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The Functional and Rheological Properties the Mesocarp Layer of the Oleaster (*Elaeagnus angustifolia* L.) grown in Karaman

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ABSTRACT

The oleaster (*Elaeagnus angustifolia* L.), also known as wild olive, is a small fruit with three parts: the outer peel or exocarp layer, the edible part or mesocarp layer, and the inner seed or endocarp layer. The mesocarp layer is rich in essential vitamins and has great potential for use in various food products. The flour made from the mesocarp layer has a moisture content of 8.99%, an ash content of 2.66%, a fat content of 0.55%, a protein content of 5.99%, a crude fiber content of 3.32%, and a total dietary fiber (TDF) content of 26.36%. The TDF content is divided into insoluble dietary fiber (IDF) and soluble dietary fiber (SDF), which are 21.35% and 5.01%, respectively. The flour has color values of L*: 75.14, a*: 2.86, b*: 23.87, and a water activity value of 0.314. The water solubility, water absorption, and oil absorption are 67.33%, 4.91 g water/g sample, and 2.26 g oil/g sample, respectively. Additionally, the mesocarp layer contains minerals such as Mg, P, K, Ca, Fe, and Na. The mesocarp layer significantly affected the thermomechanical properties of wheat flour. As the substitution level of the mesocarp layer increased from 10 to 30%, the water absorption capacity, dough development time and stability time of the wheat dough significantly decreased. Specifically, the water absorption capacity dropped from 53.5% to 47%, dough development time reduced from 1.10 to 0.75 min, and stability time decreased from 8.90 to 2.25 min. Substituting a mesocarp layer in wheat flour can significantly improve product shelf-life due to slower retrogradation. The mesocarp layer is an functional ingredient in the food industry.

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Introduction

Oleaster fruit, also known as wild olive, is a small-sized fruit that belongs to the Elaeagnaceae family. Originating from Turkey, this fruit is composed of three distinct parts, namely, the peel (exocarp), the edible portion (mesocarp), and the seed (endocarp). In the field of alternative medicine, all parts of the oleaster fruit are utilized for their various health benefits. The fruit is commonly consumed in the form of tea or powder, and is known to aid in alleviating several health conditions, such as nausea, vomiting, flatulence, gastric disorders, asthma, jaundice, and muscle relaxation. Moreover, oleaster fruit is loaded with bioactive compounds that possess antibacterial, anti-inflammatory, and antinociceptive properties. These properties make it an excellent natural remedy to fight infections and relieve pain. The mesocarp layer, in particular, has caught the attention of researchers in recent years, and has been studied for its potential use in various food products. The mesocarp of oleaster fruit is rich in essential vitamins, minerals, and dietary fiber, making it a valuable ingredient in several food products. Researchers have incorporated the fruit into various products such as biscuits, breakfast cereals, yogurt, gluten-free cakes, and bread (Şahan et al., 2013; Öztürk et al., 2018; Zangeneh et al., 2021; Tatari et al., 2022; Yavuz et al., 2022). This is primarily due to its ability to enhance the nutritional, functional, and sensory attributes of the food. With its numerous health benefits, oleaster fruit is fast becoming recognized as a superfood, and is a great addition to one's diet.

Composite flour has recently emerged as a promising alternative to traditional wheat flour for food production. Its use is primarily driven by the desire to enhance the functional and technological properties of food products while also generating economic benefits. Composite flour is created by blending multiple sources, including mango peel powder, apple peel powder, and apricot flour. To summarize, a few of them are as follows. Ajila et al. (2008) studied mango peel powder, a rich source of dietary fiber with health-promoting and functional properties to increase the nutritional properties of biscuits. Adding different levels of mango peel powder (5.0, 7.5, 10.0, 15.0

