Genotip: Pearson $\chi^2$=20,357; *P*<0,001 — Açlık süresi: Pearson $\chi^2$=1,636; P=0,950

Şekil 2. Orta ve hızlı gelişen etlik piliçlerin normal (■), hafif (■), orta (■) ve şiddetli (■) düzeyde görülen odunsu görünüme sahip göğüs oranları (%)

*Figure 2. Proportions of normal (■), mild (■), medium (■) and severe (■) scores for wooden breast observed in broiler chickens (%)*




Genotip: Pearson $\chi^2$=22,873; P<0,001 — Açlık süresi: Pearson $\chi^2$=10,143; *P*=0,119

Şekil 3. Orta ve hızlı gelişen etlik piliçlerin normal (■), hafif (■), orta (■) ve şiddetli (■) düzeyde görülen beyaz çizgi oluşumuna sahip göğüs oranları (%)

*Figure 3. Proportions of normal (■), mild (■), medium (■) and severe (■) scores for white striped breast observed in broiler chickens (%)*

Çalışmada but pH değerleri kesim öncesi açlık süresinin artması ile yükselmiştir (P<0,05). Buna karşın açlık süresinin artmasına bağlı olarak göğüs pH değerinde kısmi artışlar olmasına rağmen farklılıkların önemli olmadığı bildirilmiştir (Komiyama ve ark., 2008).

Göğüs ve but etinin sızdırma ve pişirme kaybı kesim öncesi açlık süresi ve farklı gelişme düzeyine sahip genotiplere göre önemli farklılıklar göstermemiştir (Çizelge 5). Wu ve ark. (2024), kesim öncesi açlık sürelerinin etin sızdırma kaybını etkilediğini, ancak pişirme kaybında farklılık olmadığını belirtmiştir. Benzer olarak Schneider & Gewehr (2023), 4, 8, 12 ve 16 saatlik açlık sürelerinde 42 günlük yaşta göğüs etinde pişirme kayıpları arasında farklılık olmadığını ve %21,9-25,4 arasında değiştiğini bildirmişlerdir. Komiyama ve ark. (2008), 4, 8, 12 ve 16 saatlik açlık uygulamalarında sızdırma ve pişirme kaybının değişmediğini belirtmişlerdir. Araştırmacıların verdiği sonuçlar çalışmamızdaki değerlerden kısmen daha yüksek bulunmuştur (sızdırma kaybı: %3,63-4,95; pişirme kaybı: %26,27-29,65).

Orta gelişen genotipte göğüs etlerinin %56,7'si normal ve %43,3'ü hafif odunsu görünüme sahipti. Hızlı gelişen genotipte ise odunsu görünümün şiddeti belirgin düzeyde artış göstererek göğüs etlerinin %16,7'sinde şiddetli odunsu görünüm belirlenmiştir (P<0,05; Şekil 2a). Kesim öncesi açlık süreleri odunsu göğüs oluşumunu önemli düzeyde etkilemese de (Şekil 2b), 16 saatlik açlık periyodunda 8 ve 12 saate kıyasla normal görünümlü göğüs eti oranında artış ile şiddetli odunsu göğüs oluşumunda azalma trendi gözlenmiştir.

Göğüs etinde beyaz çizgi oluşumu genotipler arasında farklılık göstermiş, hızlı gelişen genotip daha yüksek değerlere sahip olmuştur (P<0,05; Şekil 3a). Orta gelişen genotipteki göğüs etlerinin %76,7'sinde beyaz çizgi oluşumu gözlenmemişken, hızlı gelişen genotipte ise bu değer %16,7 bulunmuş ve göğüs etlerinin %6,7'sinde şiddetli beyaz çizgi oluşumu belirlenmiştir (P<0,05; Şekil 3a). Kesim öncesi açlık süreleri göğüs etinde beyaz çizgi oluşumunu önemli düzeyde etkilememiş ve aynı zamanda 12 saatlik açlık süresinin şiddetli beyaz çizgi oluşumuna neden olmadığı belirlenmiştir (Şekil 3b). Oral Toplu ve ark. (2021), büyütmenin farklı dönemlerinde kısıtlı yemleme yaptıkları ve kesim dönemi 12 saat açlık uyguladıkları etlik piliçlerin göğüs etinde beyaz çizgi oluşum düzeylerinin farklılık gösterdiğini, orta şiddetli beyaz çizgi oluşumunun serbest yemlenenlerde %57,5 olmasına karşın, kısıtlı yemlemeye bağlı olarak bu oranın düştüğünü (%12,5'e kadar) ve farklılıkların önemli olduğunu belirtmişlerdir. Araştırmacılar aynı şekilde orta düzeyli odunsu göğüs oluşumunun serbest yemlenenlerde %50 olmasına karşın, kısıtlı yemleme düzeyine bağlı olarak azalmalar olduğunu (%7,5'e kadar) ve farklılıkların önemli olduğunu bildirmişlerdir. Trochino ve ark. (2015), farklı kısıtlı yemleme koşullarında yetiştirilen ve kesim öncesi 7 saat açlık ile 4 saat susuzluk süresi olan piliçlerde kısıtlı yemlenenlerde kısmen daha yüksek beyaz çizgili göğüs ve daha az odunsu göğüs belirlemişlerdir. Livingston ve ark. (2019) ise, etlik piliçlerde şiddetli beyaz çizgili ve odunsu göğüs oluşumunun sırasıyla %20 ile %27 düzeylerinde olduğunu; göğüs eti oranındaki artışla birlikte bu düzeylerin yükseldiğini bildirmiştir. Etlik piliçlerin büyüme hızını ve göğüs eti verimini artırmaya yönelik süregelen genetik seleksiyon, son 20 yılda göğüs kası miyopatilerinin yüksek oranda görülmesine yol açmıştır (Petracci ve ark., 2009; Kijowski ve ark., 2014; Petracci ve ark., 2015). Çalışmamızda da hızlı gelişen genotipte beyaz çizgili ve odunsu göğüs oluşumunun daha şiddetli olması bu bildirişler ile uyumlu bulunmuştur.

## Sonuç

Bu çalışmada, farklı gelişim düzeylerine sahip etlik piliç genotiplerinde (ANADOLU-T A1 ana hattı ve ROSS-308) kesim öncesi 8, 12 ve 16 saatlik açlık sürelerinin stres düzeyi, karkas özellikleri ve et kalitesi üzerindeki etkileri karşılaştırılmıştır. Sonuçlar, açlık süresinin 16 saate kadar uzatılmasının tonik immobilite testi ile ölçülen stres düzeyini önemli ölçüde artırmadığını göstermiştir. Ancak, özellikle orta gelişen genotipte uzun açlık süreleri canlı ağırlık kaybını artırmıştır. Karkas randımanı hızlı gelişen ROSS-308'de daha yüksek bulunurken, abdominal yağ oranı ANADOLU-T'de belirgin şekilde fazla olmuştur. Et kalite parametrelerinden pH, renk ile pişirme ve sızdırma kaybı açlık sürelerinden etkilenmezken, ROSS-308 genotipinde odunsu göğüs ve beyaz çizgi oluşumları daha şiddetli gözlenmiştir. Bu bulgular, kesim öncesi 12 saatlik açlık uygulamasının sindirim kanalı temizliği ve karkas kalitesi için yeterli olduğunu, ancak hızlı gelişen genotiplerde et kalite kusurlarının izlenmesi gerektiğini ortaya koymaktadır. Üretimde genotip bazlı uygulamaların optimize edilmesi, verimlilik ve refah dengesi açısından kritik öneme sahiptir.

## Beyanlar

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Bu çalışma, Ondokuz Mayıs Üniversitesi Hayvan Deneyleri Yerel Etik Kurulunun 26.01.2023 tarih ve 2023/01 nolu kararına uygun olarak yürütülmüştür.

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Makale yazarlarının her biri çalışmaya eşit katkı sağlamıştır.

### *Çıkar Çatışması*

Yazarlar herhangi bir çıkar çatışması olmadığını beyan etmektedir.

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# Effects of *Spirulina* and *Chlorella* Used as Protein Source on Growth and Digestion Enzymes of Common Carp (*Cyprinus carpio*, L., 1758)

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ABSTRACT

This study was conducted to determine the effects of using *Spirulina* and *Chlorella* instead of fish meal on the growth, feed utilization, digestibility and digestive enzyme activity of common carp (*Cyprinus carpio*). For this purpose, 25% fish meal was added to the control diet, 25% *Spirulina* to the SP diet and 25% *Chlorella* to the CL diet as the main protein source. In the 3×3 planned experiment, fish with an average weight of 1.98±0.10 g were fed with isonitrogenous and isolipidic formulated diets until satiation for 60 days. At the end of the experiment, higher final body weight and specific growth rate were obtained in the groups fed with diets containing Spirulina and Chlorella ($p<0.05$) and feed conversion was not affected by the main protein source in the diet. Lipid content in muscle tissue of fish fed with control diet was lower than that of the group fed with Chlorella containing diet ($p<0.05$). Microalgae addition to the diets significantly ($p<0.05$) increased dry matter, protein and lipid digestibility, as well as protease and lipase activity. The results obtained showed that *Spirulina* or *Chlorella* in *Cyprinus carpio* diets increased growth, nutrient digestibility and activity of digestive enzymes, and therefore, based on these parameters examined, *Spirulina* and *Chlorella* could be used instead of the entire 25% fish meal in the diet.

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## Introduction

The aquaculture sector has become the fastest growing sector of the food industry due to the increased fish consumption in recent years (Asche, 2008). Feed costs constitute the largest portion of the total operating expenses of aquaculture. The most important reason for the high cost of feed is the scarcity and expensive nature of fish meal, the main protein source of feed (Albaqami, 2025). This factor, which is challenging for the sector, pushes researchers to search for sustainable and cheaper alternative sources to fish meal. One of these sources that attracts the attention of researchers is microalgae. Microalgae appear to be a sustainable source that is partly easy to produce (Vuppaladadiyam et al., 2018; Dineshbabu et al., 2019). Adding microalgae to animal feeds increases growth, immune response, disease resistance, antibacterial and antiviral activity, reduces feed consumption and enriches animal products in terms of bioactive components (Kusmayadi et al., 2021). More than 40 microalgae species are used in aquaculture (Sathasivam et al., 2019), and the most widely researched of these species are *Spirulina* and *Chlorella* (Ahmad et al., 2020). *Chlorella* and *Spirulina* are two of the most well-known microalgae genera. While *Chlorella* is a unicellular algae, *Spirulina* is a multicellular, filamentous cyanobacteria (Andrade et al., 2018). *Spirulina* and *Chlorella* have a balanced amino acid profile and high protein content, although they vary depending on the cultivation conditions (Morita et al., 1999; Liu & Chen, 2014; El-Sheekh et al., 2023; Albaqami, 2025). Moreover, they are a good source of polyunsaturated fatty acids such as DHA and EPA (Andrade et al., 2018). They also contain bioactive compounds such as antioxidants, carotenoids and phenolic compounds with antiviral, antibacterial, antifungal, anti-inflammatory, antioxidative and antitumor properties (Michalak & Chojnacka, 2015; Yücetepe & Özçelik, 2016; Saide et al., 2021; Uzlaşır et al., 2023). Due to all these properties, they are preferred both as human food and as additives in animal feed.

*Spirulina* and *Chlorella* have been shown to increase growth and feed utilization when added to the diets of different fish species (Gouveia et al., 2002; Velasquez et

al., 2016; Alagawany et al., 2021), improve pigmentation (Sergejevova & Masojidek, 2012; Teimouri et al., 2013; Karadal et al., 2017; Sehgal et al., 2022), immune system (Zahran & Risha, 2014, Khani et al., 2017; ) and blood parameters (Quico et al., 2021, Raji et al., 2018; Raji et al., 2019; Mohammadiazarm et al., 2021), and increase antioxidant enzyme activity (Galal et al., 2018; Rahimnejad et al., 2017; Abdel-Tawwab et al., 2022). In some studies, it was determined that these microalgae could be added instead of all fish meal (Cao et al., 2018; Raji et al., 2020), while in some studies, it was determined that high levels of supplementation had no effect on growth (Xi et al., 2022; Man et al., 2020). At low doses, it was more effective on the immune system, antioxidant enzymes, blood parameters, cholesterol and digestive enzymes (Xu et al., 2014; Mohammadiazarm et al., 2021).

In this study, it was tried to determine whether *Spirulina* and *Chlorella* microalgae could be added to *Cyprinus carpio* feeds, which is an omnivorous species, instead of all fish meal, based on growth parameters, digestibility and digestive enzyme parameters.

## Material and Methods

The experiment was conducted in Fatsa Marine Sciences Faculty Aquarium Fish Laboratory. Carp fish (*Cyprinus carpio*) were obtained from a commercial enterprise. During the 3-week adaptation period, the fish were fed with a control diet. At the end of the adaptation period, the fish with an average weight of 1.98±0.10 g were placed in 9 aquariums with a capacity of 60 liters and dimensions of 60x30x35, with 35 fish in each aquarium (total 315 fish), for the experiment planned as 3 groups and 3 replications. The aquariums were continuously aerated with an air motor during the experiment. 30% of the aquarium water was changed with chlorine-free and aerated water every day. The trial lasted 60 days. A 12-hour light: 12-hour dark photoperiod was applied. Fish were fed twice daily (9:00-16:00) until satiated. During the trial, water temperature was determined as 26.1±1.2 °C, pH as 7.42±0.85 and oxygen as 6.53±0.8 mg $l^{-1}$.

### *Test Diets*

The control diet was prepared to contain 25% fish meal, the SP diet as 25% *Spirulina* and the CL diet as 25% Chlorolle. While preparing the diets, first the dry ingredients were weighed and mixed according to the formulation. Then Chromium oxide was added and mixing was continued. Oil and then distilled water were added to the mixture. The resulting dough was passed through a meat grinder and given the form of pellets. The pellets were dried at 50 °C. The cooled pellets were stored at -20 °C. The nutrient composition and formulation of the diets are given in Table 1.

### *Sampling*

Before the sampling procedures at the beginning and end of the trial, the fish were left hungry for 24 hours. Sampling was performed by applying an overdose of anesthetic (Clove oil, 100 mg/L). At the beginning of the trial, a total of 25 fish were sampled to determine the proximate analysis, hepatosomatic index, and conditional factor values. At the end of the trial, 8 fish were taken from each tank to determine the proximate analysis, hepatosomatic index, conditional factor, and digestive enzyme activity. The muscle tissue separated for proximate analysis was frozen at -20°C. The intestinal samples to be used in the measurement of digestive enzyme activity were placed on ice and sent to the tissue homogenization process.

To determine the digestibility of the feeds, feces samples were collected by siphoning from the 15th day of the trial. The aquariums were cleaned immediately after feeding. After the cleaning process, feces were collected. The collected feces were placed in sampling containers and stored at -20°C until the time of analysis.

Table 1. Ingredient and proximate composition of experimental diets

| Ingredients | Control | SP | CL |
|---|---|---|---|
| Fish meal[1] | 25 | - | - |
| *Spirulina*[2] | - | 25 | |
| Chloralla[3] | - | | 25 |
| Soybean meal[4] | 25 | 25 | 25 |
| Corn gluten[5] | 15 | 15 | 15 |
| Wheat starch[6] | 27.5 | 25.5 | 25.5 |
| Fish oil[7] | 3 | 5 | 5 |
| Vitamin premix[8] | 2 | 2 | 2 |
| Mineral premix[8] | 2 | 2 | 2 |
| Chromium oxide[9] | 0.5 | 0.5 | 0.5 |
| Proximate analysis | | | |
| Dry matter | 92.5 | 91.9 | 92.3 |
| Crude protein | 37.0 | 36.9 | 37.1 |
| Crude lipid | 6.5 | 6.6 | 6.4 |
| Ash | 1.3 | 1.2 | 1.2 |
| Nitrogen-free extract[10] | 47.7 | 47.2 | 47.6 |
| Gross energy[11] (kj $g^{-1}$) | 19.4 | 19.3 | 19.4 |

[1]Sürsan, Türkiye; crude protein, 66.43%; crude lipid, 9.27%. [2] Naturiga, Türkiye; crude protein, 65.8 %; crude lipid, 1% [3] Naturiga, Türkiye; crude protein, 66.1%; crude lipid, 1.2%. [4] AFNA GmbH, Hamburg, Germany; 46.3%; crude lipid, 3.5%. [5] Vamos Tarım, Türkiye; 60.2%; crude lipid, 2.3%. [6] İnci, Türkiye. [7] Sürsan, Türkiye. [8] Sürsan, Türkiye. [9] Sigma-Aldrich, USA. [10] Nitrogen-free extract = Dry matter − (protein + lipid + ash). [11] Gross energy was calculated based on the energy values of 23.6 kJ $g^{-1}$ for protein, 39.5 kJ $g^{-1}$ for lipid and 17 kJ g-1 for NFE.

***Analysis***

Diets, feces samples and fish meal proximate analyses (dry matter, crude protein, ash content) were performed according to AOAC (1995). Lipid content was determined by the method of Bligh & Dyer (1959). The amount of chromic oxide in feces and diets was determined using the method of Furukawa & Tsukahara (1966). Intestines sampled to measure digestive enzyme activity were homogenized on ice with buffered saline solution. Then, they were centrifuged at 10,000 g for 15 min at 4°C. The protein content of the intestine supernatants was evaluated following the method of Bradford (1976). Protease activity was assessed using a casein hydrolysis assay with tyrosine as a standard as described in Sotoudeh & Esmaeili (2022).The enzyme sample (0.1 mL) was incubated with 1% casein (0.25 mL) and buffer (0.25 mL) at 37°C for 1 hour. The reaction was stopped by adding trichloroacetic acid (8%), and absorbance was measured at 280 nm (Hidalgo et al., 1999). Amylase activity was determined by incubating 100 µL of sample with 2% starch in assay buffer (pH 6.8) for 1 hour at 37°C. After adding a color-reactive solution. Color-reactive solution was prepared by adding 16 mL sodium potassium tartrate solution and 40 mL 96 mM 3,5-dinitrosalicylic acid solution into 24 mL distilled water. The mixture was boiled and cooled on ice. Absorbance was measured at 540 nm (Yılmaz et al., 2018). Lipase activity was performed as described in Ravardishİ et al. (2021). The reaction solution was prepared by dissolving 0.53 mM p-nitrophenyl myristate in a mixture of 0.25 mM Tris-HCl, 0.25 mM 2-methoxy ethanol, and 5 mM sodium cholate buffer (pH 9.0). 5 µL of enzyme extract was added to 0.5 ml of the substrate and incubated for 15 min at 30°C. The reaction was stopped by adding 0.7 mL of acetone:n-heptane (5:2) mixture. The mixture was centrifuged for 3 min at 6080 g and 4°C. After centrifugation, the absorbance of the released p-nitrophenol was measured at 405 nm.

***Statistical Analyses***

Shapiro-Wilk and Levene tests were used to test whether the data showed normal distribution and the homogeneity of variances. The obtained data were statistically analyzed using one way ANOVA. In case of a difference, which groups were different from each other was examined with Tukey's multiple comparison test at a confidence interval of 0.05. The statistical analysis was performed using SPSS software version 23.

## Results

Data on growth and feed conversion performance of *Cyprinus carpio* fed with experimental diets for 60 days are given in Table 2. The final weight and SGR values showed a significant ($p<0.05$) increase compared to the Control group, and no difference ($p>0.05$) was detected between the groups fed with diets containing *Spirulina* and *Chlorella*. In addition, there was no statistical significance ($p>0.05$) between the groups in FI, FCR, PER, CF, HSI. There was no mortality in the groups during the experiment.

As seen in Table 3, no significant effect of *Spirulina* and *Chlorella* added to the diets on dry matter, crude protein and ash values in muscle tissue was detected ($p>0.05$). Crude lipid content decreased with *Chlorella* addition to the diet ($p<0.05$), and no significant difference was detected between the control group and the SP group ($p>0.05$).

Dry Matter, protein and lipid ADC are given in Table 4. The presence of *Spirulina* and *Chlorella* in the diet significantly ($p<0.05$) increased Dry Matter, protein and lipid ADC compared to the Control group. The lowest Dry Matter, protein and lipid ADC in the experiment was determined in the Control group.

Table 2. Growth performance and feed utilization in common carp fed diets containing *Spirulina* and *Chlorella*

| | Control | SP | CL | P value |
|---|---|---|---|---|
| Initial weight | 1.98±0.20[a] | 1.99±0.29[a] | 1.98±0.10[a] | - |
| Final weight | 16.40±0.39[b] | 17.01±0.17[a] | 17.22±0.22[a] | 0.03 |
| SGR[1] | 3.53±0.07[b] | 3.58±0.12[a] | 3.61±0.11[a] | 0.378 |
| FI[2] | 18.02±0.23[a] | 18.62±0.66[a] | 18.56±0.07[a] | 0.100 |
| FCR[3] | 1.25±0.01[a] | 1.24±0.02[a] | 1.22±0.02[a] | 0.113 |
| PER[4] | 2.16±0.02[a] | 2.19±0.03[a] | 2.21±0.02[a] | 0.166 |
| Survival[5] | 100[a] | 100[a] | 100[a] | - |
| CF[6] | 2.51±0.09[a] | 2.38±0.23[a] | 2.41±0.17[a] | 0.637 |
| HSI[7] | 1.89±0.04[a] | 1.91±0.08[a] | 1.90±0.0[a] | 0.896 |

Values are mean ± SD. Different superscripts of the values in the same row indicate significant differences ($p<0.05$). [1]Specific Growth Rate (SGR, %) = 100 × [(Ln final weight – Ln initial weight)] / days. [2]Feed intake (FI) = feed intake (g)/ number of fish. [3]Feed conversion ratio (FCR) = feed intake (g)/weight gain (g). [4]Protein efficiency (PER) = (final body weight (g) - initial body weight (g))/protein intake. [5]Survival rate (SR; %) = (number of alive fish/total number of fish) × 100. [6]Condition factor (CF) = (g/cm$^3$ ) = 100 × (body weight)/(body length)$^3$. [7]Hepatosomatik indeks (HSI, %) = (liver weight (g)/body weight (g)) × 100

Table 3. Proximate composition

| | Initial | Control | SP | CL | P value |
|---|---|---|---|---|---|
| Dry Matter | 24.32 | 25.25±0.31[a] | 25.28±0.41[a] | 25.37±0.25[a] | 0.908 |
| Crude Protein | 13.49 | 14.46±0.52[a] | 14.60±0.14[a] | 14.73±0.12[a] | 0.275 |
| Crude Lipid | 3.84 | 4.92±0.16[a] | 4.63±0.14[a] | 4.30±0.22[b] | 0.014 |
| Ash | 6.98 | 5.85±0.16[a] | 6.05±0.30[a] | 6.33±0.24[a] | 0.009 |

Values are mean ± SD. Different superscripts of the values in the same row indicate significant differences ($p<0.05$).

