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Effects of Melatonin Application on Post-Harvest Quality and Shelf Life of Kabarcık Grape Variety

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ABSTRACT
In viticulture, post-harvest is a very important period to ensure and protect grape quality and health. The storage of the Kabarcık variety, which is of great importance for Dulkadiroğlu grape growers, after harvest and its short road endurance period, constitute a real problem expressed by the growers. In this reseach, it was aimed to maximize grape cluster health and post-harvest quality protection with melatonin application. Different doses of melatonin were applied to grape clusters of Kabarcık variety obtained from Dulkadiroğlu producer vineyards in the 2023-24 production season. Fruit weight loss, brix (sugar content), pH, and titratable acid (tartaric acid) amounts were determined in the clusters subjected to melatonin doses in a total of four different periods (days 0, 5, 10, and 15). As a result, the highest weight loss was seen in the control and 6-hours 25 μmol L^{-1} melatonin applications, while the least weight loss was seen in the 3-hours 250 μmol L^{-1} melatonin application. Thus, while the 25 μmol L^{-1} dose of melatonin was seen as too low and ineffective, the 250 μmol L^{-1} dose of melatonin was determined to be an effective solution in preventing weight loss in grape berries. This study highlights the important effects of melatonin in improving fruit quality and extending shelf life.

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Melatonin Uygulamasının Kabarcık Üzüm Çeşidinde Hasat Sonrası Kalite ve Raf Ömrü Üzerine Etkileri

ÖZET
Bağcılıkta hasat sonrası, üzüm kalitesi ile sağlığının sağlanması ve korunması için çok önemli bir dönemdir. Dulkadiroğlu üzüm yetiştiricileri için büyük öneme sahip olan Kabarcık çeşidinin, hasat edildikten sonra muhafazası ve yola dayanım süresinin kısa oluşu yetiştiriciler tarafından dile getirilen gerçek bir problem oluşturmaktadır. Bu araştırmada, hasat sonrası melatonin uygulaması ile üzüm salkım sağlığı ile üzüm kalitesinin korunması ve en üst düzeye çıkartılması amaçlanmıştır. 2023-2024 üretim sezonunda Dulkadiroğlu üretici bağlarından elde edilen Kabarcık çeşidi üzüm salkımlarına farklı melatonin dozları uygulanmıştır. Melatonin dozlarına tabi tutulan salkımlar toplam 4 farklı zaman dilimlerinde (0, 5, 10, ve 15. günler) olmak üzere meyve ağırlık kaybı, briks (şeker içeriği), pH ve titre edilebilir asit (tartarik asit) miktarları tespit edilmiştir. Sonuç olarak, en fazla ağırlık kaybı kontrol ve 6 saat 25 μmol L^{-1} melatonin uygulamalarında görülürken, en az ağırlık kaybı 3 saat 250 μmol L^{-1} melatonin uygulamasında görülmüştür. Böylece, 25 μmol L^{-1} melatonin çok düşük ve etkisiz olarak görülürken, 250 μmol L^{-1} melatonin üzüm tanesinde ağırlık kaybını önlemede etkili bir çözüm olarak tespit edilmiştir. Bu çalışma, melatoninin meyve kalitesinin iyileştirilmesi ve raf ömrünün uzatılması açısından önemli etkilerini vurgulamaktadır.