and 20.0%) enhanced the dietary fiber and antioxidant capacity properties of the biscuits. Rupasinghe et al. (2008) reported that apple peel powder, a very high dietary fiber content, increased phenolic substance and antioxidant activity of the muffins. Adding up to 24% of apple peel powder preserved most physical properties except the color properties. Ozbas et al. (2014) used apple and apricot kernel flours by replacing 10-40% wheat flour in biscuit formulation to investigate the effects of fruit powders on low-fat biscuit quality. In both additive types, the total dietary fiber amount increased due to increased concentration. It was also determined that adding 30% apricot powder was a better fat substitute than apple powder. It has also been reported that apricot flour can produce low-fat bakery products. The versatility and numerous benefits of composite flour have led to its growing popularity in functional foods and nutraceuticals. As such, it has become an increasingly important area of research for the food industry, with many companies and researchers exploring new ways to develop and utilize composite flour in the production of healthier, more nutritious, and more sustainable food products.

This research delves into the characteristics of the mesocarp portion of oleaster cultivated in Karaman, focusing on its physical, technological, functional, and dough rheological properties. The study offers valuable insights into the possible applications of oleaster in various industries. The results indicate that oleaster boasts significant properties that can be utilized for a wide range of purposes. This investigation is an important step in understanding the qualities and benefits of oleaster.

Material and Methods

Materials

The oleasters (*Elaeagnus angustifolia* L.) were collected from three different locations in Karaman, Turkey, in 2021. To analyze the samples, they were mixed and dried in an oven at 22-24 °C for 48 hours (Nüve, FN055, Ankara) and blended for 2 seconds in a Waring blender. The oleaster samples were then separated into three layers using sieving, which included the exocarp (upper 1000 microns), mesocarp (under 300 microns), and endocarp layers (upper 300 microns and under 1000 microns). For this study, the mesocarp layer was used, while wheat flour was used as a control.

Methods

The proximate composition of mesocarp layer

The mesocarp layer of oleaster underwent chemical analyses to determine its proximate composition. The AACC methods 44-15A, 08-01, 46-12, 30-25, and 32-10 were followed according to the standard procedures of AACC (2002) to measure moisture, ash, protein, fat, and crude fiber, respectively. Additionally, the dietary fiber contents of the mesocarp layer were analyzed based on AOAC 960.43 using the Megazyme Dietary Fiber Determination Kit (K-TDFR-100A, Ireland). The insoluble (IDF), soluble (SDF), and total (TDF) dietary fiber contents were determined, and the amount of SDF was calculated by subtracting the IDF from the TDF (AOAC, 2005).

Color analysis

The color analyses of the mesocarp layer (L*:lightness or darkness, a*:redness or greenness, and b*: yellowness or blueness) were conducted with the colorimeter (HunterLab Color Flex, USA). The results were given as means of triplicate measurements.

Water activity analysis

The water activity of the mesocarp was determined utilizing a Novasina (Labmaster device aw, Switzerland).

Water absorption, oil absorption and water solubility indices of the mesocarp layer

The experiment was conducted to ascertain the water and oil absorption indices (WAI & OAI) of the mesocarp layer by the Sharma et al. (2014) method. A slight modification was implemented in the method. Specifically, 0.5 grams of mesocarp layer were dispersed in 10 ml of water. The mixture was then allowed to stand for 10 min at 25°C, then centrifuged at 9000 rpm for 15 min. The WAI was expressed as the amount of water retained by the mesocarp layer (g/g). The oil absorption of oleaster flour was estimated by mixing 0.5 g sample with 10 mL corn oil, allowing it to settle for 10 min at room temperature, and then centrifuging at 9000 rpm for 30 min. The oil retained by the solids was expressed as g/g. WSI was expressed as the weight of dry solids in the supernatant per dry weight of the mesocarp layer.