Table 4. Apparent digestibility coefficients (ADC, %) of dry matter, protein and lipid of *Cyprinus carpio* fed experimental diets.

| ADC[1] (%) | Control | SP | CL | P value |
|---|---|---|---|---|
| Dry Matter | 86.48±0.44[b] | 90.80±0.27[a] | 91.17±0.26[a] | 0.000 |
| Crude Protein | 91.91±0.54[b] | 93.11±0.22[a] | 94.19±0.26[a] | 0.009 |
| Crude Lipid | 85.08±0.27[b] | 86.65±0.36[a] | 86.40±0.52[a] | 0.006 |

Values are mean ± SD. Different superscripts of the values in the same row indicate significant differences ($p<0.05$). [1]Apparent digestibility coefficients (ADC, %) = 100-{100 × (% $Cr_2O_3$ in diet/% $Cr_2O_3$ in faeces) × (% nutrient in faeces/% nutrient in diet)}

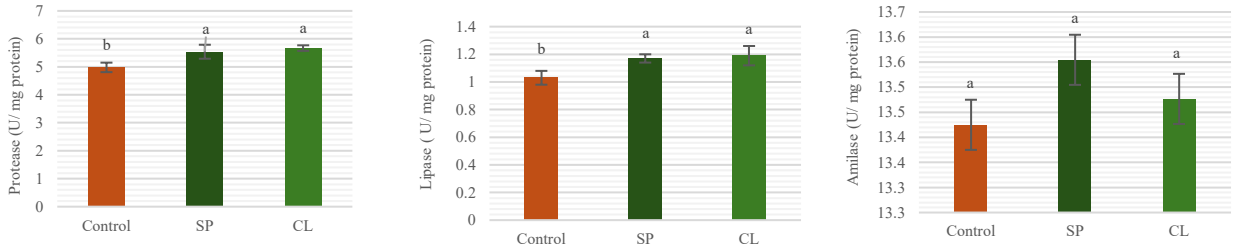


Figure 1. The digestive enzyme levels in intestine of *Cyprinus carpio* fed experimental diets. Values are mean ± SD.

The results obtained for protease, lipase and amylase activities in the experiment are given in Figure 1. No difference ($p>0.05$) was detected between the groups in terms of amylase activity. Protease and lipase activity was high in groups fed with microalgae-containing diets and the difference between these groups and the control group was found to be significant ($p<0.05$).

## Discussion

The aim of this study was to determine how the addition of *Spirulina* and *Chlorella* to common carp diets instead of all fish meal affects growth and digestive parameters. In the study, the groups fed diets containing *Spirulina* and *Chlorella* as the main protein sources showed higher growth performance. Previous studies have also found that *Spirulina* and *Chlorella* added to the diets instead of all or part of fish meal had a growth-promoting effect. In general, studies with carnivorous species show that higher levels of growth performance are obtained with smaller amounts of microalgae added to the diets. For example, Quico et al. (2021) added 3.6 to 5.0 g kg-1 to rainbow trout fry (*Oncorhynchus mykiss*) diets, Rahimnejad et al. (2017) added 10-15% to Olive Flounder (*Paralichthys olivaceus*) diets, and Chen et al. (2021) higher growth performance was obtained with 5% *Chlorella* supplementation in rainbow trout (*Oncorhynchus mykiss*) diets, Huang et al. (2024) with 10% *Chlorella* supplementation in marbled eel (*Anguilla marmorata*) diets, Xi et al. (2022) with 15.03% to 15.43% *Chlorella* supplementation in largemouth bass (*Micropterus salmoides*) diets, Teimouri et al. (2013) with 7.5-10% *Spirulina* supplementation in rainbow trout (*Oncorhynchus mykiss*) diets. In studies conducted with omnivorous fish species such as the common carp used in this study, an increase in growth was detected with both low and high amounts of *Spirulina* and *Chlorella*. For example, Adel et al. (2016) reported that maximum growth performance was achieved by adding 10% *Spirulina* to great sturgeon (*Huso huso*) diets, Bin Dohaish et al. (2018), Abdel-Tawwab et al. (2009), Velasquez et al. (2016), AlMulhim et al. (2023) by adding 5%, 5%, 30% and 5-10% *Spirulina* to Nile tilapia (*Oreochromis niloticus*) diets, respectively, and El-Sheekh et al. (2014) by adding 75% of fish meal *Spirulina* to hybrid red tilapia (*Oreochromis niloticus x Oreochromis mossambicus*) diets. Gallal et al. (2018) and Abdel-Tawwab et al. (2022) determined that maximum growth was achieved by adding 10%-10-15% *Chlorella* to Nile tilapia (*Oreochromis niloticus*) diets, respectively. In contrast to these studies, Güroy et al. (2022) reported that adding *Spirulina* to European seabass (*Dicentrarchus labrax*) diets and Yu et al. (2022) notified that adding *Chlorella* to largemouth bass (*Micropterus salmoides*) diets had no effect on growth. Unlike all these studies, the results of the current study are similar to the studies by Raji et al. (2020) in which they reported that all fish meal in the diet of African catfish (*C. gariepinus*) could be replaced with *Spirulina* and *Chlorella* and to the studies by Cao et al. (2018) in which *Spirulina* could completely replace fish meal in the diet of juvenile jibel carp (*Carassis auratus gibelio* var. CAS III). The fact that different results were obtained even in the same fish species shows that the factor affecting the amount of *Spirulina* and *Chlorella* in the diet is not only the fish species or the feeding habits of the fish, but also the size of the fish, the nutritional component of the microalgae used and the composition of the diet.

In this study, *Chlorella* added to the diet caused a significant decrease in the lipid level in the fish muscle. A decrease in lipid levels was also detected with *Spirulina* supplementation, but the difference was not found to be statistically significant. Previous studies reported that the amount of lipid accumulated in fish muscle was affected by the presence of *Spirulina* in the diet and that the lipid content decreased (Kim et al., 2013; Liu et al., 2019; Teimouri et al., 2016; Roohani et al., 2019; Cao et al., 2018; Mohammadiazarm et al., 2021; AlMulhim et al., 2023). This decrease obtained as a result of the addition of *Spirulina* to the diets was reported by Kim et al. (2013) and Liu et al. (2019) explained it with the hypolipidic activity of polyphenolic compounds found in *Spirulina*. However, it can be said that this is not an effective factor alone, and the components that make up the diet and the amount of these components are also effective factors. In particular, the presence of dietary components containing difficult-to-digest anti-nutrition factors (ANF) in plant raw materials may also affect the proximate composition of fish.

Antinutrients can disrupt the intestinal epithelial structure, which is the absorption surface of digested nutrients, or inactivate digestive enzymes (Francis et al., 2001; Krogdahl et al., 2010; Kokou & Fountoulaki, 2018; Shi et al., 2025). Indeed, Shi et al. (2017a) reported that the lipid content in fish meal increased as the amount of rapeseed meal and *Chlorella* mealin increased instead of fish meal in the diet. Shi et al. (2017b) reported that the lipid content in fish meal decreased with the increase in the amount of soy meal and *Chlorella* in the diet, but this decrease was not statistically significant.

Bioactive substances found in the structure of *Spirulina* and *Chlorella* (Abdel-Tawwab et al., 2022, Huang et al., 2024, Raji et al., 2020 may have an effect of enhancing palatability and digestive enzyme secretion (Abdel-Tawwab et al., 2022). The increase in digestive enzyme activity means increased digestibility of the feed and therefore nutrient utilization (AlMulhim et al., 2023). In this study, by adding microalgae to the diets instead of fish meal, an increase dry matter, protein and lipid ADC and also the activity of protease and lipase enzymes was detected. This increase in digestibility and digestive enzymes provided more growth in groups containing microalgae. As determined in the studies of Raji et al. (2020), in this study, over 90% dry matter and protein ADC was detected in groups containing *Spirulina* and *Chlorella*. In addition, a higher amount of lipid ADC was achieved compared to the control group.

Hassaan et al. (2021) reported that the addition of phycocyanin and β-carotene obtained from *Spirulina* platensis to the diets of *Oreochromis niloticus*, increased the activities of protease, lipase and amylase enzymes, and these compounds may have a growth stimulating effect by increasing the ability of the intestinal flora to break down indigestible feed components in the feed. In studies conducted with chlorella, it was determined that adding *Chlorella* to the diets increased the amylase, lipase and protease levels of gibel carp (*Carassius auratus gibelio*) and Koi carp, and this increase provided better utilization of the diet (Xu et al., 2014; Khani et al., 2017).

Studies have reported that adding *Spirulina* to diets improves intestinal microbiota, which helps break down indigestible feed components and therefore allows fish to benefit better from the feed (Man et al., 2020; James et al., 2006). Al-Deriny et al. (2020) reported that *Spirulina* is effective in improving intestinal morphometry indices, which increases intestinal absorption capacity and therefore accelerates growth.

## Conclusions

The importance of reducing pressure on fish meal for sustainable aquaculture is well known. In this study, chlorella and spirulina, which have economic values such as easy culture, high biomass and availability, were used as an alternative source to reduce this pressure. As a result, it has been determined that *Spirulina* or *Chlorella* added to *Cyprinus carpio* diets increases growth, nutrient digestibility and the activity of digestive enzymes, and that *Spirulina* and *Chlorella* can be used instead of all 25% of fish meal in the diet.

## Declarations

### *Ethical Approval Certificate*

Approval was obtained from Ordu University Animal Experiments Local Ethics Committee (Approval date and number: 13/05/2024-04/10) for the study.

### *Author Contribution Statement*

*Fatma Burcu Harmantepe:* Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Visualization.

*Ebru Yılmaz:* Conceptualization, Methodology, Visualization, Investigation.

### *Conflict of Interest*

The authors declare that they have no actual, potential, or perceived conflict of interest for this article.

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# Geographically Indication Ancestral Seed Ardahan Kavılca Wheat: A Comprehensive Review

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ABSTRACT

The focus on ancestral seeds and Geographical Indications (GI) reflects a growing global movement to reconnect with traditional agricultural practices and cultural heritage. These practices emphasise environmental sustainability, agricultural biodiversity and food quality. Kavılca, an old emmer wheat variety from Ardahan, Türkiye, exemplifies these practices. Known for its resilience to harsh climates, low gluten content and rich nutrient profile, Kavılca wheat supports sustainable agriculture and preserves cultural traditions. By protecting these unique products, GIs help preserve agricultural biodiversity and heritage against modern monoculture practices. Similarly, ancestral seeds such as Kavılca wheat offer climate change adaptation advantages with minimal dependence on chemical inputs. They also provide superior nutritional benefits, making them a healthier alternative to modern wheat varieties. Overall, the cultivation of ancestral seeds such as Kavılca wheat is crucial in addressing challenges such as climate change, food security and biodiversity loss. These practices represent a harmonious blend of tradition and innovation that promotes sustainable and culturally rich food systems for future generations. The resilience of this crop to environmental challenges, combined with its nutritional benefits and cultural significance, make it an invaluable asset in addressing the modern agricultural and food challenges we face. This review article is designed by narrative review method. It analyses the importance of Ardahan Kavılca wheat, a Geographical Indication ancestral seed, and its journey from the field to the consumer.

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## Introduction

In the contemporary era, there is an increasing focus on reconnecting with cultural heritage, particularly through the utilisation of Geographical Indications (GI) within the food supply chain. This approach highlights the significant interconnection between food production and the geographical, cultural, and traditional contexts from which it originates (Arfini et al., 2019). This paradigm is frequently characterised as a "back to the roots" movement, yet its scope extends beyond the mere production and consumption of food. It encompasses broader objectives, including the preservation of environmental integrity, the safeguarding of cultural heritage, and the assurance of superior food quality (Bérard & Marchenay, 2008).

A significant proportion of GI-designated products are firmly embedded in traditional agricultural practices, culinary expertise, and artisanal processing methods that have been transmitted across generations. Such methods are frequently aligned with environmentally sustainable principles and are finely adapted to local ecosystems (Vandecandelaere et al., 2021). The resurgence of interest in traditional products signifies a revitalisation of indigenous agricultural breeds and regional crop varieties, many of which have co-evolved with specific environmental conditions over centuries (WIPO, 2024).

This dynamic not only reinforces the preservation of agricultural biodiversity but also addresses the growing necessity of promoting resilience in the face of climate change (Coşkun, 2023). The production processes associated with GI products frequently prioritise sustainability, employing techniques that demonstrate respect for the local environmental context. A considerable number of GI producers have adopted practices that are following ecological principles, including traditional farming methods such as crop rotation, the minimisation of chemical inputs, and organic cultivation. Such practices not only enhance environmental sustainability but also contribute significantly to the economic vitality of rural areas. The production of GI-certified products has been demonstrated to foster the creation of local employment opportunities and stimulate economic activity, often

serving as a cornerstone of rural development (Halder et al., 2024).

Moreover, GI products exemplify the intrinsic connection between geography and gastronomy. The distinctive characteristics of these products—flavor, texture, and nutritional quality—are inextricably linked to the unique interplay of local soil, climate, and traditional production methods. Such geographical specificity serves to enhance the organoleptic properties of the food in question, while simultaneously solidifying its cultural and economic significance (Nihei, 2019). By establishing a connection between production and specific regions, GI products serve as a crucial economic resource for local communities, frequently reducing the appeal of urban migration by maintaining livelihoods in rural settings (Van Caenegem & Cleary, 2017).

In light of the aforementioned factors, the adoption and promotion of Geographical Indications represents a multidimensional strategy that intertwines cultural preservation, environmental sustainability, and economic development. These initiatives are of great importance for the fostering of an appreciation of regional heritage, the ensuring of the viability of traditional practices, and the addressing of contemporary global challenges such as climate change and food security.

In this context, the aim is to support the promotion and better understanding of this value, given that the GI 'Ardahan Kavılca Wheat' has not yet received the attention it deserves and is only partially covered in the literature.

## Geographical Indication

Geographical indications (GIs) are signs used on products that have a specific geographical origin and possess qualities or a reputation due to that origin (WIPO, 2024). They serve to identify goods as originating from a particular place, thereby highlighting the unique characteristics that are attributable to that region. Geographical indications (GIs) are signs used on products that have a specific geographical origin and possess qualities or a reputation due to that origin (Calboli, 2015). They serve to identify goods as originating from a particular place, thereby highlighting the unique characteristics that are attributable to that region. Historically, this linkage was often associated with the term 'terroir', which refers to the influence of a region's soil, climate, and other environmental factors on a product's qualities (OriGIn, 2024). As global trade expanded over time, the necessity to safeguard these distinctive regional products became apparent, giving rise to the formulation of legal frameworks for GIs.

The first international agreement to address the protection of GIs was the Paris Convention for the Protection of Industrial Property, which was signed in 1883. Subsequently, the Lisbon Agreement for the Protection of Appellations of Origin and their International Registration, established in 1958, provided a means of obtaining international protection for GIs (WIPO, 2024). Furthermore, the Agreement on Trade-Related Aspects of Intellectual Property Rights (TRIPS), which came into effect in 1995, reinforced GI protection by defining them and setting out the obligations of member states to protect them (Agdommar, 2008). A geographical indication is not merely a name or sign; rather, it represents the intrinsic connection between a product and its place of origin. This link may be based on certain qualities, reputation, or other characteristics that can be attributed primarily to geographical origin (Gangjee, 2017). To illustrate, the wheat cultivated in Kavılca, situated within the Ardahan province of Eastern Anatolia in Türkiye, is renowned for its distinctive attributes, which are attributable to the heterogeneous climatic and soil conditions prevailing in the region. It is crucial to differentiate between geographical indications and trademarks (Coombe, 2014). While both serve the function of identifying the source of goods, trademarks are distinctive signs used by businesses to identify and distinguish their products or services from those of other entities. In contrast, geographical indications are specifically designed to indicate the geographical origin of a product and the unique qualities associated with that origin (Blakeney, 2014). Geographical indication (Table 1) has different features and benefits (Calboli, 2021; Cardoso et al, 2022; Li et al, 2024; WIPO, 2024).

## Ancestral Wheats

Ancestral wheat varieties, including emmer (*Triticum dicoccum*), einkorn (*Triticum monococcum*) and Khorasan (*Triticum turgidum* ssp.), represent some of the oldest cultivated grains, with a history spanning over 10,000 years (Sertse et al, 2023; C&GA, 2024). Such grains are frequently designated as "ancestral" or "heritage" varieties, and they diverge considerably from modern wheat in both genetic composition and cultivation practices. Ancestral wheat has a rich cultural history and is deeply embedded in the agricultural traditions of numerous regions, including Anatolia, the place of its origin (Athinaiou et al, 2022; Onur & Ceylan, 2023).

Table 1. Benefits and Features of Geographical Indication (GI)

| Features | Benefits |
|---|---|
| Origin-linked Quality | Ensures products are authentic and high-quality. |
| Cultural Preservation | Protects traditional methods and local heritage. |
| Economic Growth | Enhances local economies and supports regional development. |
| Product Differentiation | Distinguishes products in global markets, adding value. |
| Sustainability | Promotes environmentally sustainable farming and production. |
| Legal Protection | Secures exclusive rights and prevents imitation or misuse. |
| Tourism Promotion | Boosts tourism by attracting interest to local specialties. |
| Consumer Trust | Builds consumer confidence in authenticity and quality. |

*Source: created by the corresponding author

### *General Characteristics of Ancestral Wheats*

It is generally accepted that ancestral wheat varieties offer a superior nutritional profile to modern wheat. This is evidenced by their higher protein levels, fibre content and essential mineral composition, including magnesium and zinc. Additionally, they are less likely to induce gluten sensitivities, as their gluten structure differs from modern hybridised wheat (Shi & Ling, 2018). These ancient grains have retained a considerable degree of genetic diversity, which endows them with the capacity to adapt to a range of climatic conditions and to withstand infestation by pests and pathogens. In contrast to modern wheat, which has been bred for uniformity and large-scale production, ancestral wheat displays greater resilience to environmental stresses, including drought and poor soil conditions (Wang et al, 2024). Many chefs and bakers are attracted to ancestral wheat due to its distinctive flavor profile. The flavor of these grains is more pronounced and robust than that of modern wheat, which has been bred for mass consumption and is therefore characterised by a neutral flavor profile. For example, einkorn has a slightly nutty taste, whereas emmer offers a rich, rustic flavor (Hastorf & Bruno, 2020; Doğan & Keserli, 2024). The cultivation of ancestral wheat contributes to the maintenance of agricultural biodiversity and the sustainability of agricultural practices. As these crops flourish in suboptimal growing conditions, they provide support to small-scale and local farmers who frequently employ low-input agricultural practices. This is in stark contrast to the vast monoculture fields of modern wheat, which rely heavily on chemical fertilisers and pesticides (Pérez-Lloréns & Brun, 2023). In Türkiye, for example, ancestral wheat varieties such as Kavılca have constituted an integral part of local diets for centuries (Mısır & Alp Baltakesmez, 2024). These grains are not only a staple food but also represent cultural heritage. In recent times, many regions have begun to recognise the importance of preserving these ancient varieties to safeguard both their agricultural heritage and food sovereignty (Demir, 2020; Aydar, 2022). The growing interest in these ancestral grains reflects a broader movement towards sustainable and organic farming practices (Uralçin, 2024). In light of mounting concerns regarding the environmental impact of contemporary agricultural practices, there has been a growing recognition of the ecological benefits, nutritional value, and cultural significance of ancestral wheat (Ríos & Palmera, 2024). In recent years, there has been a notable resurgence in the utilisation of ancestral seeds. To safeguard these ancient grains from extinction, movements such as seed-saving initiatives and community seed banks have been established. A collaborative effort between scientists, indigenous communities, and farmers is underway to reintegrate these crops into mainstream agricultural practices (Aydar, 2022). This resurgence in interest in ancestral wheat is aligned with the Slow Food movement and efforts to combat the loss of biodiversity caused by modern agricultural practices (Slow Food, 2024). The resurgence of ancestral wheat varieties signifies not only a return to traditional agricultural practices but also an endeavor to confront global challenges such as climate change and food security. These grains, with their long historical precedent, are being acknowledged as essential instruments for the future of sustainable agriculture (Sanchez-Garcia, & Bentley, 2019).

### *Importance of Ancestral Seeds*

Ancestral seeds play an important role in environmental sustainability. These non-genetically modified seeds can be produced naturally by adapting to local climate and soil conditions (Mısır & Alp Baltakesmez, 2024). Unlike the uniform seeds used in modern agriculture, traditional seeds support agricultural diversity. Ancestral seeds, which do not require chemical pesticides and fertilisers, promote environmentally friendly agricultural practices (Seçer & Boğa, 2016). Products derived from ancestral seeds tend to have higher nutritional value and are important for healthy food production. For these reasons, studies on ancestral seeds in Türkiye are considered critical for protecting cultural heritage and supporting sustainable agricultural practices (Onur & Ceylan, 2023).

Compared to modern bread wheat (*Triticum aestivum*), ancestral wheats are nutritionally dense, with higher levels of protein, essential amino acids, and micronutrients such as magnesium, zinc, and selenium. They also contain high amounts of dietary fiber and bioactive compounds like carotenoids and polyphenols, which are associated with antioxidant properties (Hidalgo & Brandolini, 2014). However, their gluten content is lower, which, while unsuitable for celiac patients, makes them more tolerable for individuals with gluten sensitivity.

Traditional foods made from ancestral wheats include flatbreads, porridges, and pilafs, which remain staples in various cultures. The revival of these grains aligns with a global movement to reconnect with traditional diets and artisanal food production, as they are perceived to offer superior flavor and texture compared to their modern counterparts (Nash, 2023).

Ancestral wheats contribute to agricultural biodiversity and are essential for preserving the genetic resources needed to breed future wheat varieties with improved resilience to climate change. Their cultivation supports sustainable farming practices by reducing dependency on chemical inputs and promoting soil health through crop rotation (Aloisi et al., 2023).

### *Ancestral Seed Studies in Türkiye*

Studies on ancestral seeds and wheat varieties in Türkiye are of great importance for the protection of agricultural heritage and the promotion of sustainable agricultural practices. Ancestral seeds are seeds that are adapted to local conditions, are not genetically modified and are grown using traditional methods (Altıkat et al., 2018). These seeds differ from modern hybrid seeds, which are generally used in industrial agriculture because they can be grown in natural cycles without the need for chemical intervention. Türkiye is a country with great agricultural biodiversity. Due to different climatic and soil conditions, there are many local seed varieties, especially in staple crops such as wheat. However, the widespread use of modern agricultural techniques has reduced the use of these local seeds over time, so the protection of traditional seeds and local varieties has become an important agenda (Latief & Zhang, 2023). Wheat varieties in Türkiye, especially in rural areas, have evolved naturally over thousands of years and are best adapted to the conditions of the region (Araus et al., 2007; Aksoy et al,. 2017). The most common wheat varieties grown in Türkiye are

Karakılçık wheat one of the best-known ancient varieties, has high nutritional value and low glycaemic index. Yellow wheat is mainly grown in Central Anatolia, this variety is resistant to dry conditions and is known for its hard structure. Siyez wheat, which has a history of around 10,000 years, is a wheat variety ground in stone mills (Çakır & Arıcı, 2023). It is grown mainly in the Kastamonu region. Kavılca wheat, which has a history of about 10,000 years, is grown mainly in the Ardahan region (Karagöz, 2014; Anatolia Foundation, 2024).