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INTRODUCTION

In Turkiye, approximately 4165000 tons of grapes were produced from a total area of 384537 ha in 2022 (FAOSTAT, 2024). In Kahramanmaraş, a total of 64681 tons of grapes were produced in 2022, especially in Dulkadiroğlu (25265 tons), Onikişubat (17304 tons), Pazarcık (12860 tons) districts (TUIK, 2024). Kabarcık, which is the most produced by farmers in Kahramanmaraş, is a variety with a very short shelf life after harvest. Numerous plant species contain melatonin, also known as N-acetyl-5-methoxytryptamine, a multifunctional signaling chemical that is widely dispersed (Ze et al., 2021). Plants use melatonin for a variety of physiological processes, such as fruit development (Wang et al., 2016), ripening (Sun et al., 2015), and senescence (Li et al., 2019). The senescence and degeneration of post-harvest fruit, including pear (Liu et al., 2019), sweet cherry (Wang et al., 2019), and peach (Gao et al., 2016), were greatly delayed during storage when exogenous melatonin administration was applied. Melatonin, a harmless and safe substance, thus shows promise for use in the fruit supply chain after harvest. Applying a melatonin solution by soaking or spraying is currently the main postharvest management method. Rapid soaking or spraying appears to have more potential for large-scale use in the postharvest fruit during storage and marketing, based on the viability of commercial technology (Ze et al., 2021). Improving shelf life and preserving nutritional levels mostly depends on reducing the senescence processes after harvest. Berry deterioration following harvest is primarily caused by components oxidizing, respiration consuming compounds, and softening of cell walls (Shehata et al., 2019). It has been found that exogenous melatonin is helpful in some plant tolerance to a range of environmental stresses such as toxic chemicals, salt, and drought (El-Bauome et al., 2022). Melatonin has been reported to greatly affect crop ripening in broccoli (Cano et al., 2022) and some other horticulture crops (Ze et al., 2021). Additionally, melatonin application externally postponed the fruit senescence, softened some fruits, loosing on the total fruit weight by decreasing losing water status, postponing the decay and respiration proportions (Wang et al., 2019; Liu et al., 2018; Onik et al., 2021; Aghdam & Fard, 2017; Yang et al., 2019). It has been discovered that melatonin applied postharvest can effectively delay ripening and senescence. The study regarding grape berries examined the effects of two postharvest melatonin doses (50 and 100 µmol) on the quality, bioactive components, and enzyme activities of grape berries cv "Crimson," which were kept for 35 days at 0 ± 1 °C and 90% relative humidity (RH). According to their findings, adding melatonin to berries prolongs their shelf life by preventing weight loss and preserving titratable acidity (TA), total soluble solids (TSS), berry adherence strength, and firmness (Nasser et al., 2022). In order to promote improvements in sustainable practices and a more complex knowledge of agricultural systems, this research study aims to explain the complex interactions between melatonin and grapevine physiology. In the face of changing environmental challenges and market demands, this research attempts to fill fundamental knowledge gaps and open up access to new methods that can boost grapevine productivity, durability, and post-harvest quality maintenance. Therefore, this study is aimed at improving the self-life of Kabarcık cultivar berries by exogenous melatonin applications at room temperature (22 ^{0}C) were examined to maintain berry quality in this study.

MATERIALS AND METHOD

Plant material

The research was carried out at the Kahramanmaras Sutcu Imam University, Hortıculture Department laboratory during the period from 2023-2024 growing season. The selected grape cultivar for this study was the Kabarcık grafted onto Rupestris du Lot rootstock, and clusters were collected from a trained system with a spacing of 3 m x 2 m vineyard. Although the Kabarcık is suitable for wine and must, it is also widely consumed as a table grape in the region. Another reason for choosing this variety is that the berry shell is thin and the interaction between the application of melatonin and the extension of the shelf life of the variety will be better understood.

Experiment Design and Treatments

The study employed a randomized complete block design for its execution, comprising three replicates, each consisting of 3 clusters per replicate. Clusters were harvested at a ripe stage (17-18 Brix) in September free from mechanical damage, insect damage, and any outer decay. Clusters were randomly divided into five groups of similar color, size, and form, then clusters were immersed in control (distilled water), 25 µmol L^{-1} melatonin for 3-hours, 250 µmol L^{-1} melatonin for 3-hours, 25 µmol L^{-1} melatonin for 6-hours, 250 µmol L^{-1} melatonin for 6-hours. Each cluster was left until its surfaces dried at room temperature at 23 ± 5 °C.