Extraction of free and bound (hydrolyzable) phenolics and antioxidants

The extraction of free and bound (hydrolyzable) phenolics and antioxidants from the mesocarp layer was carried out using the method of Kaya et al. (2017). The total phenolic and antioxidant content values were calculated by summing up the free and bound phenolic and antioxidant contents. The free phenolics and antioxidants extraction procedures were followed as described. The 0.2 g sample was mixed with acetone: methanol (20 ml, 1:1, v/v) and concentrated HCl (13µL) and shaken in a water bath (200 rpm, 1 hour) at room temperature and then centrifuged (7800 rpm, 10 min) to extract the bioactive component. The supernatant was transferred to a rotary evaporator until the methanol and acetone were completely evaporated in the sample at 45°C. The sediment was dissolved with 1 mL of ethanol, 1 mL of acetone, and 4 ml of distilled water and filtered through a 0.45 µm membrane filter. It was stored at -18°C until analysis. The bound phenolics and antioxidants extraction procedures were followed as described. The process involved hydrolyzing the residue that remained in the free form, using NaOH (10 mL, 4N) in a water bath, maintained at 200 rpm for 3 hours at room temperature. The pH was then adjusted to 1.5-2.0 using concentrated HCl. The extracts were passed through coarse filter paper funnels and extracted using ethyl acetate (60 ml). The resulting extract was concentrated using a rotary evaporator at 45°C. The sediment portion of the sample was dissolved in ethanol, acetone, and distilled water and then passed through a 0.45 µm membrane filter before being stored at -18 °C for analysis.

The analysis of phenolic content

The phenolic content of the sample was analyzed using the Folin-Ciocalteau method, which involved measuring the free, bound, and total phenolic substances expressed as mg of gallic acid equivalents over the gr of dry weight (dw), described by Spanos and Wrolstad (1990).

The 100 µL sample extract, 900 µL distilled water, 5 mL Folin-Ciocalteau solution (0.2 N), and 4 mL sodium carbonate solution (75 g/L) were mixed and incubated for 2 hours in a dark room temperature, were measured in a spectrophotometer (Shimadzu, UV-1800, Japan) at a wavelength of 765 nm. The standard curve of the gallic acid curve was y=0.0011x+0.0135 (R^2=0.99). The results were based on the average of three replicates.

The analysis of Trolox Equivalent Antioxidant Capacity (TEAC)

The Trolox equivalent antioxidant capacity (TEAC) was determined using the Re et al. (1999) method with modifications. The ABTS radical solution was prepared by dissolving 0.0384 g of ABTS in distilled water and mixing it with 12.25 mM (2 mL) of potassium persulfate solution. The solution was then completed with distilled water to a final volume of 10 ml, wrapped in aluminum foil, and kept in the dark at room temperature for 12-16 hours before analysis. The ABTS+ solution was diluted with saline phosphate buffer (PBS, pH:7.4) to absorb 0.700±0.020 at a wavelength of 734 nm before analysis. Four different concentrations (5, 10, 15, and 20 µL) were added to 1 mL ABTS radical, and measurements were performed at a wavelength of 734 nm after 6 min. The calibration curve of Trolox absorbance versus concentration spanned from 5-20 µmol, and the results were expressed as µmol Trolox Equivalent per g sample.

The analysis of EC50 with DPPH assay

In a study conducted by Pellati et al. in 2004, various antioxidant activities were analyzed using the DPPH assay. The free, bound, and total antioxidant activity was assessed by Pellati et al. (2004) method by dissolving DPPH+ radical (0.03943 g) in methanol within a 100 ml flask. The sample extract was mixed with varying volumes (20-40-60-80-100 µl) of DPPH+ solution (600 µl) and brought to a total volume of 6 mL with methanol. The samples were then vortexed and incubated for 15 min at room temperature without light before being measured using a Shimadzu spectrophotometer (UV-1800, Japan) at a wavelength of 517 nm. The percentage inhibition was calculated using formula (1), and the results were expressed as the EC50 value. This value was determined as the concentration of the antioxidant substance required to inhibit 50% of the DPPH radical in the medium. The EC50 values were presented as the mean of duplicate analyses and reported as 'mg dw.'

$$\% \text{ inhibition} = [(A_{\text{DPPH}} - A_{\text{Extract}})/\text{A}_{\text{DPPH}}] \times 100 \quad (1)$$

Mineral analysis of mesocarp layer

The mineral content analysis of the mesocarp layer (Mg, P, K, Ca, Fe, Zn, Na and Cu) was conducted through the use of inductively-coupled plasma spectroscopy, specifically ICP-OES from the Vista series. Before analysis, the samples were treated using a closed vessel microwave digestion oven (MARS 5, CEM Corporation, USA) with concentrated nitric and sulfuric acid. The resulting mineral component concentrations were determined through ICP-OES, utilizing the methodology proposed by Skujins in 1998.

