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Reducing respiration rate and increasing chemical stability of mulberry fruits by using postharvest putrescine and melatonin

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Abstract

Background Plant growth regulators have been widely used to extend the cold storage period of fruits and preserve quality. These phytohormones help mulberry fruit in maintaining its postharvest quality and soft texture, prevent from spoiling during cold. For this purpose, mulberry fruit were stored in cold for 5, 10 and 15 day (0.5 °C and 90% RH) and the impacts of melatonin (1.0 mM) and putrescine (1.0 mM) on quality parameters, phenolic compounds (gallic, protocatechuic, catechin, syringic, *p*-coumaric, chlorogenic, *o*-coumaric, ferulic, phloridzin, rutin and quercetin) and organic acids (malic, citric, succinic, oxalic, tartaric, fumaric and ascorbic) were investigated.

Results The combined application of putrescine and melatonin maintained the weight loss (4.35%), decay rate (9.48%) and 29.89% lower respiration rate (6.07 mg CO₂ kg⁻¹ h⁻¹) in mulberry fruits stored for 15 d as compared to the control. Phenolic compounds (especially rutin) and organic acids were found to be more affected by melatonin and putrescine applications compared to other findings in this study. However, melatonin treatment is more effective in maintaining the level of organic acids and phenolic compounds in mulberry fruit during storage than the putrescine. It was determined that soluble solids content, weight loss, decay rate and pH values had a positive relationship with each other. Also, it was also seen that phenolic compounds and organic acids had a linear relationship.

Conclusion The combined application of melatonin and putrescine was found more effective in maintaining the quality parameters of mulberry fruits during cold storage.

Keywords *Morus*, Storage, Polyamine, Melatonin, Phenolics

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Background

Türkiye, which has a long history of fruit growing, is among the homelands of mulberry (*Morus sp.*). Mulberry is a type of berry fruit that can adapt to a wide range of climate conditions and is produced in temperate, subtropical and tropical climate regions. The most common types in Türkiye are black mulberry (*Morus nigra*), purple mulberry (*Morus rubra*) and white mulberry (*Morus alba*) and are grown in different regions for different purposes [1]. Mulberry fruits are used in the production of molasses, fruit pulp, jam and dried fruit. Mulberry fruit is consumed by adults and children because it is very beneficial for health. Fruit is used as an antipyretic, mouth and tooth sore treatment and showing anti-inflammatory properties [2]. Due to its usage in health improvement [2] and its consumption in different ways, there is an increase in studies to extend the marketability and storage period of mulberry fruit. Kim et al. [3] stated that the storage conditions and mulberry cultivars were affecting the shelf life of fruits. Some researchers have reported that weight loss in mulberries is reduced under ozone-enriched cold storage conditions [4]. Yang et al. [5] stated that weight loss and decay were less, and anthocyanins were higher in mulberry fruits stored for 18 days with chitosan-g-caffeic acid application.

Plant growth regulators are compounds used in modern cultivation techniques and affect many physiological events in plants. Polyamines (spermidine, putrescine and spermine) and melatonin are growth regulators whose use has increased in recent years. Melatonin, which is also produced endogenously in plants, is defined as indolamine. Also, putrescine is expressed as an organic compound with an amine group. Putrescine affects important activities such as cell growth and division, plant growth and flowering in the plant body [6]. It has been stated that during storage, putrescine reduces lipid peroxide and preserves phenolic compounds in plums [7], reduces weight loss and preserves firmness in bitter melon [8], reduces rotting and delays softening in tomatoes [9]. Melatonin, on the other hand, is involved in physiological events such as germination, photosynthesis, fruit ripening, plant growth and leaf fall [10]. In addition, it has been reported that melatonin delays fruit softening in mango, preserves firmness and the amount of phenolic compounds during storage [11], reduces rotting in strawberries [12], increases antioxidant activity in spaghetti squash and reduces cold damage [13].

After harvest, one of the most important factors to extend the life of fruits is cold storage. In mulberry fruit, the texture is preserved by cold storage, decay is slowed down, and the quality of the fruit is preserved [3, 4]. Compounds such as 1-methylcyclopropene (1-MCP), natamycin, and ozone application affects the storage duration and quality of the fruit. Öz and Ulukanlı [14]

reported that browning reduced in storage with the application of 1-MCP and calcium chloride. Putrescine [15] and melatonin [16] are also important plant growth regulators that affect storage time and fruit quality. Also, putrescine [17] and melatonin [18] extend the storage time and shelf life by reducing ethylene synthesis in fruits. It is known that these chemicals strengthen the cell wall structure and play a role in reducing color deterioration in fruits [19, 20]. Many studies have been conducted on the use of putrescine and melatonin for preservation in different fruits. However, studies on their combined use in preservation are insufficient.