Determination of Cluster Weights, Brix (sugar content), pH, and Titratable Acidity

The cluster weights, Brix (sugar content), pH, and titratable acidity values of the control group without melatonin application and melatonin applied samples, stored at 23°C room temperature, were determined on the 0, 5th, 10th, and 15th days. The weights of the cluster were measured every 5 days using a Radwag brand AS 310 R.2 model precision scale and the weight values were recorded. Water loss of berries was determined by the formula;

$$\text{Weight loss}(\%) = \frac{(\text{Harvest Weight} - \text{Last Weight})}{\text{Harvest Weight}} * 100$$

Brix, pH, and titratable acidity values of the samples were calculated using the juices obtained from grape berries. Brix values of the samples were measured with an Atago brand PAL^{-1} model digital handheld refractometer, and the pH value of the fruit was measured with a Hanna brand HI-2550 model pH meter. 5 ml of the sample and some pure water were taken into an erlenmayer and 2 drops of 1% phenolphthalein indicator were added to the solution and titrated with 0.1 N NaOH solution. % titratable acidity was calculated using the formula below.

$$\%\, Titretable\ Acidity = \frac{SxNxExF}{C} x100$$

S is the amount of NaOH used in titration (ml),

N= Normality of NaOH used in titration,

E= Equivalent value of the relevant acid

F= Factor of NaOH used

C= Amount of sample used (ml)

Statistical Analysis

All descriptive analyses were carried out in R Studio using the agricolae package. The significance of treatments and days and their interactions with berry characteristics (weight loss, brix, pH and titratable acidity) were analyzed through Analysis of Variance (ANOVA) in R Studio. All data were checked for normality using the chi-square test before analysis. Linear models were employed to evaluate the main effects (treatments and days) on berry characteristics (weight loss, brix, pH and tartaric acid). Post-hoc analysis utilizing Tukey HSD was conducted with the agricolae package in R Studio. Principal Component Analyses (PCAs) for berry characteristics (weight loss, brix, pH and tartaric acid) datasets were carried out using ggbiplot2 within R Studio. The heatmap was generated using the pheatmap package in R Studio, providing a visual representation of relationships and variations in the analyzed datasets (R Core, 2013).

RESULTS AND DISCUSSION

Weight loss, brix, pH and titratable acidity were affected by treatments and days. About weight loss, it was statistically different in treatments (p=0.0101 *) and days (p=<2e-16 ***). Control and 6-hours 25 µmol L^{-1} treatments, (31.91% and 32.41%, respectively) were higher predominantly weight loss than treatment of 3-hours 250 µmol (24.08%). 3-hours 25 µmol L^{-1} (29.41%) and 6-hours 250 µmol L^{-1} treatments (25.75%) were not statistically different. Day 15, (54.06 %) was consistently higher weight loss than day 0 (control, 0.00%). And day 10 (36.93 %) and day 5 (22.86 %) with p=<2e-16 *** were between these treatments. Regarding Brix, it was the predominant greater (with p= <2e-16 ***) in 6-hours 25 µmol L^{-1} treatment (17.95) while 3-hours 250 µmol L^{-1} treatment (16.60) and control (16.62) were the least. In addition, 6-hours 250 µmol L^{-1} (16.90) and 3-hours 25 µmol L^{-1} (16.97) were between these treatments. Brix (with p= <2e-16 ***) ranged from day 0 (15.30) to day 15 (20.40). Day 10 (18.50) and day 5 (15.46) were between these days. For pH, it was the predominant greater (with 2.54e-08 ***) in 3-hours 250 µmol L^{-1} treatment (4.30), while control and 6-hours 25 µmol L^{-1} treatment (4.15 for both treatments) were least. And 3-hours 25 µmol L^{-1} (4.18) and 6-hours 250 µmol L^{-1} (4.23) were between treatments. pH (with p=<2.60e-11***) dramatically increased from day 0 (4.15) to day 15 (4.33). Lastly related to titratable acidity, the biggest value was obtained from the treatment of 3-hours 25 µmol L^{-1} and 3-hours 250 µmol L^{-1} (0.43 for both treatments) while the lowest from 6-hours 25 µmol L^{-1} treatment with 0.39. In titratable acidity (with p=< 2e-16 ***) greater value was obtained from day 10 with 0.51 and the lowest value was obtained from day 0 (0.35) (Table 1).