The dough reology analysis

The rheological properties of dough were measured using Mixolab® with Chopin protocol+. The methodology of Dubat (2010) was used to express the physical state of the dough during mixing and heating. Phase 1 involved initial mixing for water absorption, held at 30 °C for 8 min and achieved by 1.1 Nm (+/-0.05 Nm) torque values. Phase 2 represented the weakening of the protein at 30-60°C to indicate protein quality. Phase 3 measured the rate of starch gelatinization, with the value of C3 depending on the starch characteristics. Phase 4 represented the stability during baking to hold 90°C and define the starch gel stability. Finally, phase 5 expressed the retrogradation from 90 to 50°C. These measurements were taken for both wheat flour and composite flour containing various levels of mesocarp level.

Results and Discussion

The Functional Properties of Mesocarp Layer

Table 1 presents an extensive analysis of the mesocarp layer, covering its chemical, physical, and functional properties. The data reveals that the flour has a moisture content of 8.99%, an ash content of 2.66%, a fat content of 0.55%, a protein content of 5.99%, a crude fiber content of 3.32%, and a total dietary fiber (TDF) content of 26.36%. The TDF content is further broken down into insoluble dietary fiber (IDF) and soluble dietary fiber (SDF), which are 21.35% and 5.01%, respectively. The color values were L*: 75.14, a*: 2.86, and b*: 23.87, while the water activity value was measured as 0.314.

Tablo 1. The proximate composition of mesocarp layer

Chemical composition (%, dw)	
Moisture	8.99±0.00
Ash	2.66±0.05
Fat	0.55±0.17
Protein	5.99±0.56
Crude fiber	3.32±0.16
TDF	26.36±0.19
IDF	21.35±0.35
SDF	5.01±0.16
Physical and functional properties	
Color	L*: 75.14±0.13 a*: 2.86±0.03 b*: 23.87±0.05
Water activity (a_w)	0.314±0.004
Water solubility (%)	67.33±3.06
Water absorption (g water/g sample)	4.91±0.33
Oil absorption (g oil/ g sample)	2.26±0.07
Mineral content (mg/100 g, dw)	
Mg	30.21±0.27
P	97.11± 0.83
K	1100.58± 24.94
Ca	63.90±0.37
Fe	0.07±0.01
Zn	nd
Na	7.91± 0.57
Cu	nd

*Values represent mean±sd for triplicate measurements, nd: not detected

Table 2. The free, bound and total phenolic content and antioxidant capacity of mesocarp layer

Bioactive components of the mesocarp layer		
Phenolic content mg GAE/g, dw	Free	25.32±0.52
	Bound	4.83±0.37
	Total	30.15±0.15
Antioxidant capacity EC50 mg, dw sample	Free	0.78±0.03
	Bound	4.64±0.25
	Total	5.42±0.23
Antioxidant capacity µmol TE/g, dw	Free	263.15±3.61
	Bound	102.50±14.71
	Total	365.65±11.10

*Values represent mean±sd for triplicate measurements

Şimşek and Sufer (2020) reported that the crumb of oleaster fruits from İzmir, Aksaray, and Niğde were also analyzed, with the results showing a range of L*:78.91-82.59, a*:1.48-2.98, and b*:17.92-26.88. Additionally, the water solubility, water absorption, and oil absorption were calculated as 67.33%, 4.91 g water/g sample, and 2.26 g oil/g sample, respectively.