The following academic studies on Kavılca are worthy of note: in their study, In his master's thesis, Aydar (2022) sought to compare *Triticum dicoccum* wheat, the ancestral seed of the Kars region and also known as 'kavılca' wheat, with modern wheat Triticum aestivum in terms of quality criteria and health effects, to determine the potential of kavılca wheat as an ingredient in the food industry. In his master's thesis, Uralçin (2024) obtained biochar (a carbonaceous product) from 'kavılca' (*Triticum dicoccum Schrank*) shells by hydrothermal pyrolysis at temperatures of 130, 200, and 230°C. He then investigated the capacities of these biochars and activated carbon obtained from a suitable biochar. Consequently, a total of eight biochars were produced from the Kavılca raw material. Of these, six underwent activation, resulting in the production of activated carbon from the most efficient biochar. The objective of the study conducted by Mısır & Alp Baltakesmez (2024) was to ascertain the textural and sensory characteristics of pasta produced with varying proportions of kavılca flour. The findings of the study indicated that pastas produced with 50% and 60% kavılca flour were perceived as the most optimal in terms of texture and sensory attributes. In their study, Aslan Türk et al. (2024) compared the nutritional values and physical properties of crackers produced from Kavılca and other whole wheat flours. The study showed that Kavılca was superior to other varieties in terms of fibre ratio and protein values.

In recent years, various institutions and local administrations in Türkiye have initiated projects related to ancestral seeds. The General Directorate of Agricultural Enterprises plays an important role in the conservation of ancestral seeds in Türkiye. It carries out studies on the conservation of local seeds in gene banks and their reintroduction into agriculture (Pandır & Erol, 2024). Within the scope of Kavılca activities, an organisation was organised in Ardahan under the name of 'Promotion Event of Ardahan Kavılca, the Ancestor of Wheat' (2024). In this event, it was aimed to increase the awareness of Kavılca wheat at local and international level. In the event, dishes made from Kavılca wheat were introduced and the gastronomic potential of the product was emphasised (KÜSİP, 2024). Many municipalities organise local seed exchange festivals to bring ancestral seeds to farmers and initiate agricultural projects based on ancestral seeds. In 2020, the Ministry of Agriculture launched the "Local Seed Production and Research Programme" to protect and disseminate local seeds (Republic of Türkiye Ministry of Agriculture and Forestry, 2020).

**Kavılca Wheat**

Ardahan Kavılca wheat is a variety of the ancient seed emmer wheat (Triticum dicoccum L.) (2n=28, AABB). It is characterised by a hard husk that makes it more resistant than modern wheat varieties. The robust outer layer is beneficial in safeguarding the grain from environmental stress, pests and diseases (Lachman et al., 2012). The gluten content of Kavılca wheat is relatively low in comparison to that of modern wheat, which makes it a suitable option for consumers with gluten sensitivity. However, despite this low gluten content, it is not recommended for individuals with celiac disease. The grain's high fibre and essential amino acid content make it a nutritionally dense option for a variety of food products, including bread, pasta and bulgur. Kavılca wheat is distinguished by its health-promoting attributes. It is a rich source of dietary fibre, which plays an important role in digestive processes and the maintenance of a healthy gut microbiome (Özgören & Işık, 2023). The low gluten content of this variety makes it a suitable alternative for those seeking to reduce gluten intake. Additionally, the grain is rich in protein, iron, and antioxidants, providing essential nutrients that support overall health. It has been demonstrated that the ingestion of ancient grains such as Kavılca can assist in maintaining stable blood sugar levels and reducing the likelihood of developing chronic diseases such as diabetes and cardiovascular disease (Figure 1).

Ardahan kavılca wheat displays remarkable resilience to adverse climatic conditions, particularly the cold and brief growing seasons typical of the Ardahan region of northeastern Türkiye (Figure 2). The wheat cultivated in the Ardahan province has a long history. The geographical border plays an important role in the region's agricultural economy. Consequently, all stages of production for wheat, which is renowned for its geographical origin, are conducted at the geographical border. (Türk Patent ve Marka Kurumu, 2024).

Figure 1. Ardahan Kavılca Spike of Ancestral Wheat

Figure 2. Production Region Boundaries of Kavılca Wheat

Table 2. Kavılca Wheat from Field to Table Prepared With Traditional Methods

| Steps | Description |
|---|---|
| 1. Seed Selection | Farmers begin by choosing ancestral Kavılca wheat seeds, which have been passed down through generations. These seeds are valued for their resilience, biodiversity, and historical heritage. |
| 2. Soil Preparation | The soil is prepared by local farmers in Ardahan, often without the use of synthetic fertilizers or pesticides, due to Kavılca's natural resistance to harsh climatic conditions. |
| 3. Planting | In early spring, the seeds are planted. The wheat grows best in the cold, high-altitude climates of northeastern Türkiye, where it has adapted over millennia. |
| 4. Cultivation | Kavılca wheat requires minimal irrigation and grows under rain-fed conditions. It is a hardy crop that can thrive in the short growing season typical of the Ardahan region. |
| 5. Harvesting | Harvest occurs in late summer or early autumn. The grain is manually harvested, often using traditional methods passed down through generations. |
| 6. Threshing & Processing | The wheat is threshed to remove its husk, which is harder compared to modern wheat varieties. This step often involves milling the wheat into flour, which can be used for various recipes. |
| 7. Flour Milling | Once processed, the wheat is stone-ground into flour, which preserves its nutrient content, including high levels of fiber, protein, and vitamins. |
| 8. Local Market Distribution | The flour is sold in local markets or directly from farms. With its growing popularity, Kavılca flour has started to gain attention beyond the region, especially among artisanal bakers. |
| 9. Gastronomy Use | Kavılca flour is utilized in various traditional Turkish dishes, such as bread, pilaf, and bulgur. Its earthy flavor and dense texture make it popular in both home kitchens and restaurants. |
| 10. Consumption | On the table, Kavılca wheat products are enjoyed not only for their flavor but also for their health benefits, reflecting a cultural connection to ancestral eating habits. |

*Source: created by the corresponding author.

This wheat variety is an exemplar of crop adaptability, and as such, represents a crucial component of sustainable agricultural practices. The capacity of this wheat variety to flourish in such challenging conditions, without the need for excessive irrigation or chemical inputs, underscores its potential contribution to climate-resilient agriculture (Ilhan, 2021). Farmers who cultivate Kavılca wheat enjoy the benefits of sustainability, as the crop necessitates a reduced reliance on pesticides and herbicides, following environmentally conscious agricultural methodologies (Table 2). Kavılca wheat is a notable ingredient in the culinary arts, distinguished by its rich, earthy flavor and dense texture (Gallop, 2022). These qualities render it a highly sought-after ingredient in traditional Turkish dishes, including bulgur, pilaf and bread. Additionally, it aligns with the broader global movement of reintroducing these grains into contemporary gastronomy, where chefs and home cooks are combining traditional ingredients for their distinctive flavors and nutritional benefits. Wheat's low gluten content and distinctive flavor profile permit bakers to produce hearty and palatable baked goods. Due to its low gluten content, the flour produced from Kavılca wheat is subjected to a series of blending processes during the manufacturing process (Mısır & Alp Baltakesmez, 2024). This is done to optimise the utilisation of the product in terms of its flavor profile and texture. From a socio-cultural perspective, Ardahan Kavılca wheat plays an invaluable role in the preservation of local traditions and agricultural heritage (Mısır & Alp Baltakesmez, 2024). Kavılca wheat, which has been cultivated by the same families for generations, is not only a fundamental component of the regional diet in Ardahan but also a symbol of the region's cultural identity. The recent resurgence of interest in ancient seeds such as Kavılca wheat is part of a broader global movement to reconnect with traditional foodways. This movement underscores the significance of

biodiversity conservation and the maintenance of food sovereignty, particularly in rural communities where agriculture is a central aspect of the way of life (Demeulenaere, 2014). The designation of Ardahan Kavılca wheat as a Geographical Indication (GI) serves to reinforce its socio-cultural significance by safeguarding it from commercial exploitation and ensuring that its production remains closely aligned with the region. The legal protection afforded by GI status helps to maintain local agricultural practices and strengthens the economic potential of small farmers, ensuring that these communities can continue to benefit from traditional farming methods (Rangnekar, 2004). This multifaceted perspective on Ardahan Kavılca wheat underscores its significance not only as a crop but also as a pivotal element in diverse domains, including health, agriculture, gastronomy, and cultural heritage (Berkes et al., 2000). The resilience of this crop to environmental challenges, coupled with its nutritional benefits and cultural significance, renders it an invaluable asset in addressing the modern agricultural and food issues that we face.

## Conclusion

Ardahan Kavılca wheat, a variety of the ancient emmer wheat (*Triticum dicoccum L.*), is an invaluable crop with a rich history and cultural heritage. This wheat is characterised by a hard husk, low gluten content and high nutritional value, including increased levels of fibre, protein, iron and antioxidants. Cultivated in the cold, high-altitude Ardahan region of north-eastern Türkiye, Kavılca wheat shows remarkable resilience to harsh climatic conditions and requires minimal irrigation and chemical inputs. These attributes position it as a model for sustainable and climate-resilient agriculture.

In addition to its agricultural benefits, Kavılca wheat has significant culinary and health benefits. It is used in traditional Turkish dishes such as bread, bulgur and pilaf, and is valued for its earthy flavor and dense texture. Its low gluten content makes it suitable for those reducing gluten in their diet, although it is not recommended for those with celiac disease. In addition, its consumption supports gut health, stabilises blood sugar levels and may reduce the risk of chronic diseases such as diabetes and cardiovascular disease.

Kavılca wheat's Geographical Indication (GI) status highlights its socio-cultural importance, protects it from commercial exploitation and supports traditional farming practices. This recognition promotes food sovereignty, preserves biodiversity and sustains the livelihoods of local farmers. The resurgence of interest in ancient grains underscores their relevance in addressing modern agricultural, health and cultural challenges. Ardahan Kavılca wheat is a testament to the enduring value of traditional crops in modern food systems. Promoting and preserving Kavılca wheat to a wider audience is critical not only for regional development but also for the conservation of agricultural biodiversity and environmental sustainability. Increasing academic research on Kavılca wheat will provide a better understanding of the agricultural, nutritional and industrial potential of this crop. Effective promotion of the registration of Kavılca wheat as a geographical indication at national and international levels can increase the market value of the product. Promotion of Kavılca wheat products (e.g. pasta, bread and flour) in different market segments can contribute to the region's economic growth. Training farmers to cultivate Kavılca wheat could encourage local communities to adopt the crop. Events highlighting the gastronomic potential of Kavılca wheat can support regional tourism. Ardahan Kavılca wheat should be seen not only as a heritage of the past but also as an asset that can play an important role in the sustainable agricultural practices and healthy food strategies of the future. Multifaceted approaches to the conservation and promotion of wheat can contribute to the region's development and environmental sustainability.

## Declaration

The authors declare no conflict of interest in this paper.

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# Agricultural Utilization of Biochar: A Review of Production Technologies

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ABSTRACT

Biochar production has gained significant attention lately due to its potential to sequester carbon, improve soil fertility and mitigate climate change. Various production technologies have been developed to convert biomass into biochar, each with its unique characteristics and advantages. This review provides a comprehensive overview of the current biochar production technologies aiming to synthesize existing knowledge and identify research gaps with a focus on their potential to contribute to the United Nations Sustainable Development Goals (SDGs) 2, 12, 13 and 15. The scope of this review encompasses various biochar production techniques including slow pyrolysis, fast pyrolysis, gasification and torrefaction. The effects of production conditions such as temperature, residence time, and feedstock types on biochar properties and yields are discussed. The prospects of using biochar in the agricultural system were discussed. Additionally, challenges and opportunities associated to scaling up biochar production technologies are highlighted. The findings of this review have implications for the development of sustainable biochar production practices and environmental management strategies.

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## Introduction

Surging global demand for sustainable agricultural practices, climate change mitigation and environmental management has led to a surge of interest in biochar production technologies (Roberts et al. 2023).

Biochar, a carbon-rich material produced from the thermal decomposition of biomass, has been recognized for its potential to sequester carbon, improve soil fertility and support sustainable agriculture (Sohi et al., 2010). Biochar has a long history dating back to ancient civilization where it was used to improve soil fertility and support agriculture (Saleem et al., 2023). The modern concept of biochar production, however has evolved significantly over the past two decades, driven by advances in thermal conversion technologies and growing concerns about climate change (Khan et al., 2021).

Today, biochar production involves various technologies including slow pyrolysis, fast pyrolysis, hydrothermal carbonization and gasification (Kumar et al., 2020). The evaluation of biochar processes has shifted from a primary focus on charcoal production to a more holistic approach, considering the interplay between biochar properties, soil biota and ecosystem services (Sohi et al., 2010). Recent advances in biochar production technologies have enabled the production of high-quality biochars with tailored properties, optimized for specific applications (Vaghela and Kapupara, 2024).

This review synthesizes the current state of knowledge on biochar production technologies, highlighting their principles, advantages and limitations. By examining the evolution of biochar production and the impact of modern technologies, this review aims to provide a comprehensive understanding of the field and its potential to contribute to the achievement of the United Nations Sustainable Development Goals (SDGs) 2, 12, 13 and 15 by supporting sustainable agriculture, climate change mitigation and environmental management.

Torrefaction, slow pyrolysis, gasification and fast pyrolysis are the thermochemical conversion processes primarily utilized for biochar production under different operational factors (Wang et al., 2020). The chemical components and the physical state of the biomass are irreversibly changed to form biochar in the absence or oxygen-limited at specified temperatures and pressure conditions. The biomass chemical constituents go through the cross-linking process, decomposition and depolymerization transforming feedstocks/biomass into a carbon-rich solid product known as biochar and other byproducts including bio-oil or tar, combustible gases and additional compounds depending on the reaction

conditions (Chakhtouna et al., 2022) Biochar has the propensity to add to the economic viability of developing cellulosic bioenergy production systems, as shown by its multiple advantages (Ren et al., 2022; Qin et al., 2022; Ezz et al., 2021). In contrast, biochar addition can sustainably store carbon (C) in the soil and decrease the net emissions of greenhouse gases (Han et al., 2022; Shakoor et al., 2021), soil physicochemical and biological characteristics (Khan et al., 2022; Rashid et al., 2021), reduces sediments, pollutants and nutrient loss (Yuan et al., 2022; Wu et al., 2021). Biochar addition to the soil do not store only C but also rebuild critical organic matters lost during the removal of biomass from the agricultural system for the production of energy.

Therefore, biochar has the potential to boost agricultural productivity and environmentally sustain the generation of a biomass system. Biochar can also boost bioenergy sustainability economically in corporations by balancing feedstock costs with revenues generated from selling biochar. Though, biochar influences the soil, agronomic and environmental factors have not been examined thoroughly. Although biochar can generate some income and boost agricultural and environmental sustainability, the bioenergy and food production sectors will remain unwilling to invest in biochar unless the exact implications on soil characteristics and crop yields are demonstrated.

To fully generate biochar on a commercial base, specific advantages to soil qualities and crop yields must be shown and these benefits must be linked to biochar characteristics, its utilization and financial potential. The most critical component to achieving this possibility is comprehending how biochar is produced and how the production process influences its functionality. Their advantages to crop productivity, soil and the environment will be compromised unless they are repetitive and reliable.

Therefore, the purpose of this review was to evaluate biochar production technologies and correlate the methods to biochar yield and characteristics, and also link biochar characteristics with their advantages to the agricultural systems. This review evaluated biochar production technologies such as gasification, slow pyrolysis, torrefaction and fast pyrolysis. The utilization of biochar in the agricultural system and its influences on soil health and plant development were summarised. The drawbacks of existing biochar research were discussed.

## Biochar Production Technologies

Biochar has distinct physicochemical characteristics that rely on the thermochemical working conditions and the inherent character of biomass. Numerous modules and pyrolyzers have been created for biomass production to increase the quantity and product quality. The concepts of these pyrolyzers are analogous, however, they vary in oxygenation, rate of heating, and final temperature application, which could influence the quality and quantity of final products. The different categories of thermochemical processes used in biochar production include slow pyrolysis, fast pyrolysis, torrefaction and gasification (Figure 1). The quantity and biochar quality generated in these production processes vary greatly depending on the varying reaction settings, notably the amount of oxygen supplied.

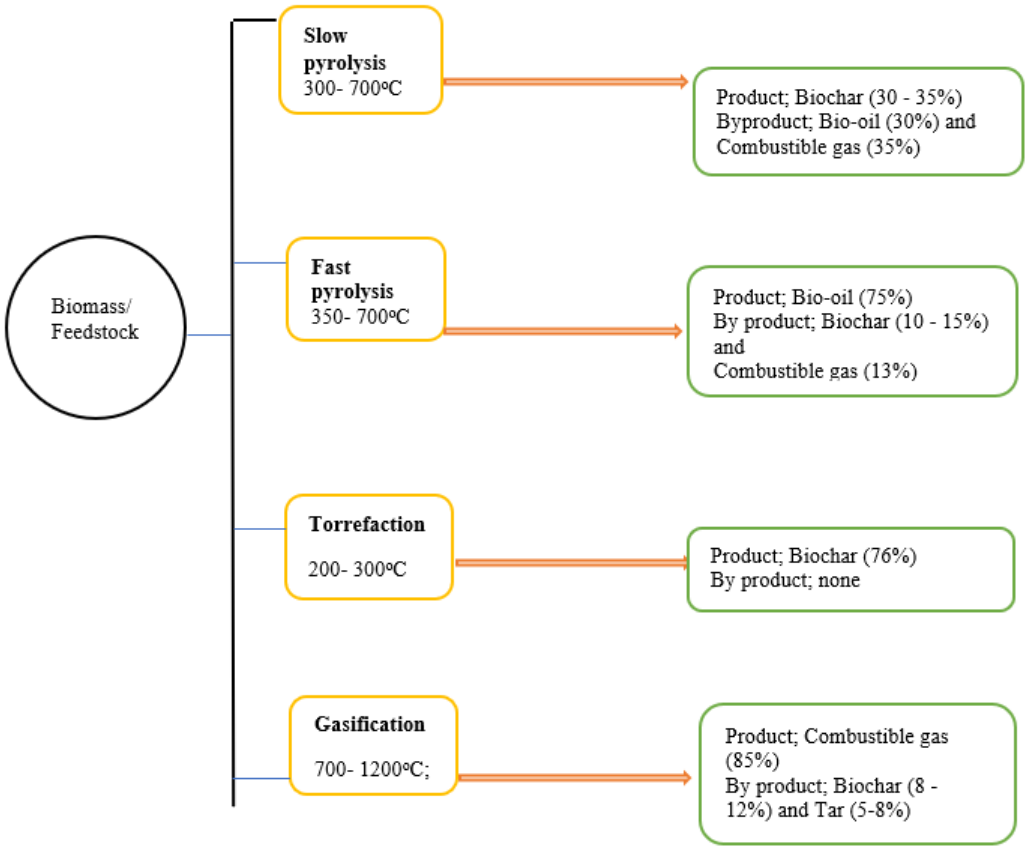


Figure 1. Biochar production technologies (modified from Wang et al., 2020).

***Slow Pyrolysis Process***

Biochar can be generated from different organic and non-organic materials including agricultural residue, forest residue, algal biomass and industrial waste has been widely used as the source of biochar via slow pyrolysis (Aishwarya et al., 2022; Wang et al., 2020). In this process, biomass is decomposed at 350–500 °C to provide adequate residence time for biomass pyrolysis vapour and surges its subsidiary cracking stages. The quality of biochar is mostly associated with its pH level, nutrient content, carbon content, specific surface area and porosity but is closely linked to its carbon content (Shackley et al., 2014; Yao et al., 2018). The higher quality biochar consists of high carbon content obtained from the relatively high pyrolysis temperature, longer residence time and lower heating rate (Table 1). For instance, Mousa et al. (2016) reported that wood-derived biochar at 750–900 °C and > 30 min residence time is highly preferred. The carbon content of redcedar heart wood biochar generated at 500 °C and 6 °C/min heating rate was 88.88% however the increased heating value of biochar attained 32.95 MJ/kg indicating that biochar quality was better (Yang et al., 2016).

In slow pyrolysis, a higher pyrolysis temperature is critical to boosting biochar quality so that more volatiles are extracted from biochar, thereby increasing its carbon content. Furthermore, decreasing the heating rate encourages better heating conduction, which favours the carbon deposition reaction and consequently increases the production of biochar (Veses et al., 2015). The particle size, the existence of a catalyst, and the pyrolysis atmosphere are the other operating factors of biomass slow pyrolysis that directly influence the quality and quantity of biomass (Veses et al., 2015). Furthermore, feedstock has a significant impact on the quantity and quality of biochar. Using forest plants as the precursor, at 500 °C, 60 min residence time and 10 °C/min heating rate biochar yield is around 30% (Solar et al., 2016). In comparison, biochar derived from lignin contained 45.69 % biochar yield emphasising that the lignin content is an essential factor for biochar quantity (Farrokh et al., 2018). Studies have illustrated that biochar yield depends on the lignin and ash contents of feedstock (Wan et al., 2020; Sun et al., 2017; Lee et al., 2013).

In addition to biochar production, the slow pyrolysis process at a relatively high temperature could also produce bio-oil as a product or a byproduct while the pyrolysis vapours released from the feedstock consist of condensable and non-condensable components. Wood vinegar also known as condensable components could be extracted as bio-oil owing to the existence of acetic acid. The bio-oil collected mostly comprises acids, phenols, esters and ketones (Qing et al., 2022; Setter et al., 2020). The bio-oil consists of different chemical contents that could be collected and utilized for its value-added bioproducts (Mora et al., 2022; Norrrahim et al., 2022; Vigneshwar et al., 2022). Generally, the earth or metal kilns are employed as fixed bed pyrolysis reactors for the production of biochar during feedstocks are loaded and heated for several hours or days in an airtight kiln (Pelaez-Samaniego et al., 2022). A kiln is an oven type often constructed from clay or metal that generates enough heat in the slow pyrolysis process. The solid reactants in these fixed-bed pyrolysis reactors may not consistently be heated and the exchange of gas-solid in a fixed-bed reactor is weak. In industries, auger pyrolysis reactors are widely utilized owing to their not demanding efforts in construction and operations (Brown et al., 2021).

Furthermore, by controlling the screw's spinning speed, the residence time of biomass in the auger pyrolysis reactor is regulated and biochar is continuously produced (Pal et al., 2022). Garcia-Perez et al. (2007) reported that the batch and continuous auger reactors in slow pyrolysis had the same biochar yield of 30 and 31 wt%. Thus, indicating that both pyrolysis rectors had similar reactions resulting in the biochar production. In addition to the auger pyrolysis reactor, the continuous pyrolysis reactor comprising the bubbling fluidized bed has been investigated for biomass slow pyrolysis. Patel et al. (2019) used the slow pyrolysis process to generate biosolid in a bubbling fluidized bed reactor at 60 min of residence time. Although pyrolysis is an endothermic process, the vapour produced during the slow pyrolysis process frequently is uncondensed yet burns immediately to supply heat for the operating process, regardless of the reactor type utilized.