All variables are shown, different color circles representing the method are distributed along the vertical axis of PC1. The purple circle represents control treatment and has a great contribution to Weight loss and Brix while 3-hours 0.25 µmol L^{-1} treatment has the lowest to total variance. The other treatments were between control and 3-hours 0.25 µmol L^{-1} cultivars and have contributions differently (Fig. 2A). This shows that treatments and days provide the greatest contribution to Weight loss (%), Brix, pH and Titratable acidity (as Tartaric acid). On the other hand, day 10 and day 15 were more widely distributed than days 0 and 5 along the horizontal axis, indicating that in these days had a greater effect on Weight loss and Brix than the other days. This shows that day 0 and day 5 have the lowest contribution to the Weight loss and Brix, while three bud spurs represents a significant portion of the total variance (Fig. 2B). For the variables, Dim1 and Dim2 states for 60.7% and 22.3% of the variance, respectively. Weight loss, Brix and pH were located on the positive side Dim1, having stront correlation, while tartaric acid was on the negavive side of Dim1 (Fig.2C). Furthermore, a robust positive association was discovered among pH, brix, and weight reduction, as indicated by the dark blue circles. Conversely, tartaric acid was found to have a pronounced negative correlation with weight loss, brix and pH, as indicated by the light blue circles (Fig. 2D).

Table 1. Weight loss (%), Brix, pH and Titratable acidity (as Tartaric acid) of grape berry of Kabarcık in EL-38 phenological stage.

Çizelge 1. EL-38 fenolojik döneminde Kabarcık üzümü salkım ağırlık kaybı (%), SÇKM (Suda Çözünebilir Kuru Madde), pH ve Titre edilebilir asitliği (Tartarik asit olarak).