The mineral content of the mesocarp layer contained as ppm 30.21 Mg, 97.11 P, 1100 K, 63.90 Ca, 0.07 Fe, and 7.91 Na, while Zn and Cu were not detected. A study conducted in Bursa reported that the oleaster mesocarp flour with pericarp contained 73.5% dry matter, 0.5% crude fat, 2.22% ash, 4.69% protein, and 4.06% crude fiber. The water solubility and water absorption capacity of peeled and unpeeled oleaster were also analyzed, with the results ranging from 90.33-96.01% and 372.74-430.33%, respectively. The total fiber content of the peeled and unpeeled oleaster flour was 23.55-30.65 g/100 g. Furthermore, the study reported that the mineral content of the oleaster flour collected from Bursa was as follows: Fe 11.59 ppm, B 7.43 ppm, Zn 3.85 ppm, Mn 3.56 ppm, and Cu 3.45 ppm (Cansev et al., 2011). It is worth noting that the physical, chemical, and bioactive properties of the oleaster fruit may vary depending on the soil, climate, and ecological conditions in which it is grown. This was highlighted by a study by Saboonchian et al. (2014), which found differences in the characteristics of oleaster grown in different regions.

Table 2 provides information on the bioactive properties of the mesocarp layer, including the free, bound, and total phenolic content as well as the antioxidant activity. The free, bound, and total phenolic contents were found to be 25.32, 4.83, and 30.15 mg GAE/g, dw, respectively. The antioxidant activity was expressed as EC50, which is the concentration required to inhibit 50% of the DPPH radical in the medium, and Trolox Equivalent Antioxidant Capacity (TEAC). The mesocarp layer had 0.78 EC50 mg dw sample and 263.15 µmol TE/g, dw for the free, 4.64 EC50 mg dw sample and 102.2 µmol TE/g, dw for the bound, and 5.42 EC50 mg dw sample and 365.65 µmol TE/g, dw for the total. Recent scientific research has extensively explored phenolic contents and the antioxidant capacity of various components of oleaster fruits, including flour, shell, core, peel, pulp, crust, and crumb. According to Hassanzadeh and Hassanpour's research in 2018, the average value of total phenolic contents for both the peel and pulp of Iran-grown oleaster was found to be 518.07 and 480.16 mg GAE/100 g fresh weight, respectively. On the other hand, Karkar and Şahin's study in 2022 showed that the phenolic contents varied depending on the extraction method used. For instance, the phenolic contents ranged from 0.13 to 34.89 mg GAE/g of oleaster flour, from 0.37 to 36.16 mg GAE/g of oleaster shell, from 1.12 to 158.73 mg GAE/g of oleaster core, and from 2.36 to 205.26 mg GAE/g of oleaster flower. Comparing our findings to previous studies, our results align with Karkar and Şahin (2022) but are higher than those reported by Hassanzadeh and Hassanpour (2018). Karkar and Şahin (2022) conducted a study that revealed the diverse range of antioxidant capacity in oleaster flour, shell, and core, which was highly dependent on the method used for extraction. For instance, the values ranged from 11.60 to 39.71 mg TE/g for flour, 14.48 to 34.42 mg TE/g for shell, and 23.72 to 67.55 mg TE/g for core. On the other hand, Hassanzadeh and Hassanpour (2018) discovered that the mean antioxidant capacity of peel and pulp was 74.71% and 53.76%, respectively. Another study by Faramarz et al. (2015) reported that the antioxidant capacity measured by the DPPH method was 86.95% and 91.78%, respectively. Moreover, Şimşek and Sufer (2021) researched the antioxidant activity of crust and crumb extracts from oleaster fruits, which were found to be in the range of 6.28-14.05 µmol TE/g DM and 5.01-11.56 µmol TE/g DM, respectively. It is crucial to bear in mind that several factors can influence the phenolic contents and antioxidant capacities of oleaster fruits, including cultivars, genotypes, climate conditions, and geographical locations, as reported in other studies (Hassanzadeh & Hassanpour, 2018; Şimşek & Sufer, 2021).