Table 1. Biochar physicochemical characteristics and yield produced from slow pyrolysis

| Feedstock | Temperature (°C) | Residence time (min) | Heating rate (°C/min) | Yield (%) | Biochar composition (%) | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | C | H | N | S | |
| CH | 350 | 30 | 0.5 | 39.82 | 69.96 | 3.63 | 3.58 | 0.24 | Setter et al., 2020 |
| CM | 300 | 120 | 10 | 58.00 | 51.30 | 4.52 | 1.70 | - | Yu et al. (2017) |
| PW | 350 | 30 | 15 | 34.70 | 72.36 | 4.7 | - | - | Ronsse et al., 2013 |
| PS | 500 | 60 | 10 | 35.5 | 60.12 | 9.21 | 0.42 | 0.92 | Qurenshi et al., 2019 |
| WNS | 500 | 60 | 15 | ~ 30.0 | 77.97 | 3.22 | 1.13 | - | Gupta et al., 2019 |
| CST | 530 | several | 30 | ~ 24.0 | 92.83 | 1.49 | 0.84 | 0.06 | Delgado et al., 2013 |
| A | 500 | 60 | 10 | ~ 32.0 | 77.97 | 3.22 | 1.13 | - | Gupta et al., 2020 |
| WS | 475 | 180 | 8 | - | 69.90 | 2.50 | - | - | Heikkinen et al., 2019 |
| L | 500 | 480 | 5 | 45.69 | 85.90 | 3.56 | 1.23 | 0.12 | Furrokh et al., 2018 |
| RW | 500 | 20 | 10 | 24.25 | 87.17 | 1.23 | 0.40 | - | Halim et al., 2016 |
| NPSC | 450 | 60 | 20 | 38.30 | 52.39 | 2.57 | 2.23 | 0.12 | Dhanavath et al., 2019 |
| RHW | 500 | 30 | 6 | 21.00 | 88.88 | 2.6 | 0.35 | 0.4 | Yang et al., 2016 |
| HC | 500 | 60 | 10 | 23.3 | 85.79 | 3.89 | 0.23 | - | Yu et al., 2019 |
| RSW | 500 | 30 | 6 | 30.90 | 85.80 | 2.40 | 0.35 | 0.35 | Yang et al., 2016 |

Notes: CH, coffee husk; CM, cow manure; PW, pine wood; PS, palm shell; WNS, walnut shell; CST, corn straw; A, algae; WS, wheat straw; L, lignin; RW, rubber wood; NPSC; neem press seed cake; RHW, redcedar heart wood; HC, hinoki cypress; RSW, redcedar sap wood.

Table 2. Biochar physicochemical characteristics produced from fast pyrolysis using different pyrolyzers

| Feedstock | Pyrolyzer | Temperature (°C) | Yield (%) | Biochar composition | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | C | H | N (%) | S | O | |
| RH | CSB | 500 | 26.40 | 45.20 | 1.5 | 0.40 | - | - | Alvarez et al., 2015 |
| CS | FBR | 550 | - | 73.39 | 4.34 | 1.19 | 0.92 | 20.16 | Wang et al., 2014 |
| WS | ATTSR | 550 | 26.56 | 56.00 | 2.30 | 1.00 | - | - | Funke et al., 2019 |
| PSD | FBR | 500 | - | 71.63 | 4.16 | 3.14 | 0.25 | 20.82 | Peng et al., 2012 |
| CC | BFB | 550 | 19.40 | 73.60 | 3.15 | 0.87 | 0.02 | 22.36 | Mullen et al., 2010 |
| YP | FBR | 550 | 6.21 | 77.30 | 3.31 | 0.76 | - | 18.63 | Hwang et al., 2015 |
| DF | BFB | 500 | 12.23 | 76.86 | 2.57 | 0.36 | 0.12 | 20.09 | Wu et al., 2016 |
| LN | LSP | 450 | 15.87 | 69.59 | 2.93 | - | - | 11.61 | Ghysels et al., 2019 |
| SS | FBR | 550 | 26.82 | 66.03 | 2.38 | 0.52 | - | 31.07 | Yin et al., 2013 |
| B | HCC | 550 | 25.45 | 80.77 | 4.40 | - | - | 14.83 | Kajita et al., 2010 |
| BM | BFB | 400 | 54.12 | 33.42 | 3.12 | 2.63 | - | 60.83 | Choi et al., 2017 |

Note: RH, rice husk; CS, corn stalk; WS, wheat straw; PSD, pine sawdust; CC, corn cob; YP, yellow poplar; DF, douglass fit; LN, lvory nut; SS, sweet sorghum; B, bamboo; BM, brown macroalga, CSB, cornical spouted bed; FBR, fluidised bed reactor; ATTSR, air tight twin screw reactor; BFB, bubbling fluidised bed; LSP, lab-scale pyrolyser; HCC, horizontal crew conveyor

***Fast pyrolysis process***

In contrast to the slow pyrolysis process, fast pyrolysis is generally produced in batch reactors and occurs in a continuous system. This process incorporates 1000 °C/min heating rates to reach a pyrolysis temperature of about 500 °C with < 2s residence time (Papari et al., 2021). The biomass particles swiftly decompose in the fast pyrolysis process, producing pyrolysis vapours and 10-15 wt% biochar yields. The condensable substance is extinguished and gathered in the pyrolysis vapours and a dark-brown liquid is collected known as bio-oil and the byproduct is biochar. The higher pyrolysis temperature conditions decrease biochar yields by encouraging the emission of gaseous volatile matters, whereas higher heating rates have an analogous effect. The feedstocks are swiftly heated and the pyrolysis vapours generated are speedily transferred from the pyrolysis reactor during higher heating rates. In high-temperature regions the pyrolysis vapours consist of shorter residence time, thereby decreasing the deposition amount of carbon content. For example, Angin (2013) reported that increasing the heating rate from 10-50 °C/min declined safflower seed biochar yield by 3–8%.

Chen et al. (2016) also reported that by surging the heating rate from 10- 50°C/min at 400 °C the poplar wood biochar yield reduced from 34.83 to 31.95 wt%. Similarly, studies by Aguado et al. (2000) showed that biochar yield declined from 38.8-26.4% by surging the heating rate from 5 to 40 °C/min. Also, increasing the pressure could boost biochar yield by extending the vapour residence time inside the feedstock particles accelerating the char-forming processes (Thengan et al., 2022). For instance, high-pressure reactors can lead to 41-62% of biochar yield (Antal et al., 1996**)**. Wang et al. (2013) observed that pyrolyzing pine sawdust in a closure fixed bed reactor surged biochar yield from 24.9 wt% to 27.5 wt%. Furthermore, (Table 2) summarises the various fast pyrolysis factors and pyrolyzer designs on the biochar yield and biochar quality varying extensively depending on the feedstock utilized.

The emission of volatile substances from the biomass particles at higher pyrolysis temperatures increases biochar's carbon content and specific surface area. Zhao et al. (2018) reported that rapeseed stem-derived biochar from 200 to 700 °C increased in specific surface area from 1 to 45 $m^2$ /g. Furthermore, Peng et al. (2012) reported that pinewood-derived biochar from 550 to 750 °C increased in carbon content from 70.68% to 78.75%. In fast pyrolysis operations, the heating rate consists of complex implications on biochar quality. The explanation by Onay (2007) shows that biochar generated at a higher heating rate exhibits higher carbon levels and specific surface area than biochar generated at a low heating rate owing to the differences in heating rates resulting in changes in the devolatilization rate and thereby modifying biochar structure. Similarly, Chen et al. (2016) discovered that boosting the heating rate surged biochar carbon levels whereas the BET surface area of biochar initially increased and gradually decreased. Conversely, some studies have shown that high heating rates decrease biochar-specific surface area and pore volume due to the swift depolymerization at the biochar surface (Anand et al., 2022; Mohan et al., 2014; Toledano et al., 2014). These findings suggest that high heating rates boost biochar carbon levels yet have no influence on the BET-specific surface area of biochar.

Many pyrolysis reactors such as the auger or screw reactors, bubbling fluidized bed, rotary cone, circulating fluidized bed and ablative reactors have been proven useful for producing higher bio-oil yield in a fast pyrolysis process (Kapoor et al., 2022; Raza et al., 2021; Qureshi et al., 2018). To avert pyrolysis vapour cracking reactions, biochar should be segregated from the pyrolysis vapours instantaneously. Bridgwater (2012) reported that the fluidized bed reactor, rotary cone, or ablative reactors produce about 15 wt% byproduct biochar during pyrolysis however, Raclavska et al. (2015) observed that the auger/screw reactor can surge biochar yield up to 25 wt%.

***Gasification Process***

Gasification typically occurs at temperatures ranging from 700 to 1000 °C, during which biomass is incompletely burned with different gasifying agents including air, pure oxygen, or steam and oxygen to form a gas product. Han and Kim (2008) reported that in the biomass gasification process, there is a need to concentrate on how to boost the quality and quantity of syngas by minimizing pollutants such as nitrogen oxides, tar, sulfur dioxide and fly ash. Shackley et al. (2014) reported that the carbon content of biochar generated by biomass gasification is directly linked to its quality.

Table 3. Biochar physicochemical characteristics produced from gasification using different pyrolyzers

| Feedstock | Pyrolyser | Temperature (°C) | Composition of biochar (%) | | | | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | C | H | N | S | O | |
| WC | DSG | 950 | 79.67 | 2.72 | 0.27 | - | 17.34 | Benedetti et al., 2018 |
| CCS | FBG | 850 | 88.76 | 1.45 | 0.41 | - | 9.38 | Millan et al., 2019 |
| RS | DFBR | 800 | 64.76 | 0.67 | 1.98 | 0.11 | 32.48 | Xu et al., 2019 |
| DMG | LSDT | 1200 | 56.57 | 3.45 | 1.55 | 0.37 | 38.06 | Hernendez et al., 2020 |
| WP | NPTR | 750 | 84.43 | 1.90 | 0.18 | - | 13.49 | Muvhiiwa et al., 2019 |
| BB | BDFB | 800 | 77.54 | 0.67 | - | - | 21.79 | Morin et al., 2016 |
| P | RCC | 750 | 86.34 | 1.09 | 0.35 | - | 12.22 | Patuzzi et al., 2016 |
| JC | HQT | 950 | 95.45 | 0.76 | 0.53 | - | 3.26 | Bai et al., 2014 |
| PI | FBR | 850 | 84.36 | 2.81 | 0.45 | 0.18 | 12.20 | Huang et al., 2013 |

Notes: WC, wood chip; CCS, coconut shells; RS, raw straw; DMG, Dealcoholized marc of grape; WP, wood pellet; BB, beech bark; P, pellet; JC, Japanese cedar; PI, pine; DSG, dual stage gasifier; FBG, Fluidized bed gasifier; DFBR, dual fixed bed reactor; LSDT, lab-scale drop tube; NPTR, nitrogen plasma torch reactor; BDFB, batch dense fluidized bed; RCC, rising co-current; Horizontal quartz tube; FBR, fixed bed reactor

The feedstock properties, equivalence ratio (ER), pressure and gasifying agent influence biochar quality. Benedetti et al. (2018) stated that the ER value is the most critical parameter that influences the gasification process, and based on the biomass physicochemical characteristics the optimum value is from 0.25–0.28. Mostly, surging ER increases gasification temperature affecting biochar quality (Table 3). In recent times biochar yield and quality as a function of ER have been widely studied. Yao et al. (2018) found that surging ER from 0.1-0.6 reduced biochar yield from 0.22 to 0.14 kg/kg biomass and slightly decreased the carbon content of biochar generated from 88.17% - 71.16%.

A report from Muvhiiwa et al. (2019) showed that biochar produced at 700 °C reduced its carbon content from 89% to 80% and at 900 °C carbon content of biochar reduced from 93% to 86% after increasing the oxygen flow rate from 0.15 to 0.6 kg/h. These findings illustrate that in the gasification processes increasing ER decreases biochar carbon content and biochar yield. The more oxygen is added to the gasifier the higher the ER value resulting in both positive and negative influences on biochar quality. From one point of view, the heterogeneous reactions are enhanced to transform extra carbon from the solid state to gaseous species thereby promoting the development of micropores and surging biochar-specific surface area (Zhang et al., 2021). From another point of view, additional oxygen molecules during the gasification process could result in biochar ablation thus surging its ash content and decreasing its quantity and mechanical strength.

The fluidized bed gasifier also consists of the bubbling fluidized bed gasifier and the circulating fluidized bed gasifier have all been built. Recently, Thomson et al. (2020) reviewed the development of these biomass gasifiers and their performances. The small-scale gasifiers employ air as the gasifying agent and are generally autothermal and atmospheric. The different gasifier designs slightly influence biochar characteristics and quantity as compared to ER. Many studies revealed that the biochar carbon content primarily depends on the ER rather than the gasifier types (Lu et al., 2021; Mishra and Upadhyay, 2021; Hernández et al., 2020). James et al. (2018) constructed the top-lit updraft gasifier to produce 39.3% of biochar yield from rice hulls. Furthermore, Adeniyi et al. (2019) constructed a top-lit fixed-bed updraft gasifier to produce 14.29 wt% biochar yield from elephant grass, and the biochar-specific surface area was 475 m /g.

***Torrefaction Process***

Torrefaction is another emerging thermal-chemical process primarily employed for the production of char products that could be utilized as a soil amendment and/or fuel (Abhishek et al., 2022). Generally, the torrefaction process involves heating biomass feedstock at temperatures between 200 and 300 °C in an inert atmosphere < 50 °C/min and between 20–120 min residence time (Bolan et al., 2022). About 30% of some highly reactive volatile chemicals are converted into torrefied vapour during this process (Afailal et al., 2023; Isemin et al., 2022; Osman et al., 2021). In this process, the target product is the dark brown solid fuel comprising 90% initial energy content and about 1.3% of the torrefied biochar and energy densification could be accomplished (Saha et al., 2022). The torrefied biochar could have an energy density comparable to coal (22–23 MJ/kg) for heating and the production of power (Lin et al., 2021). Torrefaction typically requires the burning of volatile substances in a gas combustor to provide the necessary energy.

The high temperatures and extended residence time in the torrefaction process are necessary for the torrefaction process to produce torrefied biochar with a high energy density; however, these factors also reduce the torrefied biochar quality and energy yield. A report by Niu et al. (2019) illustrates that maintaining the solid yield between 60–80% could represent the optimum torrefaction condition for biomass to produce biochar with a moderately high heating value, energy yield and mass-energy density. Szwaja et al. (2019) stated that the physicochemical characteristics of biomass comprising ash content, moisture content and higher heating value influence torrefied biochar quality. Niu et al. (2019) explained that moisture content is the most critical variable in the torrefaction process as it mainly controls the energy input.

Biomass feedstock is widely recognized constituting lignin, cellulose and hemicellulose. Several studies have been conducted on the torrefaction of these three essential components to determine the critical parameter for torrefied biochar yield. Chen et al. (2019) reported that the biochar yield from hemicellulose torrefaction recorded the lowest among the three essential components. Wang et al. (2018) reported that surging torrefaction temperature and residence time increases the lignin content and decreases hemicellulose and cellulose contents in the torrefied

biochar. Though biomass residence time is critical for torrefied biochar quality, the torrefaction temperature has a greater effect than the duration (Kai et al., 2019). Increasing torrefaction temperatures for different feedstock types led to higher carbon content and lower hydrogen content of the biochar product (Table 4). The explanation by Pala et al. (2014) illustrates that the primary degradation reactions such as dehydration and decarboxylation significantly contribute to mass loss in the torrefaction process. Moreover, many studies have been conducted on biomass torrefaction processes using different agents such as air or $N_2$. Brachi et al. (2019) reported that the torrefied biochar mass and energy yields in an oxidative torrefaction are low compared to non-oxidative treatments.

In comparison, the torrefied biochar consists of reduced moisture content and volatile content owing to the earlier biomass feedstock only undergoing mild pyrolysis at 200 °C for 20 min. The feedstock may quickly be dried nor yet exposed to different chemical processes at a low torrefaction temperature. Nonetheless, due to its benefits, torrefied biochar continues to receive a lot of interest. For example, the bulk of the moisture in biomass feedstock may be extracted, reducing transportation costs and increasing feedstock storage duration. The energy density of the torrefied biomass is increased by decomposing the reactive hemicellulose component from the feedstock (van der Stelt et al., 2011). The torrefied biomass is easier to crush into fine powders to be utilized in pulverized coal-fired power plants than fresh feedstocks (Barskov et al., 2019).

## Biochar Applications In Agricultural Systems

### *The Effects of Biochar on Soil Physicochemical Properties*

The effects of biochar on soil physical characteristics have been widely studied, for example, the addition of biochar in the soil mixtures can enhance soil bulk density, porosity, packing and surge soil aeration and the net soil surface area (Chetri and Reddy, 2021; Munawar et al., 2021). Furthermore, biochar addition alters soil-water connections by enhancing water infiltration, water holding capacity, soil aggregate stability and soil-preparation workability (Haq et al., 2021; Abukari, 2019). Many studies have revealed that reducing bulk density and surging soil porosity may assist in transferring water, gases and heat in soils and enhance soil quality (Ahmad et al., 2022; Alkharabsheh et al., 2022; Almendro-Candel et al., 2018). The variation in soil physical properties could be ascribed to biochar's large surface area and low bulk density as a result of its extensive poor size dispersion (Leng et al., 2021; de Jesus Duarte et al., 2019).

Biochar application to soil improves soil structural quality and soil aggregation while also influencing soil chemical parameters. The addition of biochar to the soil could modify its pH (Abukari & Cobbinah, 2024). In light of the alkaline composition of many biochars, the positive influence is more noticeable in acidic soils (Palansooriya et al., 2019). Dai et al. (2017) reported that soil acidity is improved through (1) the alkalinity of biochar, cation release including K, Ca, Mg and Na are the primary parameters for the surge in pH; (2) mineral elements such as Ca, K, Mg, Na, and Si in feedstocks produce oxides or carbonates in the pyrolysis process reducing exchangeable acidity and surging pH by reacting with $H^+$ and monomeric Al species in acid soils; (3) functional group including –COO– and –O– significantly contributes to the alkalinity of biochar; (4) high pH buffering capacity owing to the higher cation exchange capacities (CECs) releases cation including K, Ca, Mg, and Na from biochar primarily increases pH. The application of biochar to soil can modify soil pH which results in a change in nutrient solubility thus modifying nutrient availability. Zahedifar and Moosavi (2020) reported that the addition of biochar surges soil pH leading to higher availability of primary and secondary nutrients such as K, P, Ca and Mg. Biochar addition also reduces Al toxicity in acidic soils due to the increased pH of biochar (Abukari et al., 2022; Das and Ghosh, 2020; Shetty and Prakash, 2020). Soil CEC is an important attribute of soil fertility. The addition of biochar enhances soil CEC.

Table 4. Biochar physicochemical characteristics and yield produced from torrefaction

| Feedstock | Temperature (°C) | Mass yield (%) | Energy Yield (%) | Biomass composition (%) | | Biochar Composition (%) | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | | C | H | C | H | |
| B | 210 | 95.34 | 97.36 | 46.12 | 6.11 | 48.54 | 6.08 | Ma et al., 2019 |
| CSV | 200 | 97.10 | 98.52 | - | - | 45.8 | 5.5 | Medic et al., 2012 |
| PC | 225 | 89.00 | 94.00 | 47.21 | 6.64 | 49.47 | 6.07 | Phanphanich and Mani, 2011 |
| PT | 230 | 82.00 | 91.00 | 52.09 | 5.79 | 59.00 | 5.49 | Krysanova et al., 2019 |
| OPP | 260 | 94.50 | 94.50 | 54.93 | 6.33 | 57.31 | 6.33 | Brachi et al., 2019 |
| SCB | 200 | 79.00 | 98.00 | 32.50 | 5.01 | 34.50 | 4.98 | Kanwal et al., 2019 |
| SCG | 200 | 97.00 | 98.07 | 52.99 | 7.29 | 53.94 | 7.28 | Zhang et al., 2018 |
| LR | 210 | 92.00 | 99.30 | 42.5 | 6.41 | 44.50 | 6.41 | Xin et al., 2018 |
| RSW | 200 | 94.35 | 98.52 | 42.57 | 5.84 | 45.06 | 5.46 | Kai et al. (2019) |
| SBK | 225 | 90.40 | 96.93 | 49.09 | 6.06 | 55.40 | 5.53 | Wang et al. (2017) |
| BCP | 230 | 86.00 | 90.50 | 48.78 | 6.27 | 50.06 | 6.09 | Wang et al. (2017) |
| EFB | 200 | 87.50 | 90.30 | 43.00 | 6.00 | 46.20 | 5.50 | Lam et al. (2019) |
| PP | 250 | 77.00 | 88.00 | 46.50 | 5.10 | 56.40 | 6.00 | Cardona et al. (2019) |
| MAR | 200 | 89.35 | 91.98 | 36.49 | 6.12 | 41.27 | 5.95 | Zhang et al. (2018) |

Notes: B, bamboo; CSV, corn stover; PC, pine chips; PT, peat; OPP, olive pomace pellets; SCB, sugar cane bagasse; SCG, Spent coffee grounds; LR, licorice residues; RSW, rice straw; SBK, spruce bark; BCP, biomass chips, EFB, empty fruits bunches; PP, plant parts; MAR, macroalga residues.

The increased CEC of biochar-amended soils could be due to the increased specific surface area of biochar, aromatic carbon oxidation and carboxyl groups development in the biochar, and superiority of negatively charged surface functional groups (Ji et al., 2022; Palansooriya et al., 2019). Increased soil CEC improves soil nutrient retention and enhances nutrient availability to plant roots (Abukari and Duwiejuah, 2019; Laird et al., 2010). Furthermore, as explained previously, cations such as K, Ca, Mg, and Na released from biochars owing to enhanced CEC are primary contributors to higher soil pH. El-Nagger et al. (2019) reported a positive correlation between the increase in soil CEC, application rate and biochar ash content following its addition.

***The Effects of Biochar on Soil Nutrition And Fertility***

Biochar can serve as a source or sink for nutrient availability following its addition to the soil (Bolan et al., 2022; Hossain et al., 2020) since biochar nutrients are derived from the feedstock types (Palansooriya et al., 2019). The addition of biochar into agricultural soil demonstrates to be a sustainable approach for the enhancement of nutrient cycling, facilitating the interaction of biochar and plant roots thereby influencing the development of roots and the general performance of plants (Bolan et al., 2022; Gujre et al., 2021; El-Naggar et al., 2019; Purakayastha et al., 2019). Similarly, when exogenous nutrients are loaded on biochar, it could be utilized as a slow-release fertilizer for releasing nutrients (Mahmoud et al., 2022; Yang et al., 2021). Besides the nutrients such as N, P, K, Ca, Mg, S, Fe, Mn, Cu, Zn, and Si produced from feedstock, macro and miro nutrients such as $Cu^{2+}$, $Fe^{2/3+}$, $Mn^{2+}$, $Zn^{2+}$ can also be absorbed. Owing to biochar's large surface area and porous microstructure, biochar-bound nutrients are gradually released (Tahery et al., 2022; Jia et al., 2021). The porous networks inside the biochar generate structural impediments such as physical wrapping or chemical sorption allowing nutrients with slow desorption to be absorbed by plants (Anwari et al., 2020; Yu et al., 2019; Xiao et al., 2018).