Mulberry fruit is seen as a problem as it has a short lifespan of 8–10 days after harvest and plant growth regulators are used to extend this period. Although melatonin and putrescine are effective compounds in storage when used separately, the number of studies on the effects of combination in mulberry fruit is insufficient. This study was conducted to increase the storage period of mulberry by taking advantage of the positive effects of these compounds and to reduce losses by preserving fruit quality during cold storage.

Material and method

Mulberry fruits were harvested by hand picking from the garden established at 4×4 m distances in Yalova (Türkiye). When fruits had 15.2% soluble solids content (SSC) and transported laboratory of Bolu Abant İzzet Baysal University Faculty of Agriculture by keeping in cold. In order to determine the status at harvest time, all analyzes were performed, and the remaining fruit were subjected to melatonin and putrescine application. Some fruit was reserved for the control group while others were subjected to application of 1.0 mM melatonin (Mel), 1.0 mM putrescine (Put) and the combined application of melatonin and putrescine (MelxPut). The fruits were immersed in water containing this compound for 10 min. Only distilled water was applied to the control fruit. The fruits were dried and placed in containers. Fifty fruits were placed in perforated plastic containers and stored in cold storage (0.5± °C and 90± % RH) for 5, 10 and 15 d in 3 replications. The fruit quality and biochemical attributes were examined at 5-day intervals during the storage period. In order to determine the weight loss, another set of mulberries was used in a separate container at 0.5 °C for 15 days and weighed every 5 days.

Respiration rate

To determine the respiration percentage, fifty mulberry fruits were placed in 2-liter containers with lids. A CO₂ sensor (Testo 535 CO₂ Meter, Germany) was inserted through the hole in the lid, all surface areas were covered with parafilm. Then the amount of CO₂ in the container was determined in this sensor after 1 h. The amounts

were measured under room conditions. Results were expressed as $\text{mg CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ [21].

Weight loss and decay rate

For weight loss, initial weights of mulberry fruits were taken and weights at the end of 5, 10 and 15 days were taken and results were calculated as percentage. Initially sound fruits were placed in storage and the decay rate was determined during 5, 10 and 15 days of storage and finally the decay percentage was calculated. A small amount of mycelium growth on the fruit peel was considered as rot and was proportioned according to the number of fruits in the box.

SSC, pH and acidity

In 20 mL of fruit juice obtained from mulberry, the amount of soluble solid content was determined as % with a refractometer and pH value was determined with a pH meter. This mulberry juice was titrated with 0.1 N NaOH to pH 8.1 and the value of acidity was measured with the titration method as % (malic acid).

Organic acids

Five g of mulberry fruits were taken, homogenized with 0.009 N sulfuric acid and centrifuged at 15,000 rpm for 15 min. Extract was passed through a 0.45 μm membrane filter (Millipore Millex-HV Hydrophilic PVDF, Millipore, USA) and SEP-PAK C18 cartridge. The acids were analyzed using high performance liquid chromatography (HPLC) with Aminex HPX – 87 H, 300 mm x 7.8 mm column. Organic acids were detected DAD detector at both 254 nm and 280 nm wavelengths. Flow rate adjusted to 1 ml min^{-1} and injection volume was 20 μL . As the mobile phase, 0.009 N H_2SO_4 was passed through a 0.45 μm filter membrane [22].

Phenolic compounds

The method reported by Rodriguez-Delgado et al. [23] was used for the determination of phenolic compounds. Five g of mulberry sample was homogenized, diluted with distilled water and centrifuged at 15,000 rpm for 15 min. The extract was filtered with a 0.45 μm millipore filter and injected to HPLC. Chromatographic separation was performed on Agilent 1100 HPLC system, using a DAD detector and a 250*4.6 mm, 4 μm ODS column. The mobile phase solvents in water with a flow rate of 1 $\text{mL}^{-1} \text{min}$ and 20 μl injection volume were used for spectral measurements taken at both 254 nm and 280 nm: as mobile phase solvent A, methanol-acetic acid-water (10:2:88) and Solvent B, methanol-acetic acid-water (90:2:8).

