

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X │www.agrifoodscience.com │ Turkish Science and Technology Publishing (TURSTEP)

Influence of Grape Heterogeneity on Berry Quality Traits in Table Grapes A Study on the 'Alphonse Lavallée' Variety

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Introduction

Grapes are among the most extensively cultivated crops globally, with an annual production of approximately 80 million tons harvested from 7.3 million hectares of land (Anonymous, 2023). Of this total production, 46% is dedicated to winemaking, 42% is consumed fresh as table grapes, 8% is processed into raisins, and the remaining portion is used for must and juice production (Crespo, 2024). In the past two decades, global table grape consumption has doubled. This significant increase is largely driven by the development of new grape varieties characterized by larger berries with firmer flesh, extended shelf life, and improved accessibility to diverse markets (Anonymous, 2016; Anonymous, 2021; Crespo, 2024).

In recent years, consumers have increasingly prioritized the quality and health benefits of the food they consume over mere quantity. Grapes stand out as a fruit rich in bioactive compounds, including antioxidants, phenolics, anthocyanins, and flavonoids, all of which have been extensively validated for their health-promoting properties in numerous studies. Moreover, grapes are an excellent source of essential vitamins, minerals, and dietary fiber, contributing significantly to daily human nutrition (Liu et al., 2016; Gouot et al., 2019).

The yield and quality of grapes are profoundly influenced by a multitude of factors related to the vine's positioning within the vineyard. These factors include elevation, soil composition, temperature, humidity, and vine density. Additionally, the specific location of the grape cluster on the vine, and even the positioning of individual berries within the cluster, play critical roles in determining grape quality and yield (Haselgrove et al., 2000; Smart et al., 1985). Berry size, in particular, has been found to have a strong correlation with the composition and chemical properties of the grapes, impacting attributes such as flavor, texture, and nutritional content (Roby and Matthews, 2004; Roby et al., 2004). These relationships underscore the complex interplay between environmental conditions and grape development, highlighting the importance of meticulous vineyard management to optimize grape quality.

While influenced by genotype and clonal variation, berry size is not solely determined by these intrinsic factors (Fernandez et al., 2006; Houel et al., 2013). External factors such as environmental conditions and viticultural practices also play a crucial role in determining berry size (Matthews and Anderson, 1988; Roby and Matthews,

2004; Petrie and Clingeleffer, 2006; Holt et al., 2008). Key factors influencing berry size include inadequate vineyard irrigation (Kennedy et al., 2002; Roby et al., 2004), high crop load (Bravdo et al., 1985), pruning practices (Holt et al., 2008), and the choice of rootstock (Keller et al., 2011).

Berry size is a key determinant of fruit and wine quality (Gil et al., 2015; Melo et al., 2015). This importance stems from the relationship between berry size and the ratio of skin surface area to fruit flesh volume, which affects the quantity of skin relative to berry size (Roby and Matthews, 2004; Roby et al., 2004). Different-sized berries within a heterogeneous cluster exhibit distinct chemical and phytochemical compositions. Smaller berries, with their higher surface areato-volume ratio, contain more soluble solids per skin unit, as many essential compounds concentrate in the skin. On the other hand, larger berries have a higher solvent-to-solute ratio, leading to easier dilution of the soluble substances extracted from the skins (Matthews and Anderson, 1988). In recent years, the diversity of berry sizes at harvest has been increasingly recognized (Tarter and Keuter, 2005; Dai et al., 2011). Moreover, previous studies have highlighted not only the size heterogeneity but also the wide range of ripeness among grape berries at harvest (Singleton and Rossi, 1965; Tarter and Keuter, 2005; Kontoudakis et al., 2011).

This study aimed to investigate the induced heterogeneity in the 'Alphonse Lavallée' grape variety, a table grape with significant importance both globally and nationally, by selecting berries of varying densities. The research focused on assessing the resulting physical, chemical, and phytochemical variations in the berries.

Material and Methods

Material

The study was carried out on 6 year old vines of Alphonse Lavallée grape variety (*Vitis vinifera*) grafted on 1103 P rootstock in a vineyard of the Tokat Gaziosmanpaşa University Agricultural Research and Application Center. Vines had been planted as a spacing of $1.5 \text{ m} \times 3 \text{ m}$ and trained to cordon with double T support, and were spur pruned to about 20 ± 2 nodes per grapevine. This variety is known for its berries, which contain 1-4 seeds, have thick skins, and exhibit a dark purple color with a characteristic waxy layer. The berries are notably large and visually appealing. The grape clusters are also distinctive, being large, branched, and conical in shape (Çelik, 2006).