Biochar-based slow-release fertilizer surges nutrient bioavailability, boosts nutrient use efficiency and crop yield, and decreases leaching and runoff (Das and Ghosh, 2021; Zhou et al., 2021). The utilization of biochar directly interferes with agricultural soils and contributes to the critical nutrient cycling processes through physicochemical interactions and microbial activities (Garbuz et al., 2022; Kumar et al., 2022; Nguyen et al., 2022; Xiong et al., 2021). Nielson et al. (2018) suggested that the porous characteristics and the heterogeneous surface functional groups of biochar can contribute to ligand exchange reactions, surface complexation and the diffusion-controlled adsorption of elements thereby controlling the dynamics of plant available nutrients in soils. For instance, the nitrogen (N) cycle in soils is influenced indirectly following the addition of biochar leading to a reduction in N leaching and surges in N fertilizer. This is a result of inorganic forms of N adsorption onto biochar reduces ammonia and nitrate losses from the soil and permits nutrient retention and also releases nutrients gradually (Tsai and Chang, 2020). Although biochar is C-rich containing a high C/N ratio, its addition to agricultural soils promotes the decomposition of native soil organic matter by microorganisms. Because of the priming effect, N is essential throughout this process (Kuzyakov et al., 2019).

Biochar addition into agricultural soils boosts N utilization efficiency, and surges the total and available N but reduces the build-up of N efficiency by regulating the mineralisation of organic N, nitrification/denitrification, ammonia and volatilization (Cordovil et al., 2021). Mandel et al. (2018) reported that following the application of biochar modifies the cation and anion exchange capacities of soils thus influencing the retention of N. Biochar also influences soil phosphorus (P) transformation since it acts as a C source. For example, Xu et al. (2018) reported a positive correlation with improved soil microbial activity, decreased soil acidity, or improved CEC following the decreased $NaHCO_3^-$ extractable P concentrations owing to high C:P ratios of biochar in P immobilization. Furthermore, Liang et al. (2006) suggested that biochars that consist of high ion exchange capacity could modify the availability of P by influencing cations activities that interact with P or boost anion exchange capacity. Besides, the utilization of certain biochars can surge soil pH and modify $Al^{3+}$, $Fe^{3+}$, $Fe^{2+}$, $Ca^{2+}$ and $Mg^{2+}$ concentrations that are accountable for altering its availability and producing P complexes. Purakayastha et al. (2019) reported that biochar is rich in K and can retain K in the soil owing to its high CEC. The addition of biochar can indirectly contribute to the retention of soil nutrients founded on its properties including specific surface area, pH, porosity and CEC (Neogi et al., 2021; Diatta et al., 2020). Consequently, biochar addition to soils offers different extra advantages for nutrient recycling of plants including decreasing leaching and surging retention and use efficiency thus boosting the fertility levels of soil (Ndoung et al., 2021).

***The Effects of Biochar on Plant Development***

The addition of biochar to agricultural soils influences soil physical characteristics that in due course influence plant development. The efficiency of biochar utilization in boosting the productivity of crops in fertile soils is generally marginal in degraded and nutrient-poor soils (Abukari et al., 2020; Laghari et al., 2016; Hussain et al., 2017). The factors such as the supply of nutrients by biochar, increased fertilizer utilization efficiency, soil pH, moisture retention, nutrient retention and bioavailability, decreased soil tensile strength and improved soil structure. Also, they encourage favourable rhizosphere conditions for the earthworm population and microbiota can contribute to a surge in plant development after biochar to nutrient-deficient soils (Abukari, 2019; Yu et al., 2019, Yuan et al., 2019; Gwenzi et al., 2017; Abukari, 2014). In general, root establishment and development are major challenges for plants growing in poor soils. The improved soil characteristics affect the root area and stimulate root growth. The plant root in soil that increases in volume aids in capturing extra nutrients and increases plant development (Hallett et al., 2022; Abukari et al., 2018**)**.

The most common concern in agricultural settings is plant stress. Reports have shown that biochar has a promising possibility for reducing both biotic and abiotic plant stresses (Ahluwalia et al., 2021; Kavitha et al., 2018). For example, Kavitha et al. (2018) reported that the addition of biochar to soil boosts the antioxidant response of quinoa in solving the multiplex drought and salt build-

up conditions through surging plant-promoting hormones. The addition of biochar to saline and sodic soils has the advantage of lessening the adverse effects of salts due to the more surface charges on biochar could replace Na, K, Ca, and Mg thereby reducing exchangeable sodium percentage levels (Tan et al., 2022). Furthermore, biochar addition can stimulate microbial activities to reduce plant pathogenicity which hinders plant survival. The discharge of volatile organic compounds from microbial inhibitors deters soil pathogens thus increasing the development of plants (Russo et al., 2022).

## Limitations of Biochar Utilization

Despite its potential benefits, biochar utilization is not without limitation. One major constraint is the high production cost, which can make it economically unviable, particularly when using advanced technologies or high-quality feedstocks (Meyer et al., 2011). Additionally, biochar production requires significant energy inputs which can lead to greenhouse gas emissions and negate some climate change benefits (Woolf et al., 2010). Furthermore, the availability and quality of feedstocks can be limited by factors like land use, water availability and biomass quality (Lehmann &Joseph, 2009).

The storage and handling of biochar can also be challenging due to its powdery nature and potential for dust explosions (Kumar et al., 2020). Moreover, the lack of standardization in biochar production can lead to inconsistent quality and properties (IBI, 2014). There are also concerns about potential environmental impacts of biochar production and application, including soil contamination, water pollution and altered ecosystem processes (Sohi et al., 2010).

Scalability and commercialization of biochar production are also significant challenges, as the industry is still in its infancy (Meyer et al., 2011). Limited public awareness and acceptance of biochar can also hinder its adoption (Lehmann &Joseph, 2009). Furthermore, the regulatory framework surrounding biochar production and use are still evolving and can vary by country or region (IBI, 2014). Finally, despite growing research interest, there are still significant gaps in our understanding of biochar's properties, behaviours and impacts on ecosystems (Sohi et al., 2010).

## Conclusion

Biochar yield and quality produced via biomass thermochemical conversion processes widely vary owing to changes in the amount of oxygen supply, heating rate, and reaction temperature. In general, biochar yield reduces as the heating rate or the amount of oxygen supply increases. The advantageous effects of biochar utilization in the agricultural system, including increased soil quality and plant development, have been extensively described however varied or the existence of contradictory results, consequently, the advantages of biochar additions frequently limit biochar type, application dosage, soil type and conditions, and the type of crop. Systematic research is required to understand the links between biochar production processes, biochar properties, and biochar performance in agricultural settings.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

***Authors contributions***

Both authors contributed.

***Data availability statement***

All data supporting this study are available within the paper

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# Antidiabetic Effects of Milk-Derived Bioactive Components

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ABSTRACT

Type 2 diabetes (T2DM) is a chronic metabolic disease characterized by the body's inability to effectively use the hormone insulin, leading to high blood sugar levels. Milk is a rich source of important nutritional components that have been shown to have positive effects in the management of T2DM. Bioactive components derived from milk are molecules derived from milk proteins, fats, and other components, that exhibit various biological activities in the body and provide positive effects on health. These components (proteins, peptides, fatty acids) are formed during the consumption of dairy products or as a result of the digestion and hydrolysis of milk proteins. Recent intervention studies have shown that bioactive proteins, peptides, and fatty acids derived from milk provide beneficial effects in the prevention and management of T2DM. Milk bioactive components include casein, casein-derived peptides, whey proteins, and whey protein-derived peptides. These bioactive components exhibit anti-diabetic effects through various mechanisms. These mechanisms include increasing insulin sensitivity, regulating glucose metabolism, and reducing inflammation. Intervention studies in humans have shown that these bioactive components derived from milk reduce fasting blood sugar levels and increase insulin sensitivity. This study comprehensively reviews recent studies investigating the anti-diabetic effects of bioactive compounds derived from milk (proteins, peptides, and fatty acids) and the mechanisms of action of these compounds in the management of T2DM. Thus, it provides a perspective on the potential benefits and clinical applications of milk bioactive components in T2DM.

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# Süt Kaynaklı Biyoaktif Bileşenlerin Antidiyabetik Etkisi

MAKALE BİLGİSİ

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Biyoaktif Bileşenler
Tip 2 Diyabet Mellitus
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Protein İzolatı

ÖZ

Tip 2 diyabet (T2DM), vücudun insülin hormonunu etkili bir şekilde kullanamamasıyla karakterize edilen ve yüksek kan şekeri seviyelerine yol açan kronik bir metabolik hastalıktır. Süt, T2DM yönetiminde olumlu etkileri olduğu belirtilen önemli besin bileşenleri bakımından zengin bir kaynaktır. Sütte kaynaklı biyoaktif bileşenler, süt proteinlerinden, yağlarından ve diğer bileşenlerinden türeyen, vücutta çeşitli biyolojik aktiviteler gösteren ve sağlık üzerinde olumlu etkiler sağlayan moleküllerdir. Bu bileşenler (proteinler, peptitler, yağ asitleri), süt ürünlerinin tüketimi sırasında ya da süt proteinlerinin sindirilmesi ve hidroliz edilmesi sonucunda ortaya çıkmaktadır. Son dönemde yapılan müdahale çalışmaları, süt kaynaklı biyoaktif proteinlerin, peptitlerin ve yağ asitlerinin T2DM'nin önlenmesi ve yönetiminde yararlı etkiler sağladığını göstermektedir. Süt biyoaktif bileşenleri arasında kazein, kazein türevi peptitler, peynir altı suyu proteinleri ve peynir altı suyu proteini türevi peptitler yer almaktadır. Bu biyoaktif bileşenler, çeşitli mekanizmalar aracılığıyla anti-diyabetik etkiler göstermektedir. Bu mekanizmalar arasında insülin duyarlılığının artırılması, glukoz metabolizmasının düzenlenmesi ve inflamasyonun azaltılması yer almaktadır. İnsanlarda gerçekleştirilen müdahale çalışmaları sonucunda, süt kaynaklı bu biyoaktif bileşenlerin açlık kan şekeri seviyelerini düşürdüğünü ve insülin duyarlılığını artırdığı ortaya koyulmuştur. Bu çalışma, sütten elde edilen biyoaktif bileşiklerin (proteinler, peptitler ve yağ asitleri) anti-diyabetik etkilerini ve bu bileşiklerin T2DM yönetimindeki etki mekanizmalarını inceleyen güncel çalışmaları kapsamlı bir şekilde ele almaktadır. Böylece, süt biyoaktif bileşenlerinin T2DM üzerindeki potansiyel faydaları ve klinik uygulamaları hakkında bir bakış açısı sunmaktadır.

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## Giriş

Diyabet, kompleks ve kronik bir metabolik hastalık olup, dünya genelinde büyük bir halk sağlığı sorunu teşkil etmektedir. Diyabet Mellitus, tip 1, tip 2 ve gestasyonel diyabet olmak üzere üç ana tipe ayrılır ve bunlar arasında en yaygın olanı Tip 2 Diyabet Mellitus (T2DM) olup, tüm vakaların %90'ından fazlasını oluşturmaktadır. T2DM'de vücut, insüline etkili bir şekilde yanıt veremediğinde insulin direnci oluşur ya da yeterli insülin üretemez ve yüksek kan şekeri seviyelerine sebep olmaktadır (Lacroix ve Li-Chan, 2016; Patil ve ark., 2015).

T2DM'nin küresel yaygınlığı giderek artmaktadır. 2000 yılında dünya genelinde yaklaşık 171 milyon diyabetli olduğu, 2030'da bu sayının 366 milyona çıkacağı öngörülmektedir (WHO, 2006). Uluslararası Diyabet Federasyonu (International Diabetes Federation-IDF) tarafından yayımlanan '10. Diyabet Atlası'nda 2021 yılı itibarı ile dünyadaki diyabetli birey sayısı 537 milyon iken bu sayının 2030 yılında yaklaşık %20 oranında artarak 643 milyona ve 2045 yılında ise (2021 yılına göre) %46 oranında artarak 783 milyona ulaşacağı öngörülmektedir (IDF, 2021).

T2DM, insülin salgılanmasındaki bozukluk ve/veya insülin etkisi nedeniyle kan şekeri seviyelerinin yükselmesiyle karakterize heterojen bir klinik sendromdur (Bailey ve Flatt, 1995). Kronik yüksek kan şekeri, kalp krizi, böbrek yetmezliği, sinir hasarı ve göz hastalıkları riskini artırmaktadır (Yang ve ark., 2020). T2DM'nin tedavisinde tiazolidinedionlar, metformin, saksagliptin, linagliptin, sitagliptin ve vildagliptin gibi çeşitli sentetik ilaçlar sıklıkla kullanılmaktadır. Ancak, bu ilaçlar ağırlık artışı, kemik kaybı, alt ve üst solunum yolu enfeksiyonları gibi yan etkilere yol açabilmeleri nedeniyle ilaçların kullanımı sınırlı olmaktadır (Lacroix ve Li-Chan, 2016; Patil ve ark., 2015). Bu durum, araştırmacıları T2DM'nin yan etkisiz tedavi ve önleme yöntemleri için güvenli alternatifler aramaya yönlendirmektedir.

Diyet bileşenleri, T2DM'nin yönetimi ve önlenmesinde önemli bir rol oynamaktadır. Son dönemde, proteinler, peptitler, yağ asitleri ve polifenoller gibi gıda kaynaklı biyoaktif bileşikler; antioksidan, antiinflamatuar, antihipertansif, antikanser ve immünomodülatör etkileri nedeniyle büyük ilgi görmektedir (Chalamaiah ve ark., 2018). Hem tip 2 diyabetli (T2D) hem de T2D'siz bireylerde postprandiyal (yemek sonrası) glikoz seviyelerinin kontrolü açısından, yüksek kan glikoz seviyelerine uzun süreli maruziyeti azaltmak büyük önem taşımaktadır(Gerich, 2003).

Süt, besin açısından oldukça zengin bir gıdadır ve ayrıca, antioksidan, antiinflamatuar, antihipertansif, antikanser ve immünomodülatör özelliklere sahip biyoaktif bileşenler olan proteinler, peptitler ve yağ asitlerinin en önemli kaynağıdır (Jayathilakan ve ark., 2018). Bu biyoaktif özelliklerin yanı sıra, son dönemde gerçekleştirilen çok sayıda çalışma, sütten elde edilen biyoaktif bileşiklerin (proteinler, peptitler ve yağ asitleri) T2DM'nin tedavi ve yönetiminde olumlu etkiler sağladığını göstermiştir (Lacroix ve Li-Chan, 2016; Patil ve ark., 2015). Süt proteinleri, özellikle kazein ve peyniraltı suyu (PAS), insülin salınımını artırabilir ve vücut hücrelerinin glikoz alımını etkileyerek yemek sonrası kan şekeri dalgalanmalarını kontrol etmeye yardımcı olabilmektedir (Akhavan ve ark., 2010). Pek çok sistematik inceleme ve meta-analiz, süt ve süt ürünlerinin daha yüksek tüketiminin T2DM'nin daha düşük görülme sıklığı ve riski ile bağlantılı olduğunu ortaya koymaktadır (Alvarez-Bueno ve ark., 2019). Bu makale, süt kaynaklı biyoaktif bileşiklerin antidiyabetik etkilerini inceleyerek, bu bileşiklerin T2DM'nin yönetimindeki potansiyel rolünü ve mekanizmalarını derlemeyi amaçlamaktadır.

## Süt Kaynaklı Biyoaktif Bileşenler

Süt, memelilerin meme bezleri tarafından üretilen ve besin açısından zengin olan bir sıvıdır. İnek, manda, keçi, koyun, deve, eşek gibi hayvanlar tarafından üretilmektedir. İnsanlar için önemli bir gıda maddesi olan süt, kaliteli bir besin kaynağıdır ve süt proteinlerinin %80'ini oluşturan kazeinler ile geri kalan %20'sini oluşturan PAS proteinleri gibi çeşitli proteinleri içermektedir (Zhou ve ark., 2021).

Kazein (α-, β-, γ- ve κ-kazein) ve PAS (β-laktoglobulin, α-laktalbümin, serum albümini, immünoglobulinler, laktoferrin ve proteaz-pepton fraksiyonları), sütte bulunan ve antioksidan, antiinflamatuar, immünomodülatör ve antikanser gibi çeşitli sağlık yararları sağlayan önemli biyoaktif bileşenlerdir (Nongonierma ve FitzGerald, 2015). Kazein ve PAS proteinlerinden elde edilen çeşitli peptidlerin, antioksidan, antiinflamatuar, immünomodülatör, antimikrobiyal ve antihipertansif gibi birçok biyolojik özelliğe sahip olduğu kanıtlanmıştır (Chalamaiah ve ark., 2018). Süt proteinlerinde bulunan biyoaktif peptit dizileri, sağlık yararlarının sağlanmasında önemli bir rol oynamaktadır. Bu peptitler, genellikle süt proteinlerinin pepsin, tripsin ve pankreatin gibi çeşitli proteolitik enzimlerle hidrolize edilmesiyle elde edilmektedir (Lacroi ve Li-Chan, 2013).

Çizelge 1'de süt biyoaktif bileşenleri ile ilgili klinik çalışmaları ve Çizelge 2'de *in vitro*/hayvan deneyleri ilgi ilgili çalışmalar bulunmaktadır.

## Süt kaynaklı biyoaktif bileşenlerin antidiyabetik özellikleri

PAS ve kazein alımının artışı, insülin salgısını tetikler. PAS proteini, misel kazeinine göre daha hızlı bir insülin yanıtı sağlar. Ancak kazeinin hidrolizi, amino asitlerin emilim sürecini hızlandırırken, aynı zamanda misel formundaki kazeine göre insülin salgısını da artırır (Nilsson ve ark., 2007).

Hidrolize süt proteinlerinin tüketimi, genellikle hidrolize edilmemiş proteinlere göre daha fazla *in vivo* insülin salgısını tetikler (Power ve ark., 2009). Ayrıca, PAS'dan elde edilen biyoaktif bileşik, β-laktoglobulinin hidroliziyle ortaya çıkan İzolosin-Prolin-Alanin (Ile-Pro-Ala) adlı bir tripeptittir. Bu bileşik, dipeptidil peptidaz-4 inhibitörü olarak işlev görerek glikoz seviyelerini azaltmakta ve insülin salgısını artırmaktadır (Tulipano ve ark., 2011).

Son araştırmalar, T2DM'nin yönetimi ve önlenmesinde sütten elde edilen belirli biyoaktif proteinlerin (kazein, peyniraltı suyu) ve peptitlerin faydalarını ortaya koymuştur. Çeşitli in vitro, in vivo ve klinik çalışmalar, süt proteinlerinin ve peptitlerinin anti-diyabetik etkiler

gösterdiğini kanıtlamaktadır. Bu etkiler, birçok moleküler mekanizma aracılığıyla ortaya çıkmaktadır. Bu mekanizmalar arasında, dipeptidil peptidaz-IV (DPP-IV) enziminin inhibisyonu ve IRS/PI3K/Akt sinyal yolunun aktivasyonu yer almaktadır. Ayrıca, süt proteinleri kan glikozunu düşürmekte, insülin direncini iyileştirmekte ve HbA1C (glikolize hemoglobin) seviyelerini azaltmaktadır. Adenozin monofosfat ile aktive olan protein kinaz (AMPK) sinyallemesinin aktivasyonu, fosfoenolpiruvat karboksikinaz (Pck1) ve glukoz 6 fosfotaz (G6PC) enzimlerinin düzenlenmesi gibi etkiler de bu proteinlerin faydaları arasındadır. Ayrıca, süt proteinleri glikoz tüketimini ve insülin salgılanmasını artırırken, açlık plazma glikozunu azaltır ve pankreas β hücrelerinin işlevini iyileştirmektedir. Son olarak, glukoz taşıyıcı tip 4 (GLUT-4) seviyelerinin yükseltilmesi de bu mekanizmalardan biridir. (Derosa ve ark., 2020; Gong ve ark., 2020).

Kazein ve PAS proteinlerinden elde edilen protein hidrolizatları/peptitler, güçlü DPP-IV( Dipeptidil Peptidaz-IV) inhibitörü kaynaklarıdır. Bu protein hidrolizatlarından çeşitli DPP-IV inhibitör peptidleri tanımlanmış ve izole edilmiştir. Süt proteininden türetilen peptitlerin DPP-IV inhibitör etkileri, peptitlerin dizilişi, bileşimi ve uzunluğuna bağlıdır (Lacroix ve Li-Chan, 2012). Ayrıca, N veya C terminalindeki belirli amino asitlerin varlığı, sütten elde edilen peptitlerin DPP-IV inhibitör aktivitesinde önemli bir etken rol oynamaktadır (Nongonierma ve FitzGerald, 2013). Çoğu DPP-IV inhibitör peptidinin N terminalinde hidrofobik amino asitler (Triptofan, Lösin, Izolosin veya Fenilalanin), ikinci pozisyonda bir prolin veya alanin ve C-terminalinde bir prolin bulunduğunu bildirmiştir (Le Maux ve ark., 2017). Ayrıca, süt kaynaklı peptitlerin DPP-IV enziminin inhibisyonunda rekabetçi inhibisyon mekanizmasının önemli bir rol oynadığı bildirilmiştir. Kazein ve peynir altı suyu proteinlerinden türetilen peptitler, DPP-IV'ün substratı olarak işlev görür ve enzimin aktif bölgesiyle doğrudan etkileşime girerek DPP-IV'ü inhibe eder (Nongonierma ve ark., 2019; Nongonierma ve FitzGerald, 2013).