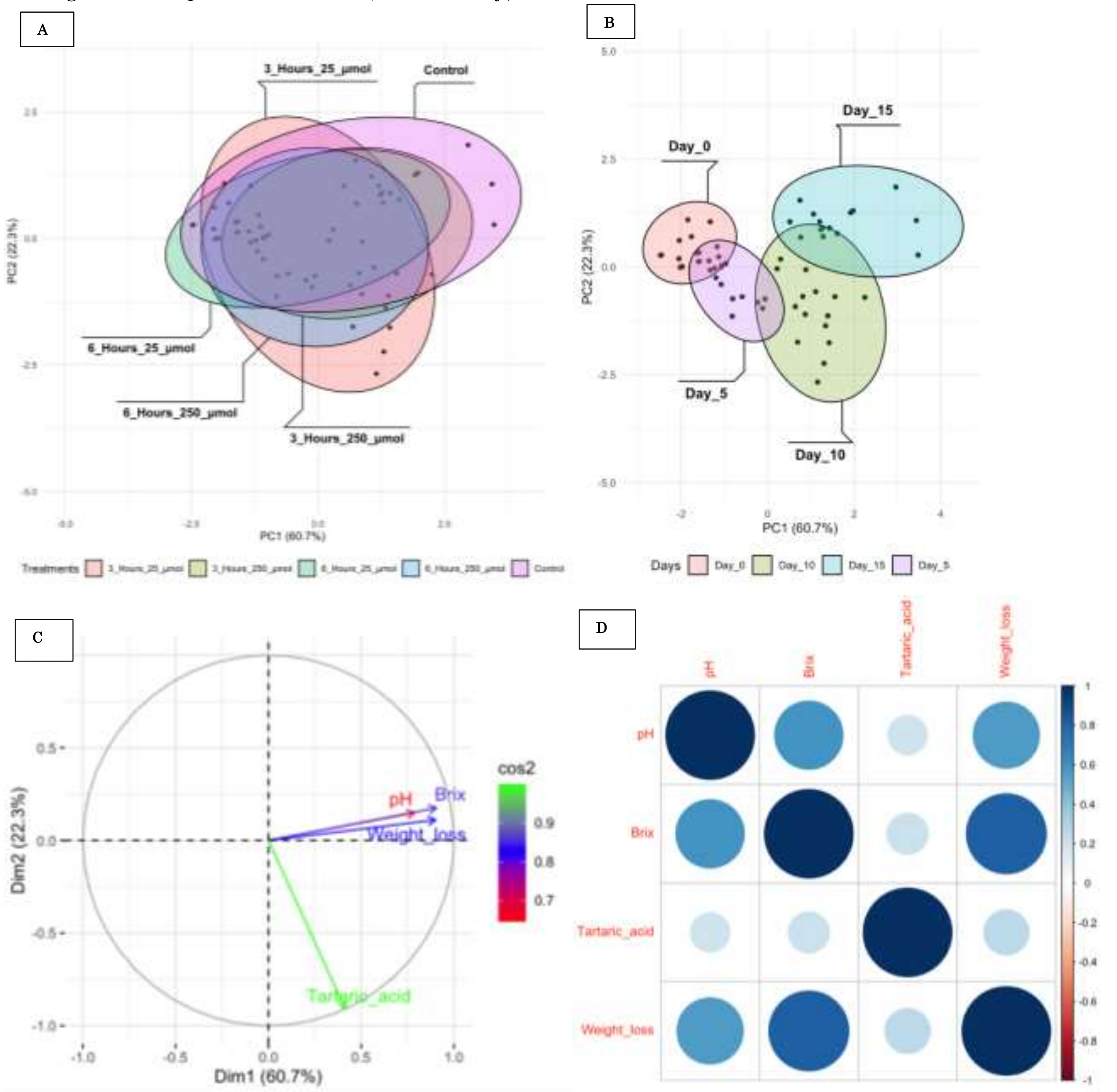
	Weight Loss (%) *Ağırlık Kaybı (%)*	**Brix** *SÇKM (Suda Çözünebilir Kuru Madde Miktarı)*	**pH** *pH*	**Titratable acidity (Tartaric acid)** *Titre edilebilir asitlik (Tartarik asit)*
Treatments^{W} (Tr)				
Control	31.91±1.85a	16.62±0.03c	4.15±0.02c	0.42±0.00ab
3-hours 25 µmol L^{-1}	29.41±1.92ab	16.97±0.02b	4.18±0.01bc	0.43±0.00a
3-hours 250 µmol L^{-1}	24.08±1.89b	16.60±0.01c	4.30±0.02a	0.43±0.00a
6-hours 25 µmol L^{-1}	32.41±1.95a	17.95±0.03a	4.15±0.01c	0.39±0.00b
6-hours 250 µmol L^{-1}	25.75±1.55ab	16.90±0.02b	4.23±0.02b	0.41±0.00ab
Days^{T} (D)				
Day 0	0.00±0.00d	15.30±0.01d	4.15±0.01b	0.35±0.00c
Day 5	22.86±1.75c	15.46±0.02c	4.17±0.01b	0.42±0.00b
Day 10	36.93±1.90b	18.50±0.03b	4.29±0.02a	0.51±0.00a
Day 15	54.06±1.95a	20.40±0.05a	4.33±0.02a	0.37±0.00c
Significance				
Tr	0.0101 *	<2e-16 ***	2.54e-08 ***	0.0222 *
D	<2e-16 ***	<2e-16 ***	2.60e-11 ***	< 2e-16 ***
Tr x D	0.8559	<2e-16 ***	0.368	7.1e-11 ***

W, Mean separation in Treatments; D, Mean separations in Days. Tr, Treatments; D, Days; Tr x D, interactions; For a given factor (different letters within a column represent significant differences (Tukey test, *, Significant at *p*-value <0.05; ***, Significant at *p*-value < 0.001). Data are stated as averages of the data and their standard deviations.