Methods

When the grapes reached 18-20 °Bx TSS, they were harvested and hulled without damaging the berries and made ready to be used in the study. To assess the ripening heterogeneity of the harvested grape berrys, NaCl solutions were prepared with concentrations ranging from 100 to 130 g/l, corresponding to densities between 1075 and 1094 $kg/m³$ (Fournand et al., 2006). These solutions were designated as Y1 (100 g/l NACI solution), Y2 (110 g/l NACI solution), Y3 (120 g/l NACI solution), and Y4 (130 g/l NACI solution). Grape berries that floated in these salt solutions were considered to have the same density as the solution and were preserved for subsequent analyses. The control group, labeled as Y0, was created by randomly selecting grape berries from the harvest. Various physical, chemical, and phytochemical analyses were then

performed on the berries categorized by their density, and the specific analyses conducted are detailed below.

Berry Dimensions (mm) and Weight (g): The width and length of the berries were measured with a digital caliper with an accuracy of 0.01 mm, and the weights were recorded using a precision scale with a sensitivity of 0.01 g. These measurements were conducted on 10 berries per replicate, across 3 replicates for each treatment.

Berry Firmness (N): Firmness measurements were performed on the same berries used for dimension and weight analysis. A Zwick Z 0.5 Universal Testing Machine was employed to determine firmness, with results expressed in Newtons (N).

Berry Skin Color (L, a*, b*):* Skin color measurements were measured using a Minolta Konica CR-400 chromometer, assessing 18 berries per replicate.

Soluble Solids Content (SSC, %): The soluble solids content of the grape juice, extracted by squeezing the berries, was measured using a digital refractometer.

pH and Titration Acidity (g/l): The pH of grape berries with varying densities was measured using a benchtop pH meter, while titration acidity was determined by titrating grape juice samples with 0.1 N NaOH. The amount of NaOH required to reach a pH of 8.1 was used to calculate the total acidity, expressed as tartaric acid equivalents (0.075 equivalent value), in g/l (Cemeroğlu, 1992).

Total Phenol Content: The homogenized grape berries were incubated for 60 minutes in a solution containing acetone, water, and acetic acid. Following incubation, pure water and Folin-Ciocalteu's reagent were added to the mixture, along with 7% sodium carbonate. The solution was then incubated for two hours to develop a blue color, and its absorbance was measured at 750 nm. The total phenol content was reported as gallic acid equivalents (µg GA g-1 fresh wieght)(Singleton and Rossi, 1965).

Total Antioxidant Capacity (TAC): The total antioxidant capacity was measured using the TEAC method, with absorbance readings taken at 734 nm using a spectrophotometer. The results were calculated based on a Trolox standard curve (ranging from 10 to 100 μ mol/L) and expressed as µmol Trolox equivalents per gram of fresh weight (Özgen et al., 2006).

Total Monomeric Anthocyanin Content: The total monomeric anthocyanin content in the grape berries was assessed using the pH differential method, with the results expressed as µg malvidin-3-glucoside equivalents per gram of fresh weight (Giusti et al., 1999).

Statistical Analysis

The obtained data were statistically analyzed using the SAS software package. Following an analysis of variance (ANOVA), Duncan's multiple range test ($p \le 0.05$) was employed to assess the significance of differences between density groups.

Results and Discussion

Physical Characteristics: Berry Width, Length, Weight, Firmness, and Color Values

The physical properties of Alphonse Lavallée grape berries were analyzed across different density groups (Y0 to Y4), focusing on berry width, length, weight, and firmness (Table 1).

*Means with the same letter are not significantly different from each other ($p<0.05$).

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The results revealed significant variations in these physical attributes, depending on the density group. The physical heterogeneity observed in grape berries is strongly influenced by the cultivar, climatic conditions, and vineyard management practices (1; 2). In the 'Alphonse Lavallée ' grape variety, the study examined physical properties such as berry width, length, firmness, weight, and color across berries of varying densities. Among the density groups, all parameters except for berry width exhibited statistically significant differences. The Y0 (control) group, yielded the highest values for berry length, firmness, and weight in the parameters where significant differences were observed (Table 1).