Çizelge 1. Süt kaynaklı biyoaktif proteinlerin ve peptitlerin insanlarda antidiyabetik etkileri
*Table 1. Antidiabetic effects of milk-derived bioactive proteins and peptides in humans*

| Etken madde | Çalışma tasarımı | Örneklem | Doz ve süre |
|---|---|---|---|
| PAS protein izolatı | Rastgele, paralel kollu | Ortalama yaşı 48,4 olan, kilolu ve obez 70 erkek ve kadın | 54g/gün, 12 hafta |
| Kazein hidrolizatı | Rastgele, çift kör, kısmi çaprazlama | 50-70 yaş aralığında 36 T2DM hastası | 17,6 gr'lık tek doz |
| Kazein hidrolizatı | Rastgele, plasebo kontrollü, çift kör | 58 yaşında T2DM'li 13 hasta | 12 gr |
| PAS protein izolatı ve hidrolizatı | Rastgele, plasebo kontrollü, çift kör | Tip 2 diyabetli 10 erkek hasta | 0, 0,1, 0,2 ve 0,4 g /kg |
| PAS proteini | Rastgele, çaprazlama | 10 sağlıklı genç adam | 10 ve 20 gr |
| Kazein ve hidrolizatı | Rastgele, çift kör, çapraz geçişli | 60 T2DM hastası | 0,3 gr/kg vücut ağırlığı |
| PAS proteini ve hidrolizatı | Rastgele, tek kör, çaprazlama | 54,9±2,3 yaşlarında T2DM'li 11 erkek | 15 gr 3 sabah makro besin kahvaltısı ve öğle yemeğinden önce |
| Kazein protein hidrolizatı | Randomize, tek merkezli, çift kör plasebo kontrollü | Gebelik yaşı 20-35 hafta arasında olan hafif GDM'li 50 hasta | 8,5 gr/gün, 8 gün |
| PAS protein izolatı | Rastgele, plasebo kontrollü | Tip 2 diyabetli 18 yaş üstü 120 beyaz erkek ve kadın | 5 gr/gün, 3 ay |
| PAS'dan peptidler β-laktoglobulin ve α-laktalbümin | Rastgele, çift kör, plasebo kontrollü, 3 yönlü çapraz geçişli | Prediyabetik rahatsızlığı olan 21 denek (8 erkek ve 13 kadın) | 1400 mg veya 2800 mg /gün, 6 hafta |
| Etken madde | Etkiler | | Kaynaklar |
| PAS protein izolatı | Azalmış açlık insülin seviyeleri ve HOMA-IR skorları gözlenmiştir. | | Pal ve ark., 2010 |
| Kazein hidrolizatı | T2DM hastalarında plazma glikozunun azalması respite edilmiştir. | | Geerts ve ark., 2011 |
| Kazein hidrolizatı | Artan insülin seviyeleri ve azalan glikoz seviyeleri gözlenmiştir. | | Jonker ve ark., 2011 |
| PAS protein izolatı ve hidrolizatı | Arttırılmış insülin salgılanması ve plazma insülin konsantrasyonları izleniştir. | | Goudarzi ve Madadlou, 2013 |
| PAS proteini | Hem insüline bağımlı hem de insüline bağımsız mekanizmalar yoluyla yemek sonrası glisemiyi azalttığı saptanmıştır. | | Akhavan ve ark., 2014 |
| Kazein ve hidrolizatı | T2DM hastalarında postprandiyal insülin salınımının arttırdığı belirlenmiştir. | | Manders ve ark., 2014 |
| PAS proteini ve hidrolizatı | Kahvaltıdan sonra toplam postprandiyal glisemiyi %13 oranında azalttığı, Kahvaltıdan sonra erken glikozu azalttığı ve insülin konsantrasyonunu artırdığı bulunmuştur. | | King ve ark., 2018 |
| Kazein protein hidrolizatı | Gestasyonel diyabet hastalarında plazma glikoz düzeylerinde orta düzeyde azalma gözlenmiştir. | | Saleh ve ark., 2018 |
| PAS protein izolatı | Açlık plazma glikozunu düşürdü ve lipid profilini iyileştirdiği belirlenmiştir. | | Derosa ve ark., 2020 |
| PAS'dan peptidler β-laktoglobulin ve α-laktalbümin | Glukozun kademeli postprandiyal glisemiyi azalttı. Küçük bir insülinotropik etki gösterdi ve HbA1c değerlerini önemli ölçüde azalttığı bildirilmiştir. | | Sartorius ve ark., 2019 |

Çizelge 2. Süt kaynaklı biyoaktif protein ve peptitlerin in vitro ve in vivo antidiyabetik etkileri
*Table 2. In vitro and in vivo antidiabetic effects of milk-derived bioactive proteins and peptides*

| Etken madde | Araştırma türü | Yöntem |
|---|---|---|
| PAS protein izolatı hidrolizatı | In vitro | DPP-IV inhibitör aktivitesi |
| Kazein, PAS ve laktoferrinden hidrolizatlar | In vitro | DPP-IV inhibitör aktivitesi |
| α-laktalbumin, β-laktoglobulin, Laktoferrin ve peynir altı suyu protein izolat hidrolizatları | In vitro | DPP-IV ve α-glukozidaz inhibitör aktiviteleri |
| β-laktoglobulin açısından zengin PAS proteininden elde edilen peptit | In vitro | DPP-IV inhibitör aktivitesi |
| PAS protein izolatı ve α-laktalbümin'den elde edilen peptidler | In vitro | DPP-IV inhibitör aktivitesi |
| β-laktoglobulin hidrolizatları | In vitro | DPP-IV inhibitör aktivitesi |
| PAS proteini hidrolizatı | Wistar sıçanları | Diyet 156.41 g/kg, 9 gün |
| PAS izolatı, peynir altı suyu hidrolizatı, α-laktalbumin ve kazein proteini | Zucker Diyabetik yağlı sıçanlar ve normal Wistar sıçanları | Diyette 42.4-49.1g doz, 13 hafta |
| Süt yağı kürecik zarı | Erkek C57BL/6J fareleri yüksek yağlı diyet ve STZ ile T2D'yi indükledi | Doz 400 mg/kg, 8 hafta |
| Etken madde | Etkiler | Kaynaklar |
| PAS protein izolatı hidrolizatı | 0,075 mg/ml inhibitör konsantrasyonu ile DPP-IV inhibisyonu gözlemlenmiştir | Lacroix ve Li-Chan, 2012 |
| Kazein, PAS ve laktoferrinden hidrolizatlar | DPP-IV inhibisyonu bildirilmiştir | Nongonierma ve FitzGgerald, 2013 |
| α-laktalbumin, β-laktoglobulin, Laktoferrin ve peynir altı suyu protein izolat hidrolizatları | Çeşitli düzeylerde DPP-IV ve α-glukozidaz inhibitör aktiviteleri sergilenmiştir( | Lacroix ve Li-Chan, 2013 |
| β-laktoglobulin açısından zengin PAS proteininden elde edilen peptit | Inhibitör konsantrasyon değeri 44,7 µM olan DPP-IV inhibisyonu izlenmiştir ( | Silveira ve ark., 2013 |
| PAS protein izolatı ve α-laktalbümin'den elde edilen peptidler | 45-57 µM IC50 değerine sahip iki peptid tarafından DPP-IV inhibisyonu gözlenmiştir ( | Lacroix ve Li-Chan, 2014 |
| β-laktoglobulin hidrolizatları | 1,0-1,47 µM aralığında IC50 değerlerine sahip DPP-IV inhibitör aktivitesi izlenmiştir ( | Le Maux ve ark., 2017 |
| PAS proteini hidrolizatı | Plazma membranındaki taşıyıcı GLUT-4 seviyelerinin artırıldığı gözlenmiştir ( | Morato ve ark., 2013 |
| PAS izolatı, peynir altı suyu hidrolizatı, α-laktalbumin ve kazein proteini | HbA1c'de azalma bildirilmiştir | Gregersen ve ark., 2014 |
| Süt yağı kürecik zarı | Karaciğer ve iskelet kasında c-Jun N-terminal kinaz (JNK) sinyallemesini baskılarken fosfatidilinositol 3-kinaz (P13K)/protein kinaz B (AKT) yolunu teşvik ederek hiperglisemi ve dislipidemiyi iyileştirdi | Yuan ve ark., 2019 |

Bir randomize kontrollü klinik çalışmada, 40 Tip 2 Diyabet hastasına 3 ay boyunca her gün 500 ml deve sütü takviyesi uygulanmıştır. Bu müdahale, açlık kan şekerinde belirgin bir azalma sağlamış ve insülin doz gereksinimini %13,7 oranında azaltmıştır (Ejtahed ve ark., 2015). Son zamanlarda yapılan araştırmada, sağlıklı genç kadınlarda süt proteini matrisi takviyesinin hem insülin hem de inkretin salgısını artırdığını göstermiştir (Amigo-Benavent ve ark., 2020).

**Kazeinden elde edilen hidrolizatların ve peptitlerin antidiyabetik etkisi**

İnsanlarda yapılan çok sayıda araştırma, kazein proteinlerinin ve peptitlerinin T2DM yönetimindeki yararlı etkilerini bildirmiştir. Kazein hidrolizatının tek doz (17,6 g) uygulamasının T2DM hastalarında plazma glikoz seviyesini düşürdüğü gösterilmiştir (Geerts ve ark., 2011). Jonker ve arkadaşları (2011), kazein hidrolizatının faydalı etkilerini belirlemek için 13 T2DM hastası üzerinde randomize, çift kör, plasebo kontrollü bir çalışma yürütmüştür. Araştırmacılar, 12 g kazein hidrolizat alımının insülin seviyesini artırdığını ve glikoz konsantrasyonunu azalttığını tespit etmiş; ancak, 6 g tüketiminin insülin ve glikoz seviyeleri üzerinde herhangi bir önemli etki göstermediği gözlemlemiştir (Jonker ve ark., 2011).

Kazein ve türevi hidrolizatların, 0,3 g/kg kazein veya hidrolizatının alınmasından sonra T2DM hastalarında yemek sonrası insülin salınımını artırdığı bildirilmiştir (Manders ve ark., 2014). 50 gestasyonel diyabet (GDM) hastasında, 8 gün boyunca günde iki kez 8,5 g kazein hidrolizat uygulanması insülinotropik bir etki yaratmamıştır, ancak gestasyonel diyabet hastalarında plazma glikoz seviyelerini orta düzeyde düşürdüğü görülmüştür (Saleh ve ark., 2018).

**Serum proteinleri ve protein hidrolizatları/ peptitlerinin antidiyabetik etkisi**

Literatürdeki klinik araştırmalar, PAS proteinleri ve peptitlerinin tüketiminin tip 2 diyabetin yönetiminde olumlu etkiler sağladığını ortaya koymuştur. PAS proteinleri ve peptitleri, insülin salgılanmasını artırarak, açlık plazma glikozunu düşürerek ve HbA1C seviyelerini azaltarak T2DM hastalarında faydalı etkiler göstermiştir

(Derosa ve ark., 2020; Goudarzi ve Madadlou, 2013; Jakubowicz ve ark., 2017; Sartorius ve ark., 2019). 70 aşırı kilolu ve obez yetişkin üzerinde yapılan bir çalışmada, 12 hafta süresince PAS proteini takviyesi uygulanan grupta, açlık insülin seviyeleri ve insülin direncinin homeostatik modeli değerlendirilmesi (HOMA-IR) skorlarında kontrol grubuna göre anlamlı bir azalma gözlemlenmiştir (Pal ve ark., 2010). Goudarzi ve Madadlou (2013) yapmış olduğu bir çalışmada, tip 2 diyabet hastalarında 0,2 g/kg PAS proteini hidrolizatı ya da 0,4 g/kg PAS proteini izolatı alımının insülin salgılanmasını belirgin şekilde artırdığını rapor edilmiştir (Goudarzi ve Madadlou, 2013). Jakubowicz ve arkadaşları (2017) gerçekleştirdiği çalışmada, 12 hafta süresince kahvaltılarda günde 28 g peynir altı suyu proteini tüketiminin 56 T2DM hastasında Hemoglobin A1C (HbA1C) seviyelerini azalttığı görülmüştür (Jakubowicz ve ark., 2017). Ayrıca, 60 dakika süresince hidrolize peynir altı suyu proteininin intraduodenal infüzyonunun, 20 sağlıklı erkek üzerinde plazma insülin, glukagon, gastrik inhibitör peptid (GIP), glukagon benzeri peptid-1 (GLP-1) ve peptid-tirozin-tirozini seviyelerini artırdığı belirlenmiştir (Jensen ve ark., 2019). Sartorius ve arkadaşları (2019) yürüttüğü randomize, çift kör, plasebo kontrollü, 3 yönlü, çapraz geçişli klinik çalışmada, 6 hafta boyunca peynir altı suyu β-laktoglobulin ve α-laktalbumin kaynaklı peptitlerin tüketiminin HbA1c seviyelerinde anlamlı bir azalma sağladığı ve prediyabetik(henüz tam olarak diyabet gelişmemiş ancak kan şekeri seviyelerinin normalden yüksek olması) durumdaki çalışmaya katılanların tamamında hafif bir insülinotropik (insülin salınımını arttırıcı) etki gösterdiği tespit edilmiştir. Bununla birlikte, bu müdahalenin prediyabetik bireylerde glikoz yanıtını artırmadığı da rapor edilmiştir (Sartorius ve ark., 2019). Son dönemde gerçekleştirilen bir klinik çalışmada, 120 T2DM hastası 3 ay süresince günde 5 g PAS protein izolatı kullanmıştır (Derosa ve ark., 2020). Bu araştırmanın sonuçlarına göre, PAS izolatı takviyesi açlık plazma glikoz seviyelerinde anlamlı bir azalma sağlamış ve lipid profilinde, başlangıç ve plasebo grubu ile kıyaslandığında belirgin bir iyileşme gözlemlenmiştir (Derosa ve ark., 2020).

Klinik çalışmalardan elde edilen veriler, T2DM hastalarında PAS proteini ve peptitlerinin öğün öncesi takviyesinin olumlu etkilerini desteklemektedir. Bir başka klinik çalışmada, 10 sağlıklı erkek üzerinde yapılan denemelerde, öğün öncesinde 10 ve 20 g PAS proteini uygulamasının, hem insüline bağımlı hem de insüline bağımsız mekanizmalar aracılığıyla öğün sonrası glisemiyi azalttığı gözlemlenmiştir (Akhavan ve ark., 2014). Bjørnshave ve arkadaşları (2018) tarafından yapılan çalışmada, T2D hastalarına 15 dakika süren yağ açısından zengin bir öğünden önce 20 g PAS proteini takviyesi verilmiştir. Bu araştırma, PAS proteininin öğün öncesi tüketiminin T2D hastalarında insülin, glukagon ve GIP yanıtlarını artırdığını ortaya koymuştur (Bjørnshave ve ark., 2018). King ve arkadaşları (2018) gerçekleştirdiği randomize tek kör çapraz geçişli çalışmada, PAS proteini ve türetilmiş hidrolizatının antidiyabetik etkileri incelenmiştir. Çalışmada, 11 T2D hastasına 3 sabah boyunca kahvaltı ve öğle yemeğinden önce sırasıyla 15 g PAS proteini ve 15 g PAS hidrolizatı takviyesi verilmiştir. Araştırma, PAS proteininin toplam postprandiyal glisemiyi %13 oranında azalttığını ($P < 0,05$) ve PAS hidrolizatının kahvaltı sonrası erken glikoz seviyelerini kontrolle kıyaslandığında zayıflattığını göstermiştir. Ayrıca, her iki takviyenin de insülin konsantrasyonlarını artırdığı ($P < 0,05$) tespit edilmiştir.

**Süt yağının antidiyabetik etkisi**

Sütün yağ asidi (FA) profili, besin değerinin belirlenmesinde önemli bir rol oynar(Hirahatake ve ark., 2020). Özellikle esansiyel yağ asitleri olan linoleik asit (LA, C18:2 ω-6) ve alfa-linolenik asit (ALA, C18:3 ω-3) öne çıkar. Bu yağ asitleri, çoklu doymamış yağ asitleri (PUFA) grubuna dahildir ve insanların günlük beslenmesine katkıda bulunur. Ancak, vücut bu yağ asitlerini sentezleyemez, bu nedenle diyetle alınmaları gereklidir (Hu ve ark., 2001). Konjuge linoleik asit (KLA) izomerleri, geviş getiren hayvanların etlerinde ve sütlerinde en yüksektir düzeyde bulunmaktadır. Son zamanlarda, diyet KLA izomerlerinin bir karışımının bozulmuş glikoz toleransını normalleştirdiğini ve obez sıçanlarda hipergliseminin gelişimini önlediğini veya geciktirdiği bildirilmiştir (Ryder ve ark., 2001).

Süt, yüksek miktarda doymuş yağ asiti içeriğiyle bilinen bir kaynaktır. Süt yağ kürecikleri, trigliserit çekirdeğinin etrafını saran fosfolipitler, polar lipitler ile membrana özgü proteinleri içeren karmaşık bir yapı ile kaplıdır. Bu yapı, süt yağ küreciği zarı olarak adlandırılmaktadır (Yuan ve ark., 2019). Birçok çalışma, süt proteinleri ve peptitlerinin sağlık üzerindeki olumlu etkilerini ortaya koymuş olsa da, süt yağı ve diyabet arasındaki ilişkiye dair araştırmalar oldukça sınırlıdır. Ancak, Yuan ve arkadaşları (2019) yürüttüğü güncel bir çalışmada, T2D'li farelerde 8 hafta boyunca günlük 400 mg/kg vücut ağırlığında süt yağı küreciği zarı takviyesi uygulamıştır. Bu çalışma, süt yağı küreciği zarı takviyesinin T2D farelerinde hiperglisemi ve dislipidemiyi iyileştirdiğini, fosfatidilinositol 3-kinaz (PI3K)/protein kinaz B (Akt) sinyal yolunu güçlendirerek ve karaciğer ile iskelet kasındaki c-Jun N-terminal kinaz(JNK) sinyal mekanizmasını inhibe ederek bu iyileşmeyi sağladığını göstermiştir. PI3K/Akt sinyal yolu, karaciğer ve iskelet kasında glikoz alımını kontrol eden önemli bir mekanizmadır. Ancak, insülin direnci oluştuğunda bu sinyal yolu düzgün çalışamaz ve glikoz alımı bozulmaktadır (Yuan ve ark., 2019). PI3K/Akt sinyal yolunun, glikoz alımını düzenlemede hayati bir rol oynadığı, ancak insülin direnci durumunda bu mekanizmanın işlevselliğini yitirdiği vurgulanmaktadır. Bu bulgular, MFGM'nin diyabet yönetiminde potansiyel bir destekleyici ajan olarak kullanılabileceğini göstermektedir.

Sütteki toplam yağ asitlerinin %4'ünden fazlası dallı zincirli yağ asitleri oluşturmaktadır (Vlaeminck ve ark., 2006). Bu dallı zincirli yağ asitleri, diyabet hastalığı için önemli olan pankreas β-hücre fonksiyonunu arttırdığı bildirilmektedir (Wongtangtintharn ve ark., 2004). Ayrıca bir başka araştırma, tam yağlı süt ürünlerinin daha yüksek diyet tüketimi ile tip 2 diyabet insidansının azalması arasında bir ilişki olduğunu göstermiştir (Mitri ve ark., 2021).

## Sonuç

Birçok klinik çalışma, sütten elde edilen biyoaktif bileşiklerin(proteinler, peptitler) TDM'nin önlenmesi ve yönetiminde olumlu etkiler sağladığını göstermiştir. Sütten elde edilen biyoaktif peptitler, DPP-IV'ün inhibisyonunu ve GLP-1'in bozulmasını önleyerek, insülin salgılanmasını destekleyerek antidiyabetik etki göstermektedir. Buna ek olarak, süt yağı antidiyabetik etki göstermektedir; fakat bu alanda yapılan çalışmalar sınırlı görülmektedir. Bir diğer kısıtlama ise, klinik çalışmaların çoğunun bu biyoaktif bileşiklerin tip 2 diyabetin tedavi ve yönetimindeki etkinliğini desteklemek için yetersiz örneklem büyüklüğü ile gerçekleştirilmiş olmasıdır. Bu nedenle, süt kaynaklı biyoaktif bileşiklerin antidiyabetik özelliklerini doğrulamak amacıyla daha büyük örneklem gruplarına sahip çalışmalara ihtiyaç duyulmaktadır. Sonuç olarak, sütten elde edilen biyoaktif bileşiklerin antidiyabetik özelliklerine dair çeşitli mekanizmalar rapor edilmiştir. Ancak süt kaynaklı biyoaktif bileşiklerinin diyabet üzerindeki etkilerinin doğruluğunu ispatlamak ve güvenilirliğini test etmek adına yeni araştırmaların yapılması gerekmektedir.

## Beyanlar

Bu makale, kısmi olarak 7. Uluslararası Tarım, Gıda, Çevre ve Biyoloji Kongresinde sunulmuştur.

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# Industry 4.0 and Food Safety in the Food Sector

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ABSTRACT

Since the beginning of time, people have had the most basic need for food products (food and beverages) to survive. With the increasing human population, the need for food products has also increased, and with the developing technology, these needs have been tried to be met with mass production in the food sector. However, with the effect of globalization, the safety problem of increasing mass food products has emerged, and this has led societies to create food safety and traceability policies and to create traceability systems. In this review, the stages of the industrial revolution, starting with the 1st Industrial Revolution, which emerged with the use of steam engines in the 18th century, and up to the 4th Industrial Revolution (Industry 4.0), where machines have started to take an active role in industrial processes compared to human power, have been discussed and their effects on the food sector have been examined. Studies on the use of the basic principles and applications of Industry 4.0 in food traceability have been researched and discussed.

Türk Tarım – Gıda Bilim ve Teknoloji Dergisi, 13(3): 821-830, 2025

# Gıda Sektöründe Endüstri 4.0 ve Gıda Güvenliği

MAKALE BİLGİSİ

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İzlenebilirlik
Gıda Güvenliği
Endüstri devrimi

ÖZ

Tarihin başlangıcından beri insanların yaşamlarını sürdürebilmeleri için en temel gereksinimleri gıda ürünleri (yiyecek-içecek) olmuştur. Artan insan nüfusu ile gıda ürünlerine olan ihtiyaç da artmış ve gelişen teknolojiyle birlikte, gıda sektöründe gerçekleştirilen seri üretimlerle bu ihtiyaçlar karşılanmaya çalışılmıştır. Ancak küreselleşmenin de etkisiyle artan kitlesel gıda ürünlerinin güvenliği sorunu ortaya çıkmış ve bu da toplumları gıda güvenliği ve izlenebilirliği politikaları ve izlenebilirlik sistemleri oluşturmaya yönlendirmiştir. Bu derlemede 18. yy'da buharlı makinelerin kullanımıyla ortaya çıkan 1. Endüstri Devrimi ile başlayıp günümüzde endüstriyel süreçlerde makinelerin insan gücüne göre etkin bir rol almaya başladığı 4. Endüstri Devrimi (Endüstri 4.0) dahil olmak üzere endüstriyel devrim aşamaları ele alınmış ve bunların gıda sektöründeki etkileri incelenmiştir. Endüstri 4.0'ün temel prensipleri ve uygulamalarının gıdada izlenebilirlikte kullanımı ile ilgili çalışmalar araştırılarak tartışılmıştır.

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## Giriş

Endüstri 4.0 fikri, Almanya' nın sanayi sektörünü geliştirmek ve sanayisini daha etkili ve üretken hale getirmek için ortaya attığı bir girişim olarak bilinmektedir (Ojo ve ark., 2018).