A hierarchical clustering heatmap illustrating the relative concentrations of berry characteristics in grape samples across treatments and days. Berry characteristics are clustered at the bottom of the heatmap, revealing similarities and dissimilarities between them. Weight loss and brix emerge as closely related, indicating similar concentration patterns across samples. Conversely, compounds like tartaric acid exhibits lower concentrations in these same samples, as shown by the deep blue color. The pH was between these groups. Grape samples, labeled with days and treatments are vertically clustered on the right. It shows that the treatments were strongly correlated among berry characteristics, while day 0 (as control), day 5, day 10 and day 15 were separated in the heatmap depending on the amount of the berry features such as weight loss, brix, ph and tartaric acid (Fig. 2E).

The association of melatonin with grapevine physiology is part of a comprehensive investigation into the consequences for berry quality and postharvest protection. To make clear the intricate processes by which melatonin influences the growth and maturity of grapevines, and therefore the characteristics of fruit quality. It has been discovered that melatonin applied postharvest can effectively delay ripening and senescence. The study regarding grape berries examined the effects of two postharvest melatonin doses (50 and 100 µmol) on the quality, bioactive components, and enzyme activities of grape berries cv "Crimson," which were kept for 35 days at 0 ± 1 °C and 90% relative humidity (RH). According to their findings, adding melatonin to berries prolongs their shelf life by preventing weight loss and preserving titratable acidity (TA), total soluble solids (TSS), berry adherence strength, and firmness (Nasser et al., 2022). The findings from this study are in parallel with the studies. Results from this research indicated that from this research the control and 6-hours 25 µmol L^{-1} treatments, (31.91% and 32.41%, respectively) were higher predominantly weight loss than treatment of 3-hours 250 µmol L^{-1} (24.08%). This showed that waiting too long (6 hours) in solution with low melatonin concentration (250 µmol L^{-1}) is not affected to weight loss as it was not statistically different than control. However, waiting enough time (3 hours) with an adequate melatonin dose (250 µmol L^{-1}) had a great contribution to weight loss (Table 1).

Moreover, some other researches are supported the current study. For example, after harvest, the main issue is weight loss since it deteriorates the quality and visual appeal of horticulture crops. According to reports, the biggest causes of water loss in fresh vegetables and fruits are transpiration and respiration (El-Mogy et al., 2020). Many studies reported that the application of melatonin has reduced the water loss in crops (Nasser et al., 2022; Liu et al., 2018; Wang et al., 2020). In addition, a study was conducted to protect post-harvest grape quality in cold storage from Türkiye, which is recently reported melatonin dips, in any dosage, slowed down the loss of berry weight, and visual quality, thereby prolonging the minimally processed (stem-excised) grapes' physical and biochemical quality (Sabir et al., 2024). In addition, the melatonin treatment has increased the Brix levels compared to the control in this study (Table 1). The findings from the previous research, additionally, have reported an increase of Brix level compared to control in grapevine which could be due to a decreasing respiration and a reduction in the loss of Brix (Nasser et al., 2022; Xu et al., 2018). Furthermore, exogenous melatonin application has also reported an increase in Brix other fruits such as strawberries (El-Mogy et al., 2020) and sweet cherries (Wang et al., 2019). Another finding from this study is that the application of melatonin has reduced the loss of TA (except, for 6-hours of waiting with 25 µmol treatment which could be the same scenario in the weight loss of 6-hours waiting with 25 µmol treatment) levels in grape berries from the current results (Table 1). The reduction in the loss of TA has also been reported in grapes and guava fruits (Nasser et al., 2022; Fan et al., 2020).