Statistical analysis

The results obtained were subjected to ANOVA test in SAS Version 9.1. When the results found were significant, the averages were subjected to Tukey test. The relationship between the data investigated in the study [24] and the heatmap [25] were determined in the R program. The relationship between the applications and the examined features was determined using the R program for PCA analysis [26].

Results

Respiration rate

It was determined that storage time ($F_{\text{Storage time}}:47.35, p \leq 0.001$) and interaction of storage time with combined application of melatonin and putrescine ($F_{\text{Storage time} \times \text{Melatonin and Putrescine}}:40.55, p \leq 0.001$) significantly affected respiration rate in mulberry fruits. The respiration rate decreased as the storage period was extended (Fig. 1). However, the lowered respiration rate was recorded in the applications as compared to the control at all storage times (5, 10 and 15th day). With the combined application of melatonin and putrescine, 50.5% less respiration rate ($5.89 \text{ mg CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$) was observed on the 10th day and 29.5% less respiration rate ($6.07 \text{ mg CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$) on the 15th day compared to the control. The application of melatonin is more effective in lowering the respiration rate under all storage durations as compared to the putrescine application (Supplementary Table).

Weight loss and decay rate

Significant loss in weight occurred in mulberry fruits during storage ($F_{\text{Storage time}}:23.28, p \leq 0.001$). When only the storage period was evaluated, the weight loss was measured as 2.21% on the fifth day and 6.08% on the 15th day (Fig. 1). It was observed that the most weight loss occurred in the control fruit and the least loss occurred in the combined application of melatonin and putrescine (Fig. 1). There was less loss in mulberries applied only in melatonin compared to only putrescine application. In mulberries stored for 15 d, the least weight loss was determined as 4.35% in fruit where melatonin and putrescine were applied together (Supplementary Table and Fig. 1).

When the storage period was evaluated, it was observed that decaying in mulberry fruit increased during storage ($F_{\text{Storage time}}:23.28, p \leq 0.001$) and was determined as 12.97% at the end of 15 d (Supplementary Table). While the amount of decay increased during storage in all applications, the highest amount of decay rate was determined in the control at the end of two weeks with 18.25% ($F_{\text{Storage time} \times \text{Melatonin and Putrescine}}:18.68$). It was determined that only melatonin application reduced the amount of decay rate during storage compared to