Faydalarından bazıları; kaynak verimliliğinde iyileşme, maliyet düşürme, pazar erişimi, müşteri memnuniyeti, sistematik yönetim, gelişmiş gıda güvenliği ve şeffaflıktır. Gıda işleme sektöründe Endüstri 4.0 teknolojilerinin benimsenmesinin önündeki en büyük engeller arasında ise; sermaye, beceri ve teknoloji eksiklikleri yer almaktadır. Bu teknolojilerin tarımda uygulanması ve gıda güvenliği sorunlarını çözebilmesinde mevcut altyapının yetersizliği ve dijital okuryazarlık eksiklikleri gibi sorunlar ortaya çıkabilmektedir (Noor Hasnan & Yusoff, 2018; Oruma ve ark., 2021). Gıda israfının azaltılması ve dağıtım verimliliğinin arttırılmasında IoT (nesnelerin internet) tabanlı çözümler, gıda tedarik zincirinde kullanım potansiyeline sahiptir.

Gıda sektöründe enerji verimliliği de önemli bir konu olarak öne çıkmakta ve bu konudaki stratejilerin geliştirilmesi gerekmektedir (Clairand ve ark., 2020). Endüstri 4.0' ın sunduğu yeni fırsatlar enerji tüketiminin azaltılması ve sürdürülebilirlik hedeflerinin gerçekleştirilmesi açısından kritik öneme sahiptir. Ancak, bu stratejilerin uygulanması sırasında karşılaşılan engeller, özellikle küçük işletmeler için önemli bir sorun teşkil etmektedir.

Ülkemizde ve dünyada gıda sektöründe izlenebilirlik ve Endüstri 4.0 uygulamalarının, gıda tedarik zinciri içerisinde izlenebilirlik amaçlı kullanımına yönelik yapılan çalışma sayısı her geçen gün artmaktadır. Gıda üretiminin tedarik zinciri sektörü içerisinde gerçekleşmesi ve izlenebilirlikte Endüstri 4.0 teknolojilerinden yararlanılması çalışmaların, gıda mühendisliğinin yanı sıra endüstri mühendisliği, işletme, bilgisayar mühendisliği vb. bir çok farklı bilimsel disiplin alanında da araştırma konusu olmasını sağlamıştır. Bu nedenle konu farklı disiplinlerden çeşitli araştırmacılar tarafından incelenmiştir. Bu durum konunun multidisipliner bir yaklaşımla ele alınması gerektiğinin de önemli bir göstergesidir. Literatürde yapılan çalışmalar incelendiğinde gerçekleştirilen araştırmaların çeşitli yönlerden ele alındığı ve irdelendiği görülmektedir. Bu nedenle çeşitli yönleri ile araştırılan konu hakkında gerçekleştirilen araştırmaların bir kısmı temel Endüstri 4.0 teknolojilerinin çalışma prensiplerinden yola çıkarak bunların gıda sektöründeki uygulamalarını irdeleyen çalışmalar olmak ile birlikte, diğer bir kısmı ise gıda sektöründe izlenebilirlik teknoloji önerileri ve modellerine yönelik çalışmalardır. Sonuç olarak, Endüstri 4.0' ın gıda sektöründe sağladığı fırsatlar kadar, karşılaşılan eksiklikler ve zorluklar da dikkate alınmalıdır. Gıda sektöründeki dijital dönüşüm yalnızca, teknolojik yeniliklerin benimsenmesi ile değil, aynı zamanda bu yeniliklerin etkili bir şekilde uygulanabilmesi için gerekli olan altyapı ve eğitimle de doğrudan ilişkilidir. Bu bağlamda, gıda sektöründe Endüstri 4.0 uygulamalarının başarılı bir şekilde gerçekleştirilmesi için, hem teknolojik hem de insan kaynakları açısından kapsamlı bir yaklaşım benimsenmesi gerekmektedir.

## Endüstriyel Devrim Aşamaları

### *Birinci Endüstri Devrimi (Endüstri 1.0)*

Birinci Endüstri Devrimi dönemi (Endüstri 1.0), 18. yy'ın sonlarına doğru (1760'dan 1840'a kadar) ortaya çıkan ve buhar gücünün kullanımı ile üretimde mekanik sistemlerin kullanılmaya başlandığı dönemi ifade etmektedir. Bu dönem ile birlikte o zamana kadar el emeği ile gerçekleştirilen üretim yerini mekanik üretime bırakmış ve toplumsal iş gücünü tarımdan sanayiye yönlendirmiştir. Bu dönemde emeğin yani insan ve hayvan gücünün yerini makineler almaya başlamış tarım, madencilik, imalat ve ulaştırma gibi sektörlerde ise olumlu gelişmeler devam etmiştir (Apriliyanti, 2022; Dai ve ark., 2024; Kılıç, 2023; Raschke, 2022). Endüstri Devriminde makineleşme yoluyla, tahılların öğütülmesi 18. yy'da insan, hayvan, rüzgâr veya su gücüyle çalışan sistemlerden buharla çalışan makinelere geçişi sağlamıştır. Bu sayede, gıda üretiminde tekrarlayan görevlerin yerine getirilmesi için buhar makinesinin kullanılması ve özellikle ısıl işlem (pastörizasyon ve sterilizasyon) gibi buhar gücüne dayalı üretim süreçlerinin geliştirilmesi mümkün hale gelmiştir (Ane & Yasmin, 2019; Hassoun ve ark., 2023a).

Endüstri 1.0'ın en önemli etkilerinden biri, dünyanın ilk modern kooperatifi olan Rochdale'deki İngiliz kooperatifinin ortaya çıkmasıdır (Wardhiani ve ark., 2023).

### *İkinci Endüstri Devrimi (Endüstri 2.0)*

Endüstri 2.0, "bilimsel yönetim" veya Taylorizmin işbölümünü, montaj hatlarını ve elektrik enerjisi makinelerinin kullanımını tanıttığı 1870' ten sonraki döneme karşılık gelir (Belaud ve ark., 2019). Bu dönem elektriğin sanayide kullanılmasıyla kitlesel seri üretimin gerçekleşmeye başladığı dönemdir. Bu dönemde fizik, kimya gibi fen bilimleri alanındaki teknik gelişmelerin teknolojide kullanılması ile 1. Endüstri Devrimi'ne göre daha gelişmiş, kompleks aletler ortaya konmuştur. Örneğin, 1. Endüstri Devrimi'nde basit prensiplerle çalışan makinelerden (kasnakların kullanımı gibi) bu dönemde daha ileri teknolojinin kullanımıyla daha üstün performans elde edilmiştir. Diğer yandan ulaşım ve iletişim araçlarında görülen önemli gelişmeler sonucunda telgraf, demiryolu, kablo sistemleri, buharlı gemi gibi icatların ortaya çıkmasını sağlamıştır. Biçerdöver, dikiş makinesi, yüksek kapasiteli konserve ve paketleme aletleri bu dönemde icat edilen başlıca aletlerden bir kaçıdır (Berktaş & Oraklıbel, 2021; Lezoche ve ark., 2020; Pilevari, 2020).

Bu dönemden itibaren gıda sektörüne özgü makinelerin icadında önemli gelişmeler görülmüştür. 1930'lu yıllarda Norman Walker tarafından icat edilen meyva suyu ekstraksiyon makinesi, 1960'lı yılarda Karl Busch tarafından icat edilen vakum paketleme sistemleri, parça pastörizasyon sistemlerinden sürekli pastörizasyon sistemlerine geçiş bu dönemin gıda sektöründeki önemli gelişmelerdendir (Hassoun ve ark., 2023a).

### *Üçüncü Endüstri Devrimi (Endüstri 3.0)*

Elektronik, bilgi işlem ve robotik üretimin gelişmesiyle 20. yy, kalite ve maliyet performanslarına odaklanan endüstri 3.0 dönemi başlamıştır. Otomasyon, daha esnek, ergonomik ve daha güvenli makinelerin tasarımı yoluyla

üretim süreçlerini optimize etme ve üretkenliği artırma fırsatları sağlamıştır (Belaud ve ark., 2019).

Bu dönemde bilgi teknolojilerinin ve elektronik sistemlerin gelişimiyle üretim iyice otomatikleşmiş ve dünya dijitalleşmeye başlamıştır. İlk mikro bilgisayarların kullanımı ve Apple'ın kurulması 3. Endüstri Devriminin önemli olaylarındandır. Bu dönem ile birlikte endüstriyel işlemler büyük ölçüde bilgisayarlar aracılığıyla gerçekleştirilmeye ve 'saha çalışanı' sayısı azalmaya başlamıştır (Berktaş & Oraklıbel, 2021). 3. Endüstri Devriminde (1970'li yıllarda), mikroçiplerin geliştirilmesiyle proseslerin dijitalleştirilmesi sağlanarak gıda işleme hatalarının daha iyi kontrol edilebilmesi sağlanmıştır. Bu dönemde bilgisayar (programlanabilir ve otomatik özelliklere sahip) ve yeni ekipmanlarla sürekli ve daha kapsamlı işleme mümkün hale gelmiştir. Ayrıca robotlar ilk olarak gıda işlemede bu dönemde kullanılmıştır (yaklaşık 1990). Üçüncü endüstri devriminde aynı zamanda bitki ve baharatların mikrobiyal dekontaminasyonu için ışınlama (iyonlaştırıcı ve mikrodalga sistemleri) sistemlerinin geliştirilmesini sağlayan ilerlemeler de dikkat çekmiştir (Farkas & Mohácsi-Farkas, 2011; Hassoun ve ark., 2023a; Nayik ve ark., 2015).

***Dördüncü Endüstri Devrimi (Endüstri 4.0)***

Dördüncü sanayi devrimi olarak adlandırılan Endüstri 4.0, üretim sistemlerinin daha akıllı, özerk ve otomatik hale gelmesini sağlamıştır. Bu dönüşüm, bilgi ve iletişim teknolojilerinin olağanüstü değişimi ile karakterize edilmektedir. Geleneksel iş modellerinin yerini dijitalleşmeye dayalı yeni yaklaşımlar alırken, bu değişim yalnızca üretim süreçlerini değil, aynı zamanda tedarik zincirlerini ve toplumsal etkileşimleri de etkilemektedir (Gedik, 2021). Bilgisayarlar ve internet aracılığıyla makinelerin koordine edilerek üretim süreçlerinin otomatik olarak gerçekleştirilebildiği ve insan gücünün yerini makine/elektronik gücün almaya başladığı dönem olarak ifade edilebilir (Doğan, 2022; Hassoun ve ark., 2023a).

Endüstri 4.0'a uyum sağlamak, ülkeler ve işletmeler için hayati bir gereklilik haline gelmiştir. Türkiye gibi gelişmekte olan ülkelerde, bu uyum sürecinin nasıl gerçekleştirileceği ve hangi stratejilerin benimsenmesi gerektiği üzerine çeşitli öneriler geliştirilmiştir. Endüstri 4.0'ın temel bileşenleri arasında; siber-fiziksel sistemler, IoT, büyük veri, bulut teknolojileri ve otonom robotlar yer almaktadır. Bu teknolojilerin entegrasyonu, üretim süreçlerinin verimliliğini artırmakta ve işletmelere rekabet sağlaması bakımından önemlidir (Akben & Avşar, 2018).

Veri toplama ve analiz süreçlerinin iyileştirilmesi ile Endüstri 4.0 gıda israfının azaltılmasını, verimlilik ve gıda kalitesinin arttırılmasını sağlamaktadır. Bu yaklaşım ile gıda üretiminde şeffaflık ve izlenebilirlik sağlanmış olmaktadır. Gıda endüstrisinin şeffaflığını sağlayabilmek için izlenebilirlik ilkeleri kullanılmaktadır. İzlenebilirlik sistemleri, gıda şirketlerinin karmaşık tedarik zincirlerinde hareket etmelerine yardımcı olmakta ve tüketicilere anlaşılır bilgiler sunarak gıda israfını azaltma stratejileri geliştirmektedir (Chapman ve ark., 2022; Corallo ve ark., 2020; Hassoun ve ark., 2023a; Sadeghi ve ark., 2022).

Endüstri 4.0 ile diğer teknolojilere veya internete bağlı cihazlar ile tüm verilere erişim kolaylaşır. Ayrıca üretim alanındaki etkinlik sağlanırken makine teknolojisi insan yerine kullanılır, böylece üretim maliyetleri düşürülür. Kısa sürede, iyi kalitede ürün üretildiği için ulusal gelir artar ve bu teknolojiyi kullanmak için uzman insanlara ihtiyaç duyulur. Bu olumlu yönlerinin yanında Endüstri 4.0'de üretim sürecinde teknolojik makineler kullanıldığı için siber saldırılara karşı savunmasız olabilmesi, ekipman ve işçilere (eğitim verilmesi) büyük yatırım gerekmesi gibi olumsuz yönleri de bulunmaktadır (Wardhiani ve ark., 2023).

## Gıdada İzlenebilirlik ve Gıda İzlenebilirliğinde Endüstri 4.0 Uygulamaları

Gıda izlenebilirliği, Endüstri 4.0 çağında gıdaları bu yeni devrimi yansıtan teknikler ve teknolojilerle izlemekle ilgilidir (Hassoun ve ark., 2024a). Gıda izlenebilirliği; gıdanın kökeni, gıda bileşenleri, işlenmesi, taşınması ve depolanması koşullarıyla ilgili tüm bilgileri içerir ve gıda izlenebilirliğinin perakendecilere olası güvenlik ve tehlikelere anında yanıt verme fırsatı verebileceğini gösterir (Tao & Chao, 2024). İzlenebilirlik, gıda ürünleri ve bileşenlerinin birincil üretiminden işleme, dağıtım ve satışına, oradan da tüketiciye ulaşmasına kadar olan tüm aşamaları kapsayan, bu süreçteki belgeleme ve bağlantıların bir bütün olarak insan sağlığı için en yüksek düzeyde güvence ve kaliteyi sağlamayı amaçladığı bir süreçtir (Hassoun ve ark., 2024a; Yaralı, 2019).

Gıda sektöründe izlenebilirliğin sağlanabilmesi ve verimli bir şekilde sürdürülebilmesi ise etkin bir izlenebilirlik sisteminin kurulmasına bağlıdır. Gıda izlenebilirlik sistemleri, gıda üretim zincirindeki girdi ve ürünlerin hareketini yönetmede gıda endüstrisini destekleyen önemli araçlardır (Matloob ve ark., 2024). İzlenebilirlik sistemleri; ürünlerin takibinin yanı sıra tedarik zincirindeki paydaşlar için ürünlerin özellikleri, içeriği, geçmişi ve hareketleri ile bunların diğer ürünlerle olan ilişkileri hakkında bilgi edinilmesini sağlamaktadır (Scholten ve ark., 2016; Van der Vorst ve ark., 2005).

Gıda proteinlerinin (özellikle kas orjinli) yüksek bozulabilirlik riski ve kısıtlı raf ömrü ile ilgili sorunlar, çevresel zorluklar ve ekonomi prensipleriyle desteklenen yüksek kaliteli ve sürdürülebilir üretime yönelik tüketici talebini karşılamak üzere farklı yenilikçi işleme ve koruma teknikleri ile analitik metodolojik yaklaşımlar geliştirilmiştir. Bu gelişme, 2015'ten bu yana ivme kazanan ve bir dizi otomatik ve dijitalleştirilmiş teknolojiyle ortaya çıkan Endüstri 4.0 devam eden çağda geliştirilmiş ve artırılmıştır. Endüstri 4.0 teknolojilerine ve yüksek otomasyon ve dijitalleştirme oranlarıyla akıllı üretim elde etmedeki rollerine özel dikkat gösterilmiştir. Darbeli elektrik alanı, yüksek basınç işleme, ohmik ısıtma, nanoteknoloji, gelişmiş kütle spektrometrisi ve hiperspektral görüntüleme sensörleri gibi ortaya çıkan teknolojiler, mevcut gıda devrimi 4.0'ın temel unsurları arasındadır. Endüstri 4.0 sayısız olasılık sağlasa da, tam potansiyelini yakalamak ve mevcut zorlukları çözmek ve Endüstri 5.0' e doğru ilerlemek için daha fazla çalışmaya ihtiyaç vardır (Hassoun ve ark., 2024b).

Gıda İzlenebilirliği; gıdanın özgünlüğünü, güvenliğini ve yüksek gıda kalitesini garanti altına almak amacıyla Endüstri 4.0 teknolojilerinin uygulanmasını ifade eder (Hassoun ve ark., 2023b). Günümüzde yapay zeka, büyük veri, akıllı sensörler, 3D gıda baskısı, nesnelerin interneti,

blokzincir ve bulut bilişim gibi Endüstri 4.0'ün temel teknolojileri gıda sektöründe gıda kalitesi, güvenliği, izlenebilirliği ve sürdürülebilirliği vb. lerini sağlamak amacıyla yaygın bir şekilde kullanılmaktadır. Endüstri 4.0' ün bu uygulamaları dijital, fiziksel ve biyolojik gıda bilimlerinin birleşimiyle karakterize edilmektedir (Hassoun & Bigliardi, 2024). Endüstri 4.0 çağındaki dijital teknolojiler, gıdanın izlenme şeklini iyileştirme, gıda israfını azaltma ve dolandırıcılığa karşı hassasiyeti azaltma konusunda önemli bir potansiyele sahiptir ve daha akıllı gıda izlenebilirliği elde etmek için yeni fırsatlar sunmaktadır (Hassoun ve ark., 2023b). Akıllı gıda izlenebilirliğinin, gıda omikleriyle (yani gıdaların besin değerlerini, kalitesini ve özgünlüğünü ve ayrıca güvenliğini ve emniyetini kapsayan gıda parmak izi) ilgili küresel zorlukların üstesinden gelmeye önemli ölçüde yardımcı olduğu bilinmektedir (Meliana ve ark., 2024).

**Tanımlama ve İzleme Teknolojileri:**

İlk Evrensel Ürün Kodu barkodları; 1970'lerde uygulamaya girmiştir. Makine tarafından okunabilen optik etiketler, okuyucular ve standartlardan oluşmaktadır. Düşük maliyetleri ve kullanım kolaylıkları nedeniyle barkodlar, kolay envanter kontrolü, yeniden stok siparişi ve ödemeyi kolaylaştırmak için büyük ölçekli perakende ticaretinde ve mağazalarda giderek daha fazla kullanılmıştır. İlk önce tek boyutlu (1D) barkodlar geliştirilmiştir. Daha sonra geliştirilen iki boyutlu (2D) barkodlar, tek barkodlara kıyasla daha fazla miktarda bilginin depolanmasına olanak sağlamıştır (Ghaani ve ark., 2016).

DNA barkodlama, 2008'den beri tıbbi bitkilere uygulanan yeni bir moleküler tanımlama ve sınıflandırma teknolojisidir. Bu tekniğin uygulanması, tıbbi malzemelerin güvenliğini ve etkinliğini büyük ölçüde sağlamıştır (De Mattia ve ark., 2011; Yu ve ark., 2021). "DNA barkodlama" kavramı, bitki tanımlamasıyla ilgili birçok sorun için kapsamlı bir çözüm bulmuş ve bitkisel endüstriyi, biyolojik çeşitlilik sınıflandırmasını ve hatta taksonominin yeniden doğuşunu olumlu yönde etkilemiştir (Mahima ve ark., 2022). DNA barkodlama, biyolojik örnekleri tanımlayabilen ve hem ham maddelerin hem de işlenmiş gıdaların tanımlanmasında yaygın olarak kullanılan moleküler tabanlı bir sistemdir. Gıdaların orijinalliğinin doğrulanması çoğunlukla proteinlerin ve/veya DNA dizilerinin analizine dayanmaktadır (Galimberti ve ark., 2013). İzlenebilirliği ve etiketleme doğruluğunu araştırmak amacıyla yapılan bir çalışmada Tayland'da satılan toplam 54 balık fileto ürünlerinin türlerini belirlemek için bir DNA barkodlama sistemi kullanılmıştır (Panprommin & Manosri Citation, 2022). ABD ticari pazarında satılan çeşitli kıyma ürünlerinde olası yanlış etiketlemelerin varlığını test etmek için ayrıca sızma zeytinyağlarının özgünlüğünü, izlenebilirliğini ve sahteciliğini incelemek amacıyla DNA barkodlama kullanılmaktadır (Ben Ayed ve ark., 2022).

Radyo Frekansı Tanımlama (RFID); dost veya düşman askeri uçaklarının tanımlanması için ikinci dünya savaşı (1939-1945) sırasında ortaya çıkmıştır. Tanımlama alanındaki en önemli teknolojilerden biri olan RFID, özellikle radyo frekansı dalgaları aracılığıyla, bir nesneye takılı bir etiket ile bir sorgulayıcı arasındaki kablosuz iletişime dayanmaktadır. Bu sistem, barkodlar gibi diğer tanımlama sistemlerine kıyasla ürün tanımlama için daha uygundur. RFID etiketleri görsel temas gerektirmediği için kutulara, kaplara yerleştirilebilir, hayvanlara enjekte edilebilir ve pasaportlar gibi herhangi bir nesnede kullanılabilirler (Bibi ve ark., 2017). Özellikle çeşitli tedarik zincirlerinde nesneleri izlemek ve takip etmek için izlenebilirlik sistemlerinde kullanılabilen iyi bilinen ve yaygın olarak benimsenen otomatik bir tanımlama teknolojisidir. Ayrıca bozulabilir ürünler için gerçek zamanlı izleme ve karar destek sistemi için başarıyla uygulanmıştır (Alfian ve ark., 2017; Mehannaoui ve ark., 2023). RFID etiketleri, veri taşıyıcı bir cihazın en gelişmiş örneğidir ve küçük bir antene bağlı bir mikroçip tarafından oluşturulan bir etiket; radyo sinyalleri yayan ve karşılığında etiketten yanıtlar alan bir okuyucu; ve RFID donanımı ile kurumsal uygulamaları birbirine bağlayan bir ara yazılım (yerel ağ, web sunucusu, vb.) olmak üzere üç ana öğeden oluşur (Ghaani ve ark., 2016). Endüstri 4.0' da, tedarik zincirinin gerçek zamanlı görünürlüğünü, kontrolünü ve çeşitli üretim süreçlerinin otomasyonunu sağlamak amacıyla bu teknolojiden yararlanılmaktadır (Soori ve ark., 2024).

Endüstri 4.0'ın bir diğer kritik bileşeni akıllı sensörlerdir. Düşük maliyet ve güç kullanımı, taşınabilirlik, kendini tanımlama ve kalibrasyon gibi çeşitli özellikler akıllı sensörleri karakterize etmektedir. Günümüzde, tarımda ve gıda endüstrisinde farklı tipte optik (kolorimetrik, flüorometrik…) ve elektrokimyasal (potansiyometrik, amperometrik ve kondüktometrik) sensörler/biyosensörler ve göstergeler yaygın olarak kullanılmaktadır (Hassoun ve ark., 2024c). Biyosensördeki sensör, biyometrik sinyalin, biyosensörlerin akıllı teknolojilerle etkileşime girmesini sağlamak için kritik öneme sahip olan niceliksel bir elektrik formülüne dönüştürür. Bu durum Gıda Güvenliği 4.0'ün veri odaklı çözümlerinin önemli bir parçası haline gelmiştir. Akıllı sensörler saha testlerinde önemli bir rol oynar ve biyosensör okumalarının doğruluğu ve güvenilirliğini sağlamak için düzenli kalibrasyon ve bakım yapılması gerekmektedir. Bakım ve optimum biyosensör performansını sürdürmek için kalifiye personel gerekmektedir (Chen ve ark., 2024).