Berry size is a critical factor in determining the quality of both grapes and wine (Melo et al., 2015). The concept of berry size is rooted in the understanding that the ratio of skin surface area to fruit flesh volume influences the amount of skin relative to berry size (Roby and Matthews, 2004; Roby et al., 2004). In the study the berry length exhibited some variation, with the Y4 group (130 g/l) showing a significantly smaller length (20.67 mm) compared to the Y0 (control) group (23.04 mm), which had the longest berries. The decreasing trend in berry length as density increases may indicate a relationship between berry compactness and its ability to elongate during development (Table 1). This observation aligns with the notion that increased berry density can restrict growth in certain dimensions. In recent years, there has been an increasing recognition of the wide variability in berry sizes observed at harvest (Tarter and Keuter, 2005; Dai et al., 2011). Berry weight demonstrated a more pronounced variation across the density groups, with the Y0 group having the heaviest berries (9.03 g), while the Y4 group had the lightest (6.47 g) (Table 1). The significant reduction in berry weight with increasing density suggests that higher density groups may experience more competition for resources, leading to smaller and lighter berries. In addition to this size heterogeneity, there is also a broad range of ripeness among grape berries at harvest time (Singleton and Rossi, 1965; Tarter and Keuter, 2005; Kontoudakis et al., 2011).

Berry firmness also varied significantly across the density groups, with the highest value recorded in the Y0 group (27.50 NW) and the lowest in the Y4 group (18.13 NW). The decreasing trend in firmness with increasing density could be attributed to the reduced structural

integrity of smaller, denser berries, which may develop thinner skins and softer flesh (Table 1). Similar to the findings in this study, Rolle et al. (2012) reported an inverse relationship between berry size and density in their research on the 'Nebbiolo' grape variety.

The skin color values of Alphonse Lavallée grape berries were analyzed across different density groups (Table 2). These values provide insights into the visual and chemical properties of the grape skins, which are critical for both table grape appeal and wine production The *L** value, which represents the lightness of the berry skin, showed significant variation across the density groups. The highest L^* value (27.25) was observed in the Y4 group, indicating that these berries were the lightest in color. In contrast, the Y0 group, representing the least dense berries, exhibited the lowest *L** value (17.07), indicating a darker skin tone. This trend is consistent with previous studies that have shown smaller, denser berries to have lower *L** values due to higher concentrations of anthocyanins, which are responsible for the darker color in red grapes (Gil et al., 2015). The increased lightness in the Y4 group may be due to a lower anthocyanin concentration or thinner skin, which allows more light to penetrate the berries.

The *a** value, representing the red-green axis of color, did not vary significantly across the first four density groups (Y0 to Y3), all of which maintained similar values around 2.93 to 2.88 (Table 2). However, the Y4 group showed a significantly lower *a** value (1.95), suggesting a reduction in the red pigmentation. This decrease in redness for the densest group may be linked to reduced anthocyanin levels, as anthocyanins contribute to the red hue in grape skins. These findings align with the results of Chen et al. (2018), who found that higher density berries can exhibit less intense red coloration.

The *b** value, which reflects the yellow-blue axis, also varied significantly across the density groups. The Y0 group had the highest *b** value (1.63), indicating a stronger yellow component, while the Y4 group had the lowest b^* value (0.80), suggesting a shift towards a bluer tone. The reduction in the *b** value in the higher density group could be related to the dilution of pigments that contribute to yellow hues, or an increase in blue pigments relative to yellow ones, which is consistent with previous findings in other grape varieties like 'Cabernet Sauvignon' (Gil et al., 2015).

Figure 1. Chemical properties (pH, titration acidity, and SSC) of must from 'Alphonse Lavallée ' grape berries with different densities.

Figure 2. Total Phenolic Content (µg GAE/g FW), Total Antioxidant Activity (umol TE/g FW), and Total Monomeric Anthocyanin (µg Cy-3glu/g FW) in 'Alphonse Lavallée ' Grape Berries Across Different Density Groups

Chemical Properties: Soluble Solid Content (SSC), pH, Titration Acidity

The chemical properties of grape berries, including soluble solids content (SSC), pH, and titration acidity, are crucial indicators of grape quality, particularly in determining their suitability for fresh consumption or wine production. These properties were analyzed across different density groups in the Alphonse Lavallée grape variety to assess how berry density influences the chemical profile of the grapes.

When examining the impact of different densities on the chemical parameters of the 'Alphonse Lavallée ' grape variety, statistically significant differences were observed. The berries in the Y4 (130 g/l) density group exhibited the highest levels of SSC and pH. Conversely, the Y0 control group showed the highest titration acidity (TA) values (Figure 1). It is well known that berries of different sizes and weights, or even those with the same density, can exhibit varying degrees of physiological ripeness (Šuklje et al., 2012). Therefore, it is expected that the chemical composition of the must derived from berries of different densities would vary.