The Rheologycal Properties of Mesocarp Layer

Table 3 and Figure 1 present the rheological properties of wheat flour (control) and composite flours that contain varying percentages (10%, 20%, and 30%) of mesocarp flour. Introducing the mesocarp layer into the composite flours has a noticeable impact on the thermomechanical properties of the control flour. One of the significant changes is the decreased hydration capacity of the composite flours in all ratios. The mesocarp layer incorporation (from 10 to 30) decreased the water absorption capacity of the wheat dough from 53.5 to 47.0%. This means that the composite flours with mesocarp layer are less capable of absorbing water than the control flour (Figure 1).

This decrease in water absorption capacity indicates that mesocarp layer incorporation hinders the hydration of granular starch and wheat protein.

Tablo 3. Rheological properties of the control and composite flours containing various levels of mesocarp layer as measured by Mixolab

S	R	WA	DDT	ST	C1**	C2**	C3**	C3-C2	C4**	C3-C4	C5**	C5-C4
CF	0	53.5	1.10	8.90	1.09	0.41	1.68	1.27	1.70	-0.02	2.53	0.83
MF	10	51.9	1.07	6.15	1.06	0.30	1.28	0.98	0.99	0.29	1.38	0.39
	20	49.3	0.95	2.15	1.12	0.27	1.10	0.83	0.77	0.33	1.13	0.36
	30	47.0	0.75	2.25	1.09	0.23	0.87	0.64	0.62	0.25	0.92	0.30

S: Samples; CF: Control flourl; R: Ratio (%); WA: Water absorption (%); DDT: Dough development time (min); ST: Stability time (min); *Values represent mean for duplicate measurements, MF: Mesocarp layer flour; ** Torque (Nm)