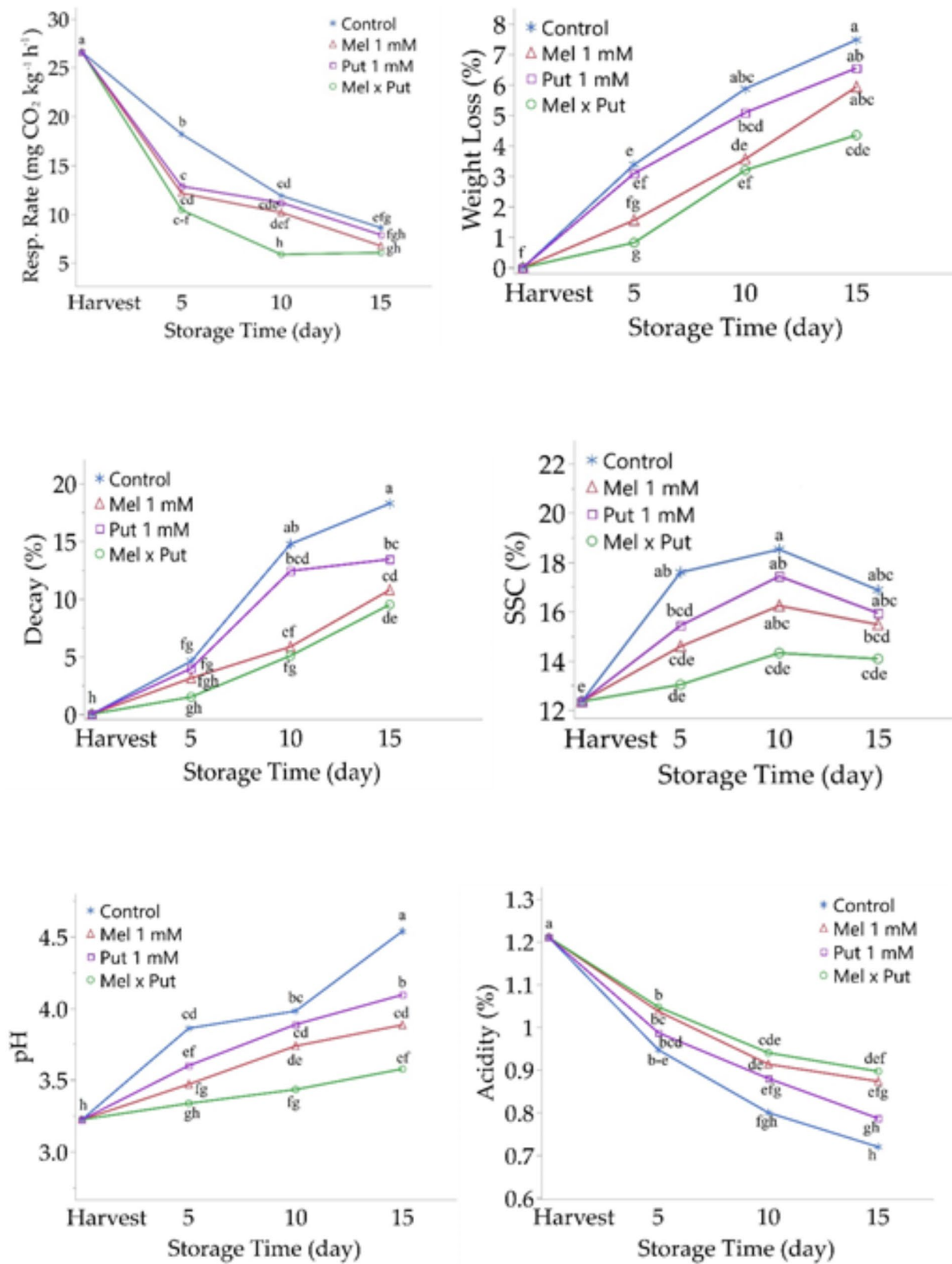


Fig. 1 Influence of postharvest melatonin and putrescine applications on quality traits of fruits. Different letters on top of the storage periods indicate significant differences at $p \leq 0.05$

only putrescine application. At the end of 15 d of storage, the least amount of decay was recorded in the combined application of melatonin and putrescine (9.48%). It was determined that this application had 48% less decay (Fig. 1) compared to the control (18.24%).

SSC, pH and acidity

The amount of soluble solids contents in stored mulberry fruit was measured and it was observed that there was an increase during storage compared to harvest time. It was found that storage time significantly affected the amount of SSC ($F_{\text{Storage time}}:3.7, p \leq 0.05$), while no statistical difference was found between 5, 10 and 15 d storage periods (Table 1). In mulberry fruits applied only putrescine, the amount of SSC was higher during storage than in fruit applied only melatonin. It was determined that melatonin and putrescine combined applications had lower SSC amounts during storage periods. As a result of two weeks of storage, the lowest SSC amount was obtained as 14.08% in the combined application of melatonin and putrescine (Fig. 1). It was determined that storage time significantly affected the pH amount in mulberry fruit ($F_{\text{Storage time}}:9.8, p \leq 0.001$) and that the pH amount (4.02 in 15th day) increased as storage time increased (Supplementary Table). In all storage periods (5, 10 and 15th day), pH amounts were recorded lower in the applications compared to the control. At the end of 15 d of storage, pH amount in mulberry fruit juice was measured highest in

1.0 mM putrescine application (4.09) and lowest in MelxPut combined application (3.58) compared to the control (Fig. 1). Titratable acidity amount was measured in mulberry fruits during storage and it was found to decrease during storage compared to harvest time (1.21%) (Supplementary Table). When storage periods were evaluated, no statistical difference was observed between 10 and 15 d of storage. The effect of storage period and putrescine and melatonin applications on acidity was found to be significant ($F_{\text{Storage time} \times \text{Melatonin and Putrescine}}:36, p \leq 0.001$). It was determined that the applications had higher acidity amounts compared to the control at all storage times. After 15 d of storage, the highest acidity amount (0.90%) was determined in MelxPut combined application. It was defined that only melatonin applications had higher acidity content compared to only putrescine applications during storage processes (Fig. 1).