Kablosuz Sensör Ağı (WSN); sabit veya mobil çok sayıda küçük sensör düğümünden oluşan ve bu düğümler arasında kablosuz iletişime sahip bir ağdır ve tedarik zincirindeki kritik gıda parametrelerinin gerçek zamanlı izlenmesi için kullanılır (Alfian ve ark., 2017; Mehannaoui & Mouss, 2019; Mehannaoui ve ark., 2023). Tedarik zincirinde bozulabilir gıda ürünlerinin kalitesinin izlenmesi için kablosuz sensör ağlarının sıcaklık izleme amacıyla kullanılması büyük önem taşımaktadır (Alfian ve ark., 2017).

**Veri Yönetim Teknolojileri:**

Veri Yönetimi Teknolojileri, veri analizi, entegrasyonu, depolama, paylaşım ve güvenliği içerir. Bunlar:

*a) Bulut bilişim;* çok sayıda sunucu makinesinin dijital bir ağ üzerinden, bütünleşik bir sistem olarak uyumlu bir şekilde çalışmasını ifade eder. 'Bulut', kullanıcıların talep üzerine erişebildiği, asgari yönetim müdahalesine sahip

ağlar, sunucular, uygulamalar, depolama alanları ve hizmetlerden oluşan sanallaştırılmış bir ortamdır (Sapkal & Kusi, 2024). Bulut hizmetleri, internet üzerinden yüksek bilişim kullanımına izin vermektedir. Bulut çözümleri ön maliyet gerektirmediği için şirketler çok fazla para harcamadan, gelişmiş bilişim kullanabilirler ve bu da yeni kurulan şirketlerin artmasını sağlamaktadır. Bulut bilişim, büyük, tek seferlik harcamaları daha küçük, düzenli maliyetlere dönüştürerek daha uygun fiyatlı hale getirmektedir. Bu durum da, küçük ve orta ölçekli işletmelerin daha önce karşılayamadıkları gelişmiş teknolojiyi kullanmalarına olanak tanımaktadır (Mistry ve ark., 2024).

*b) Blok Zincir:* Blok Zincir, verilerin dijital ortamda tarih sırasına göre birbirine zincir gibi bağlı bloklar şeklinde işlendiği ve saklandığı bir tür kayıt defteri olarak tanımlanmaktadır. Blok Zincirde kaydedilen veriler, her işlem sırasında birbirleriyle hash adı verilen çeşitli algoritmalar yardımıyla ilişkilendirildiğinden dışarıdan müdahale ile değiştirilmesi zordur. Bu nedenle taraflar için oldukça güvenilir ve şeffaf bilgiler sağlamaktadır (Sri Vigna Hema & Manickavasagan, 2024; Yu ve ark., 2024). Blok zincir, gıda izlenebilirliğinde tüketicinin bilgilendirilmesinin yanı sıra gıda sektörüne ilişkin araştırmalar için önemli bir veri tabanı sunabilen teknolojidir (Gerdan ve ark., 2020). Gıda sektöründe Blok Zincir teknolojisinin ana uygulama alanı, tedarik zincirindeki izlenebilirlik sistemiyle ilgilidir ancak bununla birlikte, çiftlikten sofraya gıda tedarik zinciri yönetim sisteminde gıda usulsüzlüklerinin izlenmesi ve saptanması ve gıda güvenliğinin doğrulanması için de önemli bilgiler sağlayabilir. Bu durum mevcut sistemlerin güvenilirliğini sorgulanır hale getirmektedir. Blok Zincir uygulaması ürünün seri numarası, üretim yeri ve tarihi, gıda güvenliği sertifikasyonu, üretim sahasının gerçek zamanlı hijyenik ve sağlığa uygunluk durumu gibi ürün verilerinin zamanında ve tahrifata uğramamış bir şekilde paylaşılması yoluyla gıda güvenliğini sağlamakta ve alıcının güvenini etkili bir şekilde teşvik etmektedir. Bilindiği üzere gıda tedarik zincirinde gıda ürünleri üretim, işleme, nakliye, dağıtım ve perakende işlemlerinden tüketiciye ulaşıncaya kadar birçok aşamadan geçmektedir (Tripoli & Schmidhuber, 2020; Patel ve ark., 2023). Blok zincir; güvenilir izlenebilirlik sistemlerini etkinleştirerek özgün, kaliteli ve güvenli gıdayı garanti eder; aynı zamanda gıda tedarik zinciri risklerini azaltır ve taleplerin daha iyi tahmin edilmesini sağlar (Hassoun ve ark., 2023b).

*c) Büyük Veri:* Büyük Veri terimi, geleneksel bilgisayar teknolojisinin potansiyellerini aşan ve karmaşık veri analiz araçları komplekslerinin yardımıyla çıkarılması gereken yönetim ve analiz teknolojilerinin devasa bilgilerini ifade eder (Mehannaoui ve ark., 2023). Orijinal verilerdeki değer bilgisinin keşfi, veri kümelerinin potansiyel değerinin çıkarılabileceği "ayrık veriler - entegre veriler - bilgi anlayışı - mekanizma çıkarma - uygulama etkisi analizi" döngüsünden geçmektedir. Gıda endüstrisindeki büyük veri uygulamasının işleme sistemi; büyük veri toplama, büyük veri işleme ve birleştirme, büyük veri madenciliği ve analizi, büyük veri görüntüleme ve büyük veri güvenliği olmak üzere beş modülden oluşmaktadır (Tao ve ark., 2021). Büyük Veri, şirketlerin sattıkları ürünlerde orijinalliği ve gıda sahtekarlığını tespit etmesini sağlamada önemli bir rol oynamaktadır (Pollard ve ark., 2019). Ayrıca geniş çapta ve etkili bilgi sunarak karar verme süreçlerini geliştirir, gıda güvenliği ve kalitesini artırır (Hassoun ve ark., 2023b).

*d) Yapay Zekâ:* Gıda izlenebilirlik sistemlerindeki yapay zekâ, kalite güvencesinde şeffaflık sağlar. Yapay zekâ ve IoT büyük verilerini kullanarak, gıda güvenliği izlenebilirlik sistemleri, yetersiz güvenilirlik ve karmaşık veri depolama gibi geleneksel izlenebilirlik sistemleriyle bağlantılı sorunları etkili bir şekilde çözebilir (Chhetri, 2024). Bu bağlantı, gıda maddelerinin soyunun sorunsuz bir şekilde izlenmesini kolaylaştırır ve gıda güvenliği ve kalitesiyle ilgili verilerin netliğini ve güvenilirliğini iyileştirmek için yöntemler sunar. Uzmanlar, gıda endüstrisine yapay zeka tekniklerinin dahil edilmesinin, gıda ürünlerinin kalitesinin değerlendirilmesine yardımcı olabileceğini ve doğru ve tutarlı değerlendirmeler için fırsat sağlayabileceğini önermektedirler (Wei ve ark., 2023; Ikram ve ark., 2024). Ayrıca otomasyon ve dijitalleşmenin gelişimini destekler, verimliliği artırır ve israfı azaltır (Hassoun ve ark., 2023b).

*e) Nesnelerin İnterneti (IoT):* IoT, ortak bir platformdaki sensörleri kullanarak çevreleri ve faaliyetleri hakkında veri toplayan ve paylaşan, birbirine bağlı cihazlardan oluşan geniş bir ağı ifade etmektedir. Ağda bulunan cihaz ve platformların bağlantısı internet aracılığıyla gerçekleşmektedir. IoT uygulaması, gıda tedarik zinciri yönetiminde tarım ve taşımacılık için kullanılan ekipman ve cihazların kontrol edilmesi, talep ve üretimin tahmin edilmesi, ürün ve ekipman akışının izlenmesinin yanında; çevreyi, mahsulü, gübreyi, hava durumunu, su yönetimini ve verimliliğini izlemek gibi amaçlarla kullanılmaktadır (Khan ve ark., 2023). Ayrıca pestisitlerin tespit edilmesini ve gıda güvenliğini sağlarken, olgunlaşma ve diğer kalite özelliklerini tahmin ederek gıda standartlarına uygunluğu temin eder (Hassoun ve ark., 2023b).

IoT'un amacı; gerçek zamanlı olarak veya gelecekte daha iyi kararlar almak amacıyla algılama ve tanımlama yoluyla verilerin toplanmasından, analiz edilmesi, işlenmesi ve depolanmasına kadar tüm işlemlerin gerçekleştirilmesi için çeşitli ve farklı akıllı cihazları insan müdahalesi olmadan birbirine bağlamaktır. Gıda izlenebilirliğinde IoT tabanlı teknolojileri; tanımlama ve izleme, iletişim ve veri yönetim teknolojileri olarak gruplandırabiliriz (Mehannaoui ve ark., 2023).

*f) Robotik Sistemler*; endüstride çeşitli görevleri yerine getirmek için kullanılan yenilikçi bir teknolojidir. Robotik, doğru uygulandığında bir şirketin faaliyetlerinde olumlu yönde değişim sağlar. Normal iş akışını olumlu etkiler, genel montaj iş akışını kolaylaştırır ve yiyecek üretmek için robotik sistemlerden yararlanılır (Javaid ve ark., 2021).

*g) 3 Boyutlu Baskı (3D) Teknolojisi:* 3 boyutlu nesnenin ilk parçası 1983 yılında 3 boyutlu baskıyı icat eden Chuck Hull tarafından basılmıştır (Yang ve ark., 2017). 3 boyutlu baskı, katmanlı üretim ve hızlı prototipleme olarak da adlandırılan tıp, gastronomi, mühendislik, imalat, sanat ve eğitim gibi sürekli büyüyen uygulama alanlarıyla araştırmacılar, endüstri ve kamuoyunun ilgisini çeken, günlük tartışmalara konu olan dijital bir teknolojidir (Dankar ve ark., 2018). Gıda sektöründe 3D baskı teknolojisinin uygulanması, birçok potansiyel avantaj sunmaktadır. Bu avantajlar arasında özelleştirilmiş gıda

tasarımları, kişiselleştirilmiş ve dijitalleştirilmiş beslenme, tedarik zincirinin basitleştirilmesi ve mevcut gıda malzemelerinin kaynağının genişletilmesi yer almaktadır. 3D gıda baskısı, geleneksel yöntemlerle elde edilmesi zor olan karmaşık ve yaratıcı gıda tasarımlarının üretilmesine olanak tanımaktadır. Bu teknoloji, aşçılar, beslenme uzmanları ve gıda tasarımcılarının mutfak bilgisi ve sanatsal becerilerini kullanarak, önceden belirlenmiş veri dosyalarına dayalı olarak herkes tarafından da kullanılabilir hale gelmektedir (Liu ve ark.,, 2017).

**Endüstri 4.0 ile İlgili Yapılan Çalışmalar**

Pal ve Kant (2018) tarafından yapılan bir çalışmada, IoT tabanlı mekanizmaların, taze gıda tedarik zincirinde merkezi veri toplama ve analitiği ile önemli iyileştirmeler sağlayabileceği ifade edilmiştir. Ancak, bu tür teknolojilerin entegrasyonu, mevcut sistemlerin uyumlu hale getirilmesi ve çalışanların bu yeni sistemlere adapte olması gerekliliği ortaya çıkmaktadır (Pal & Kant, 2018). Esmer ve Melikoğlu (2015), yapmış oldukları araştırma ile RFID teknolojisinin çalışma prensibine değinerek gıda sektöründeki uygulama alanlarını araştırmışlar ve gıda tedarik zinciri yönetimi, gıda sıcaklık ve tazeliğinin izlenmesinde kullanımına yönelik RFID tabanlı akıllı etiketlere ait deneysel ve endüstriyel çalışmaları değerlendirmişlerdir. Ediz (2021), gıdada izlenebilirliği basit ve kolay bir şekilde sağlamak amacıyla gıda şirketlerinde ortak kullanılabilecek bir algoritma ve veri tabanı önermiştir. Yıldızbası ve Üstünyer (2019), blok zincir teknolojisinin temel prensiplerini ve bu teknolojiden tedarik zincirinde nasıl yararlanılabileceğini irdelemişlerdir. Araştırmacılar blok zincir teknolojisini tedarik zinciri yönetim sürecine entegre ederek, önerdikleri model ile ülkemizde mevcutta hal yasası uygulamaları kapsamında gerçekleştirilen gıda ürünleri tedariğinin, üçüncü bir tarafa ihtiyaç duyulmadan üretici ve tüketici arasında gerçekleştirilebileceği ve gıdada izlenebilirliğin sağlanabileceğini ileri sürmüşlerdir. Keleş ve Ova (2020), yapmış oldukları araştırmada kurumsal kaynak planlaması, RFID, IoT ve blok zincir bilgi teknolojileri sistemlerini ve bunların bunların gıda sektöründe uygulamalarını irdelemişlerdir. Xu ve ark. (2020), tarımsal gıdalar için blok zincir uygulamalarını gıdada izlenebilirliği, güvenliği, kalitesi ve şeffaflığı açısından incelemişlerdir. Araştırmada blok zincir teknolojisinin çalışma prensiplerinden bahsederek blok zincirin gıda izlenebilirliğinde kullanılabilirliğini göstermek için bir şirket üzerinde vaka çalışması gerçekleştirmişlerdir. Sonuç olarak; blok zincirin tarımsal gıda sektörü için izlenebilirlik açısından umut verici çözümler ortaya koyduğu ve bazı tarımsal ürünler için hem teknik hem de düzenleyici olmak üzere birçok zorluğun çözülmesi gerektiği belirtilmiştir.

Cebeci ve Arat (2022), blok zincir teknolojisinin tarım ve gıda tedarik zincirine entegre edilmesinin artı yönlerini ve bu teknolojiden nasıl yararlanılabileceğini araştırmışlar ve gelecekteki kullanım etkisini tartışmışlardır. Indap (2022), blok zincir teknolojisinin tarım-gıda tedarik zincirinde izlenebilirlik, şeffaflık, gıda güvenliği, kaybı ve sürdürülebilirlik sorunlarını çözmedeki rolünü ve kullanılabilirliğini araştırmıştır. Araştırmacı çalışmada blok zincir teknolojisinin tarım ve gıda tedarik sektöründeki kullanımı üzerine paydaşların farkındalığını araştırmış ve sektörün dijital yatırım önceliklerini belirlemeye çalışmış ve tedarik zincirinde nasıl bir süreç modeli ile uygulanabileceğini incelemiştir. Çalışmada geliştirilen analitik çerçeve ve bulguların farklı sektörlere ve tarım-gıda tedarik zincirlerinin farklı alt sektörlerine uyarlanabilir nitelikte olduğunu belirtmiştir. Rejeb ve ark., (2022), büyük veri uygulamalarının gıda sektöründeki kullanılabilirliğini belirlemek için literatürdeki akademik araştırmaları sistematik bir şekilde incelemişlerdir ve sağlayabileceği yararları değerlendirmişlerdir. Araştırmacılar, büyük veri teknolojisinin gıda güvenliği için önemli faydalar sağlayabileceğini belirterek, ileride gerçekleştirilecek çalışmalara ilişkin önerilerde bulunmuşlardır. Yakubu ve ark. (2022), pirinç tedarik zincirinde blok zincir teknolojisinden yararlanarak tüketicilerin memnuniyet geri bildirimlerini içeren ve pirinç sektöründeki tüm paydaşlar tarafından gerçekleştirilen işlem ve etkileşimlerin takip edilebildiği bir model önermişlerdir. Araştırmacılar yaptıkları deneysel analiz sonucu; modelin güvenlik, maliyet verimliliği ve ölçeklenebilirlik açısından literatürdeki benzer yöntemlerden daha iyi bir performans gösterdiğini belirtmişlerdir. Kutyauripo ve ark. (2023), tarımsal gıda sektöründe yapay zekâ tabanlı yöntemlerin uygulamalarını incelemişlerdir. Araştırmacılar literatürdeki çalışmaları gıda tedarik süreçleri açısından sistematik bir şekilde analiz ederek, çeşitli yapay zekâ algoritmalarının gıda tedarik zincirinin tüm aşamalarında uygulanabilir olduğunu ifade etmişlerdir. Sentürk ve ark. (2023), IoT tabanlı sistemlerin temel uygulamalarını ve bu teknolojinin tarım ve gıda tedarik zinciri yönetimi vb. alanlardaki başarılı uygulama örneklerini inceleyerek son gelişmeleri değerlendirmişlerdir. Cömert ve ark. (2024), blokzincir teknolojisinin gıda tedarik zincirindeki kullanımını ve faydalarını araştırmak amacıyla ülkemizde blokzincir teknolojisini kullanan bir şirket üzerinde örnek olay incelemesi gerçekleştirmişlerdir. Çalışma kapsamında nitel araştırma vaka çalışması tasarımı kullanılarak İstanbul ve İzmir'den uzman katılımcılara gıda tedarik sürecinde blokzincir uygulamalarını da içeren önceden tasarlanan temalar hakkındaki görüşleri sorulmuş, ve toplanan verilerin betimsel analizleri yapılmıştır. Araştırmacılar çalışma sonucunda blok zincir teknolojisinin şeffaflık ve izlenebilirlik prensiplerinin, tüketiciler ve tedarik zinciri paydaşları üzerinde olumlu etkiler ortaya çıkardığını ve gıda tedarik süreçlerinde kullanımının yaygınlaştırılması gereken verimli bir sistem olduğuna işaret etmişlerdir. Hassoun ve ark. (2024b), yeni bir araştırma konusu olarak gıda izlenebilirliği 4.0 kavramından bahsederek Endüstri 4.0 teknolojilerinin gıda izlenebilirliğindeki kullanımını araştırmışlardır. Çalışmada blok zincir, nesnelerin interneti, yapay zekâ ve büyük veri uygulamalarının gıda izlenebilirliğini sağlamada ve gıda israfı ile sahtekârlığını önlemede bu teknolojilerin önündeki mevcut engellere rağmen gelecekte bu uygulamaların geliştirilmesiyle birlikte önemli fırsatlar sunabileceği ileri sürülmüştür. Ikram ve ark. (2024), yapay zekâ uygulamalarının gıda sektöründe kullanımını, avantajlarını ve dezavantajlarını değerlendirerek yapay zekânın gıda güvenliğini sağlamadaki potansiyelini araştırmışlardır. Araştırmacılar, yapay zekâ uygulamalarının gıda sektörü için izlenebilirlikte de karmaşık sorunların çözümünde önemli bir rol oynayabileceğini belirterek yapay zekâdan gıda

sektöründe daha fazla yararlanmaya yönelik çalışma sayısının arttırılması gerektiğine işaret etmişlerdir. Ghag ve ark. (2024), yapay zekanın gıda tedarik zincirine entegre edilmesinin önündeki mevcut engelleri literatürün analizi ile araştırarak bu engelleri kategorilere ayırmışlardır. Araştırmacılar sorunun çözümü için yeni bir yöntemsel yaklaşım önerisinde bulunmuştur. Ekstrüzyona dayalı baskı kullanarak kek kreması, işlenmiş peynir ve şekerli kurabiyelerin üretiminin incelendiği bir araştırmada gıda ürünlerinin 3D baskı ile nasıl özelleştirilebileceğini ve bu süreçte kullanılan malzemelerin özelliklerinin önemini ortaya konmuştur. Çalışma sonucuna göre, kek kreması gibi yumuşak gıda maddelerinin baskı sürecinde, malzemenin viskozitesi ve akışkanlık özellikleri, baskı işleminin başarısını etkileyen temel faktörler arasında yer aldığı ifade edilmiştir (Hussain ve ark., 2021).

## Sonuç

Gıda sektöründe Endüstri 4.0 uygulamaları, üretim süreçlerinin verimliliğini artırma, maliyetleri düşürme ve gıda güvenliğini sağlama konusunda büyük bir potansiyele sahiptir. Akıllı üretim sistemleri, IoT, yapay zeka, büyük veri analitiği ve robotik teknolojiler gibi Endüstri 4.0 bileşenleri, daha hızlı, güvenilir ve izlenebilir üretim süreçlerinin gerçekleştirilmesini sağlar. Bu teknolojiler, gıda ürünlerinin izlenebilirliğini ve kalitesini etkin bir şekilde kontrol etme imkânı sunmaktadır. Böylece, tüketicilerin güvenliği ön planda tutulmakta ve kontaminasyon riski en aza indirilmektedir. Özellikle sensörler ve IoT sistemleri, üretim aşamasında anlık verilerin toplanıp analiz edilmesine de olanak tanımaktadır. Bu sayede potansiyel tehlikeler erken aşamada tespit edilerek, önleyici tedbirler alınabilmektedir. Endüstri 4.0 uygulamaları ayrıca tedarik zincirindeki şeffaflık ve verimliliği artırarak, gıda ürünlerinin daha güvenli bir şekilde tüketicilere ulaşmasını sağlamaktadır.

Ancak, Endüstri 4.0'ın gıda sektörüne entegrasyonu bazı zorluklarla karşı karşıyadır. Bu teknolojilerin etkin bir şekilde benimsenebilmesi için yüksek başlangıç yatırımları, altyapı iyileştirmeleri ve sürekli eğitim gerekmektedir. Ayrıca, veri güvenliği ve gizliliği gibi önemli meseleler de dikkatle ele alınmalıdır, çünkü gıda güvenliği ile ilgili verilerin doğru ve güvenli bir şekilde yönetilmesi kritik öneme sahiptir. Küçük ve orta ölçekli şirketler arasında Endüstri 4.0 teknolojilerinin uygulanabilirliğinin tesbiti için araştırmalar ve çalışmalar yapılması önerilmektedir.

Sonuç olarak, Endüstri 4.0 teknolojilerinin gıda sektöründe uygulanması, gıda güvenliğini artırma ve genel sektörel verimliliği iyileştirme açısından önemli fırsatlar sunmaktadır. Ancak, bu süreçlerin başarılı olabilmesi için teknoloji ile insan faktörünün uyumlu bir şekilde çalışması, etkili stratejiler ve düzenlemelerle desteklenmesi gerekmektedir. Endüstri 4.0'e uyum sağlamak için siber güvenlik önlemlerinin alınması ve akıllı üretim sistemlerinin entegrasyonu gerekmektedir.

## Beyanlar

### *Etik Onay Belgesi*

Bu çalışmada etik kurul gerekmemektedir.

### *Yazar Katkı Durumları*

D.B.K: Kaynak araştırması, orjinal taslağın yazılması, inceleme, düzenleme

H.T: Kaynak araştırması, orjinal taslağın yazılması.

### *Fon Beyanı*

Çalışmada destek alınmamıştır.

### *Çıkar Çatışması*

Yazarlar arasında herhangi bir çıkar çatışması bulunmamaktadır.

## Kaynaklar

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