Previous research on the 'Cabernet Sauvignon' grape variety has investigated the effects of berry size on fruit composition and wine quality. The findings of that study indicated that as berry size increased, berry density decreased, and higher berry density was associated with increased SSC and pH levels, while TA decreased (Chen et al., 2018). These findings are consistent with the results observed in this study, where higher density (Y4) grapes exhibited elevated SSC and pH levels, while the lower density (Y0) grapes had higher TA.

In the 'Alphonse Lavallée ' grape variety, the impact of density-induced heterogeneity on the presence of secondary metabolites was evident, with the highest total phenolic content observed in the Y2 and Y3 density groups $(110 \text{ g/l}$ and 120 g/l , respectively), while the highest total antioxidant capacity (TAC) and total anthocyanin content were recorded in the Y4 (130 g/l) density group (Figure 2). These findings suggest that denser berries tend to accumulate more secondary metabolites, particularly anthocyanins and antioxidants, which are crucial for the grape's health benefits and sensory qualities.

Supporting this observation, previous research has indicated that higher anthocyanin concentrations in denser grapes are likely due to a higher skin-to-volume ratio (Canals et al., 2005). Similar results were found in a study on the 'Syrah' grape variety grafted onto the 99 R rootstock, where spectrophotometric analysis revealed that the lowest quality berries were the largest in size, aligning with the findings of this study (Hunter et al., 2015). Additionally, research on grape size variation has shown that while total anthocyanin concentration decreases as berry size increases, the content per berry actually rises, indicating a dilution effect in larger berries (Yaylak, 2022).

Multiple factors influence berry weight, leading to size variability among the berries (Shellie, 2010; Pisciotta et al., 2013). Studies have shown that total phenolics or anthocyanins per berry increase less proportionally with fresh berry weight, resulting in lower concentrations of these compounds in larger berries (Roby et al., 2004; Barbagallo et al., 2011; Wong et al., 2016). The data obtained in this study are consistent with these findings, demonstrating that smaller, denser berries are richer in secondary metabolites, which can significantly influence the overall quality of the grapes, both for fresh consumption and wine production.

Conclusion

Grapes are a vital agricultural product in human nutrition, largely due to their rich composition of primary and secondary metabolites. These compounds play crucial roles not only in the nutritional value of grape berry but also in its health benefits, making grapes an essential component of a balanced diet. In this study, we investigated the physical, chemical, and phytochemical properties of berries from the 'Alphonse Lavallée ' grape variety, focusing on how these characteristics vary across different density groups.

The results of this study are particularly noteworthy for the insights they provide into the relationship between berry density and the accumulation of secondary metabolites. Our findings reveal a significant increase in the concentration of secondary metabolites—such as total phenolics, antioxidant capacity, and anthocyanins—as berry density increases. These compounds are well-known for their health-promoting properties, including their roles in reducing oxidative stress and providing anti-inflammatory benefits. The observed increase in these metabolites with higher berry density suggests that denser berries, despite being smaller and lighter, may offer greater nutritional and health benefits compared to their less dense counterparts.

This study aligns with current trends in food consumption, where there is a growing focus on the quality of food over its quantity. Consumers are increasingly seeking foods that not only meet their nutritional needs but also contribute to overall health and well-being. In this context, the findings of our research are particularly relevant, as they highlight the potential of grape density as a factor that can be manipulated to enhance the health benefits of table grapes.

Moreover, these results underscore the importance of continued research in this area. By exploring the effects of different density levels across various grape varieties, we can gain a deeper understanding of how to optimize grape production for both quality and health benefits. This could lead to the development of new grape varieties that are specifically bred to maximize the concentration of beneficial compounds, thereby meeting the growing consumer demand for high-quality, health-promoting foods.

In conclusion, this study not only provides valuable insights into the relationship between berry density and the accumulation of secondary metabolites in 'Alphonse Lavallée' grapes but also emphasizes the broader implications for grape production and consumption. As the focus on food quality continues to grow, the ability to manipulate berry density to enhance nutritional value will become increasingly important, making this an area ripe for further research and development.

Declarations

Authors' Contribution Statement

The authors declare that they contributed equally to the article, have seen/read the final version of the manuscript ready for publication, and have approved it.

Conflict of Interest Statement

All authors declare that there is no conflict of interest regarding this study.

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