Organic acids

Organic acids were examined in stored mulberry fruit, and it was determined that storage and applications significantly affected these amounts (Table 1). The amounts of 7 organic acids, namely malic, citric, succinic, oxalic, tartaric, fumaric and ascorbic acids, were determined and it was determined that all acids decreased during storage compared to harvest time. It was recorded that melatonin and putrescine combined applications had higher acid content at the end of 15 d of storage in all determined

Table 1 Impact of different doses of melatonin and Putrescine treatment on organic acid contents of mulberry fruits during storage ($\text{mg } 100 \text{ g}^{-1}$)

Storage time		Malic	Citric	Succinic	Oxalic	Tartaric	Fumaric	Ascorbic
Harvest		11.78±0.10a	5.20±0.05a	2.73±0.14a	1.59±0.07a	1.23±0.05a	1.08±0.06a	28.30±0.52a
5th day		10.54±0.31b	4.76±0.21b	2.37±0.18b	1.35±0.12b	1.07±0.11a	0.71±0.13b	24.51±1.24b
10th day		9.70±0.42c	4.51±0.19c	1.91±0.21c	1.13±0.19c	0.87±0.15b	0.52±0.11c	21.71±1.28c
15th day		8.49±0.44d	3.98±0.24d	1.61±0.24d	0.99±0.21c	0.65±0.14c	0.45±0.10c	19.47±1.31d
Storage time × Melatonin and Putrescine interaction								
Harvest		11.78±0.10a	5.20±0.05a	2.73±0.14a	1.59±0.07a	1.23±0.05a	1.08±0.06a	28.30±0.52a
5th day	Control	10.18±0.13d	4.48±0.11d	2.14±0.10d	1.19±0.04ef	0.92±0.09ef	0.52±0.03ef	22.70±0.47de
	Mel1mM	10.58±0.06c	4.87±0.08b	2.50±0.01b	1.42±0.01b	1.14±0.05bc	0.77±0.06bc	25.28±0.14b
	Put1mM	10.44±0.10c	4.69±0.09c	2.31±0.07c	1.31±0.06 cd	1.06±0.04 cd	0.72±0.05 cd	24.40±0.51c
	MelxPut	10.96±0.13b	4.98±0.09b	2.54±0.11b	1.48±0.07b	1.17±0.05ab	0.83±0.06b	25.66±0.40b
10th day	Control	9.12±0.14 g	4.24±0.06e	1.66±0.09hi	0.85±0.08 h	0.66±0.03hi	0.39±0.03 g	19.90±0.34 h
	Mel1mM	9.90±0.08e	4.61±0.05 cd	2.00±0.06e	1.22±0.06de	0.94±0.04e	0.54±0.02ef	22.09±0.32ef
	Put1mM	9.61±0.17f	4.49±0.06d	1.79±0.04 fg	1.11±0.04 fg	0.83±0.07 g	0.49±0.04f	21.71±0.26 fg
	MelxPut	10.18±0.13d	4.69±0.09c	2.17±0.06d	1.32±0.09c	1.03±0.04d	0.67±0.03d	23.16±0.67d
15th day	Control	7.90±0.16j	3.72±0.13 g	1.28±0.06j	0.73±0.07i	0.48±0.04j	0.32±0.03 h	18.05±0.60j
	Mel1mM	8.63±0.05 h	4.05±0.10f	1.73±0.07gh	1.03±0.04 g	0.68±0.04 h	0.50±0.04f	19.77±0.22 h
	Put1mM	8.40±0.06i	3.85±0.10 g	1.55±0.04i	0.93±0.01 h	0.58±0.03i	0.42±0.03 g	18.80±0.36i
	MelxPut	9.04±0.16 g	4.29±0.07e	1.88±0.08ef	1.29±0.04 cd	0.84±0.06 fg	0.57±0.03e	21.26±0.56 g
ANOVA								
$F_{\text{Storage time}}$		87.63***	41.83***	40.34***	13.9***	27.97***	32.32***	57.05***
$F_{\text{Storage time} \times \text{Melatonin and Putrescine}}$		264.07***	82.8***	91.94***	61.43***	68.43***	85.08***	137.84***

Different letters in the same column indicates statistical differences at $p \leq 0.05$. *** indicates $p \leq 0.001$