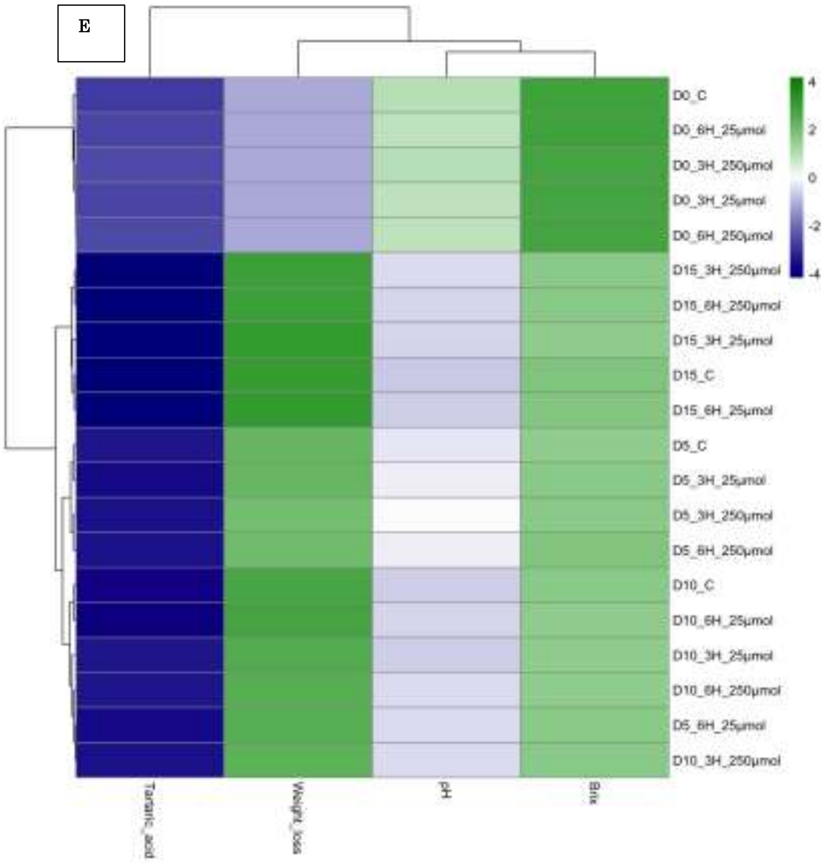


Figure 2. PCA biplot of colored by days and treatments. All application treatments (A), days (B), all variables (C), correlation of all variables (D) and heatmap of variables (E) are demonstrated.

Şekil 2. Günlere ve uygulamalara göre renklendirilmiş PCA biplot'u. Tüm uygulamaları (A), günleri (B), tüm değişkenler (C), tüm değişkenlerin korelasyonu (D) ve değişkenlerin ısı haritası (E) gösterilmektedir.

Furthermore, exogenous melatonin administration has been shown in prior research to reduce TA loss in sweet cherry and grape berries during cold storage (Wang et al., 2019). Melatonin's role in senescence control may account for some of its reduction in TA loss upon administration (Yang et al., 2019).

CONCLUSION

Post-harvest processes have an important role in grape production to preserve berry quality and health. The short shelf life of the Kabarcık variety after harvest has become a big problem for Dulkadiroğlu grape growers. In this study conducted to solve this problem, as a result, the highest weight loss was found in the control and 6-hours 25 µmol L^{-1} melatonin applications, and the least weight loss was found in the 3-hours 250 µmol L^{-1} melatonin application. As a result of this study, it was determined that keeping grape clusters in solution for a long period of 6-hours was an ineffective method and the applied dose of 25 µmol L^{-1} melatonin was found to be ineffective. However, a 250 µmol L^{-1} dose of melatonin was determined to be an effective solution in preventing weight loss in grape berries. In future studies, grape berries are required to be supported by biochemical and molecular analyses.

Conflicts of Interest Statement

The author has stated that there is no conflict of interest.

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