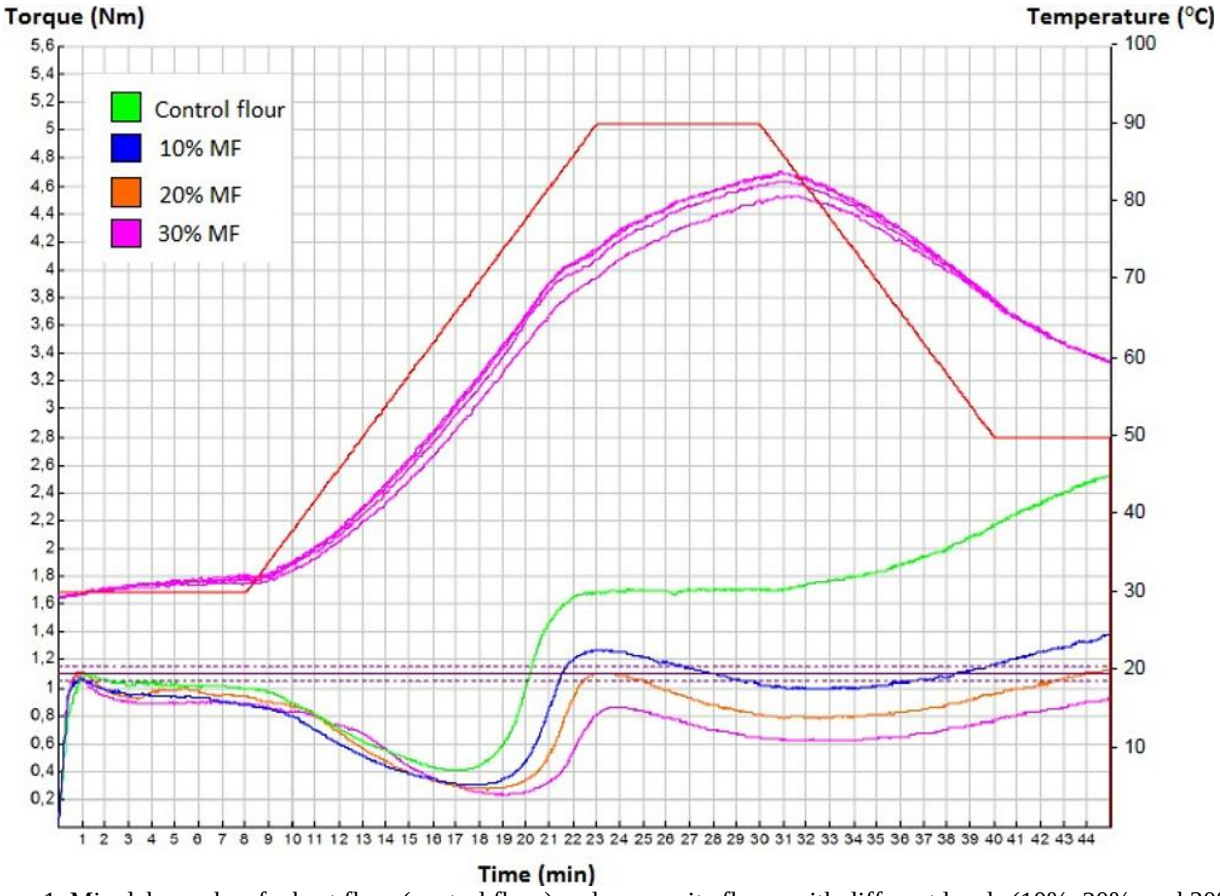

Figure 1. Mixolab graphs of wheat flour (control flour) and composite flours with different levels (10%, 20%, and 30%) of mesocarp layer

As the substitution level of the mesocarp layer increases from 0% to 30%, dough development and stability time significantly decrease from 1.10 to 0.75 and 8.90 to 2.25 min, respectively. The decrease in dough development time is likely due to the mesocarp layer being rich in total phenolics and antioxidants. These compounds may form protein-polyphenol interactions, incorporate phenolic compounds into the gluten network, and close them in the hydrophobic pockets (Sivam et al., 2010). A recent study by Welc et al. (2022) found that the presence of polyphenols in gluten can cause significant structural and functional changes in gluten proteins. These changes include aggregation and conformational alterations of disulfide bridges. Reducing these interactions is a desirable outcome, as highlighted by Pala (2012). One way to measure the strength of flour is to analyze dough stability time. A higher value indicates a stronger dough, critical for forming a three-dimensional viscoelastic structure that relies on wheat gluten (Rosell et al., 2007). However, adding the mesocarp layer, which has a protein content of 5.99%, can inhibit the development of gluten network structure due to the dilution of gluten concentration and competition for water between gluten, starch, and mesocarp layer. This results in lower dough stability time. Previous studies by Danno and Hoseney (1982) and Okada et al. (1987) have shown that free radical scavengers like phenolic acids can accelerate dough breakdown. On the other hand, Han and Koh (2011) reported that adding phenolic acids to dough can decrease kneading time, tolerance, elasticity, and bread volume. Composite flours containing mesocarp layer in all ratios are characterized by a lower C2 torque and a lower viscosity peak (C3 and C3-C2), as well as higher hot stability (C3-C4) and lower retrogradation (C5-C4). These qualities make the flour an excellent substitute for wheat flour in various food products. The changing hot viscosity (C3) of the composite flours may be attributed to a unique alteration in water distribution. Additionally, the mesocarp layer substitution in wheat flour can significantly enhance product shelf-life due to slower retrogradation (C5-C4), indicating a weaker starch gelling process.

Conclusion

In this study, the physical, technological, and dough rheological properties of the mesocarp layer of oleaster (*Elaeagnus angustifolia L.*) cultivated in Karaman were thoroughly examined, with a focus on its functional properties. The results showed that the mesocarp layer of oleasters grown in Karaman had high protein and ash content, low crude fiber, and a fat content comparable to those grown in other regions. The crumb color and dietary fiber amounts of the mesocarp layer were consistent with previous studies. However, the mineral properties of the oleaster were found to vary depending on the region, climate, and soil structure in which it is grown, leading to different characteristics. While the bioactive component properties were similar to previous studies, there were instances where they were lower, likely due to varying factors such as cultivars, genotypes, climate conditions, and geographical locations. Additionally, the inclusion of the mesocarp layer significantly affected dough rheology. The findings of this research highlight the numerous applications of oleaster in various industries, showcasing its significant properties that can be utilized for a broad range of purposes. This investigation provides valuable insights into the characteristics and potential advantages of the oleasters, contributing to our understanding of this plant's properties.

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