acids. It was also determined that only melatonin (1.0 mM) applications had higher acid content compared to only putrescine (1.0 mM) applications during storage in all acids. The effect of storage time and melatonin and putrescine applications on malic acid amounts was significant ($F_{\text{Storage time} \times \text{Melatonin and Putrescine}}:264.07, p \leq 0.001$). Malic acid amount measured as $11.78 \text{ mg } 100 \text{ g}^{-1}$ at harvest time was determined as $7.90 \text{ mg } 100 \text{ g}^{-1}$ in the control group and $9.04 \text{ mg } 100 \text{ g}^{-1}$ in MelxPut combined application with an increase of 14% at the end of 15 days. Citric acid amount was the most preserved organic acid with 82.5% preservation at the end of 15 d of storage. This acid was recorded as $4.29 \text{ mg } 100 \text{ g}^{-1}$ in the combined application of melatonin and putrescine at the end of 15 d of storage. Succinic acid amount was significantly affected by storage time and applications ($F_{\text{Storage time} \times \text{Melatonin and Putrescine}}:82.8, p \leq 0.001$). At the end of the two-week storage period, this acid was measured as $1.28 \text{ mg } 100 \text{ g}^{-1}$ in the control, while the highest amount ($1.88 \text{ mg } 100 \text{ g}^{-1}$) was measured in the MelxPut combined application. The amount of oxalic acid was determined as $1.59 \text{ mg } 100 \text{ g}^{-1}$ at harvest time. This amount was recorded as $1.32 \text{ mg } 100 \text{ g}^{-1}$ on the 10th day and $1.88 \text{ mg } 100 \text{ g}^{-1}$ on the 15th day in the melatonin and putrescine combined application. The amount of tartaric acid was significantly affected during the storage process ($F_{\text{Storage time}}:27.97, p \leq 0.001$) and was measured as $1.23 \text{ mg } 100 \text{ g}^{-1}$ at harvest. At the end of 15 d of storage, the highest content compared to the control was determined as $0.84 \text{ mg } 100 \text{ g}^{-1}$ in the combined application of melatonin and putrescine. At the end of the two-week storage period, this acid was measured as $1.28 \text{ mg } 100 \text{ g}^{-1}$ in the control group, while the highest amount ($1.88 \text{ mg } 100 \text{ g}^{-1}$) was measured in the MelxPut combined application. The amount of oxalic acid was determined as $1.59 \text{ mg } 100 \text{ g}^{-1}$ at harvest time. This amount was recorded as $1.32 \text{ mg } 100 \text{ g}^{-1}$ on the 10th day and $1.88 \text{ mg } 100 \text{ g}^{-1}$ on the 15th day in the melatonin and putrescine combined application. The amount of tartaric acid was significantly affected during the storage process ($F_{\text{Storage time}}:27.97, p \leq 0.001$) and was measured as $1.23 \text{ mg } 100 \text{ g}^{-1}$ at harvest. At the end of 15 d of storage, the highest value compared to the control was determined as $0.84 \text{ mg } 100 \text{ g}^{-1}$ in the combined application of melatonin and putrescine. Tartaric acid and succinic acid levels were maintained at 68% after two weeks compared to harvest time. The amount of fumaric acid was affected by the treatments ($F_{\text{Storage time} \times \text{Melatonin and Putrescine}}:85.08, p \leq 0.001$) and at the end of 15 d, it was measured approximately 2 times more in the melatonin and putrescine combined ($0.57 \text{ mg } 100 \text{ g}^{-1}$) application compared to the control. However, this acid was recorded as the least preserved acid during storage (52.7%). The amount of ascorbic acid was significantly affected by the application

and storage time ($F_{\text{Storage time} \times \text{Melatonin and Putrescine}}:137.84, p \leq 0.001$). The acid measured at 28.30 at harvest time was measured at the lowest value in the control group after two weeks. In 15 days of storage, 75% was preserved compared to the harvest time and the highest value ($21.26 \text{ mg } 100 \text{ g}^{-1}$) was recorded in the MelxPut combined application. In this study, it was determined that melatonin has a significant effect on preserving organic acid contents and this effect increased with putrescine application and better preserved the acid content of the mulberry fruit.

Phenolic compounds

The effects of melatonin and putrescine on the amount of phenolic compounds in 5, 10 and 15 d of storage of the mulberry fruit were investigated and are given in Tables 2 and 3. Phenolic compounds were significantly affected by the storage period. The highest amount of chlorogenic acid ($F_{\text{Storage time}}:83.79, p \leq 0.001$) was measured during storage, followed by gallic acid ($F_{\text{Storage time}}:21.95, p \leq 0.001$), rutin ($F_{\text{Storage time}}:8.39, p \leq 0.001$) and quercetin ($F_{\text{Storage time}}:121.7, p \leq 0.001$). It was observed that other phenolic compounds except for rutin decreased during storage and the lowest values were obtained at the end of 15 d of storage. The amount of gallic, protocatechuic, syringic, p-coumaric, chlorogenic, o-coumaric and ferulic acids, quercetin, phloridzin and catechin was measured in the highest melatonin and putrescine combined application at the end of 15 d of storage. Again, it was determined that only melatonin application had higher values compared to only putrescine application during storage period for these phenolic compounds (Table 3). The highest rutin amount was measured in the MelxPut combined application ($6.06 \text{ mg } 100 \text{ g}^{-1}$) and 1 mM melatonin ($6.03 \text{ mg } 100 \text{ g}^{-1}$) applications, at the end of 15 days of storage. During 15 d of storage, this phenolic compound was the most preserved (96.4%) among all phenolic compounds (Table 2). Chlorogenic acid was measured $22.42 \text{ mg } 100 \text{ g}^{-1}$ in the combined application of melatonin and putrescine and 76% was preserved at the end of two weeks and measured. While the lowest amount of gallic acid was measured in the control group at the end of 15 days of storage, the highest was measured as $20.09 \text{ mg } 100 \text{ g}^{-1}$ in melatonin and putrescine combined application (Table 2). Protocatechuic acid and syringic acid levels were preserved at 64% after two weeks of storage (1.21 and $1.41 \text{ mg } 100 \text{ g}^{-1}$, respectively). The highest level phloridzin and quercetin was measured in the combined application after 15 d. Ferulic acid varied throughout storage and was preserved by 83% in MelxPut combined application at the end of 15 d (Table 3). P-coumaric acid amount was measured as $4.0 \text{ mg } 100 \text{ g}^{-1}$ at the time of harvest and $3.17 \text{ mg } 100 \text{ g}^{-1}$ in MelxPut combined application at the end of 15 d. The least preserved phenolic compound at the end of 15 days

Table 2 Impact of different doses of melatonin and Putrescine treatment on phenolic compounds of mulberry fruits during storage (mg 100 g⁻¹)

Storage time		Gallic	Protocatechuic	Catechin	Syringic	P-Coumaric	Chlorogenic
Harvest		23.35±0.93a	1.88±0.09a	4.00±0.10a	2.17±0.10a	4.00±0.15a	29.16±0.57a
5th day		20.950.66b	1.53±0.09b	3.58±0.21b	1.78±0.11b	3.81±0.11a	27.19±0.90b
10th day		19.770.87c	1.19±0.19c	3.17±0.17c	1.38±0.22c	3.35±0.15b	22.57±1.23c
15th day		18.891.23d	0.98±0.20d	2.75±0.11d	1.13±0.23d	2.91±0.22c	20.72±1.45d
Storage time × Melatonin and Putrescine interaction							
Harvest		23.35±0.93a	1.88±0.09a	4.00±0.10a	2.17±0.10a	4.00±0.15a	29.16±0.57a
5th day	Control	20.34±0.30de	1.43±0.06 cd	3.27±0.05f	1.65±0.08de	3.66±0.07d	26.05±0.17d
	Mel1mM	21.14±0.35bc	1.55±0.05bc	3.69±0.07c	1.80±0.04bc	3.87±0.04bc	27.67±0.24bc
	Put1mM	20.62±0.15 cd	1.50±0.07c	3.55±0.07d	1.75±0.07 cd	3.79±0.06c	26.88±0.66c
	MelxPut	21.71±0.70b	1.64±0.06b	3.80±0.06b	1.90±0.06b	3.92±0.04ab	28.16±0.34b
10th day	Control	18.85±0.54 g	0.90±0.06 h	2.99±0.04 g	1.04±0.06i	3.13±0.03gh	21.25±0.59 h
	Mel1mM	20.34±0.37de	1.30±0.07ef	3.21±0.09f	1.50±0.08 fg	3.47±0.06e	22.80±0.21f
	Put1mM	19.19±0.36 fg	1.22±0.07f	3.09±0.06 g	1.43±0.10 g	3.34±0.08f	22.08±0.33 fg
	MelxPut	20.71±0.06 cd	1.36±0.06de	3.41±0.03e	1.57±0.08ef	3.45±0.08ef	24.17±0.98e
15th day	Control	17.22±0.59 h	0.74±0.08i	2.62±0.07i	0.86±0.09j	2.63±0.10j	18.89±0.25j
	Mel1mM	19.76±0.20ef	1.05±0.05 g	2.81±0.05 h	1.22±0.05 h	3.01±0.04 h	21.49±0.35gh
	Put1mM	18.50±0.24 g	0.90±0.05 h	2.70±0.05i	1.04±0.05i	2.82±0.09i	20.09±0.48i
	MelxPut	20.09±0.48de	1.21±0.16f	2.88±0.07 h	1.41±0.17 g	3.17±0.09 g	22.42±0.55f
ANOVA							
F _{Storage time}		21.95***	37.14***	70.46***	37.08***	71.48***	83.79***
F _{Storage time × Melatonin and Putrescine}		32.81***	57.88***	148.13***	61.81***	95.62***	138.63***

Different letters in the same column indicates statistical differences at $p \leq 0.05$. *** indicates $p \leq 0.001$

Table 3 Continue Table 3 (mg 100 g⁻¹)

Storage time		o-Coumaric	Ferulic	Phloridzin	Rutin	Quercetin
Harvest		2.79±0.10a	2.91±0.07a	1.83±0.08a	6.28±0.26abc	5.72±0.05a
5th day		1.90±0.35b	2.74±0.09b	1.59±0.18b	6.87±0.70a	5.08±0.18b
10th day		1.47±0.19c	2.67±0.12b	1.25±0.14c	6.47±0.43b	4.28±0.24c
15th day		1.38±0.14c	2.23±0.15c	0.97±0.13d	5.91±0.20c	3.86±0.18d
Storage time × Melatonin and Putrescine interaction						
Harvest		2.79±0.10a	2.91±0.07a	1.83±0.08a	6.28±0.26ef	5.72±0.05a
5th day	Control	1.49±0.07ef	2.62±0.07e	1.37±0.07d	7.97±0.12a	4.83±0.08d
	Mel1mM	2.10±0.06c	2.79±0.07bcd	1.71±0.06b	6.41±0.06de	5.10±0.04c
	Put1mM	1.69±0.08d	2.73±0.05 cd	1.48±0.03c	6.82±0.12c	5.10±0.10c
	MelxPut	2.31±0.09b	2.82±0.03b	1.79±0.05ab	6.30±0.06ef	5.28±0.03b
10th day	Control	1.31±0.07gh	2.52±0.04f	1.06±0.03 fg	7.07±0.09b	3.96±0.07 h
	Mel1mM	1.53±0.11e	2.71±0.05d	1.37±0.02d	6.06±0.09gh	4.44±0.08f
	Put1mM	1.34±0.08fgh	2.61±0.05e	1.20±0.04e	6.62±0.06 cd	4.17±0.06 g
	MelxPut	1.71±0.12d	2.81±0.05bc	1.38±0.07d	6.13±0.13 fg	4.55±0.05e
15th day	Control	1.25±0.08 h	2.06±0.06j	0.80±0.03i	5.65±0.19i	3.65±0.03j
	Mel1mM	1.43±0.05efg	2.27±0.04 h	1.04±0.06 g	6.03±0.07gh	3.93±0.06 h
	Put1mM	1.30±0.16gh	2.15±0.05i	0.92±0.04 h	5.89±0.15 h	3.77±0.05i
	MelxPut	1.53±0.07e	2.43±0.03 g	1.13±0.05ef	6.06±0.09gh	4.08±0.05 g
ANOVA						
F _{Storage time}		35.63***	51.8***	46.12***	8.39***	121.7***
F _{Storage time × Melatonin and Putrescine}		79.2***	92.27***	134.75***	69.64***	378.63***

Different letters in the same column indicates statistical differences at $p \leq 0.05$. *** indicates $p \leq 0.001$

of storage compared to harvest time was o-coumaric acid (Table 3).

Identification of interrelationships between storage, melatonin and putrescine applications and fruit properties by PCA and heatmap

The quality parameters of mulberry fruit varied during storage, and melatonin and putrescine applications also affected this variability. The change in these properties during storage with putrescine and melatonin applications was determined as 89.37% (PC1 + PC, Fig. 2). It was observed that melatonin (1.0 mM) and the combined application of melatonin and putrescine overlapped very little, but putrescine (1.0 mM) overlapped highly with melatonin (1 mM). It was determined that the control group generally had high values during storage and formed a wider range, but MelxPut combined had lower values and formed a narrower range. PCA graph showed

that weight loss, decay and pH formed a close group, and again, all organic acids formed a close group with each other. Harvest and 5th day were in the 1st region, 10th d was in the 2nd region and provided more intersection with the control group. It was determined that other properties except tartaric acid, fumaric acid and oxalic acid showed a positive relationship in the PCA plane (Fig. 2).

The change in phenolic compounds according to the applications during storage is given in the PCA graph (Fig. 3). In melatonin and Putrescine application, the change between phenolic compounds during storage was determined as 96.61% (PC1 + PC2). While the control group had the widest range, melatonin (1.0 mM) application was found to have the narrowest range. While the combined application (MelxPut) and melatonin application showed a high intersection, putrescine overlapped with these applications to a small extent. It was

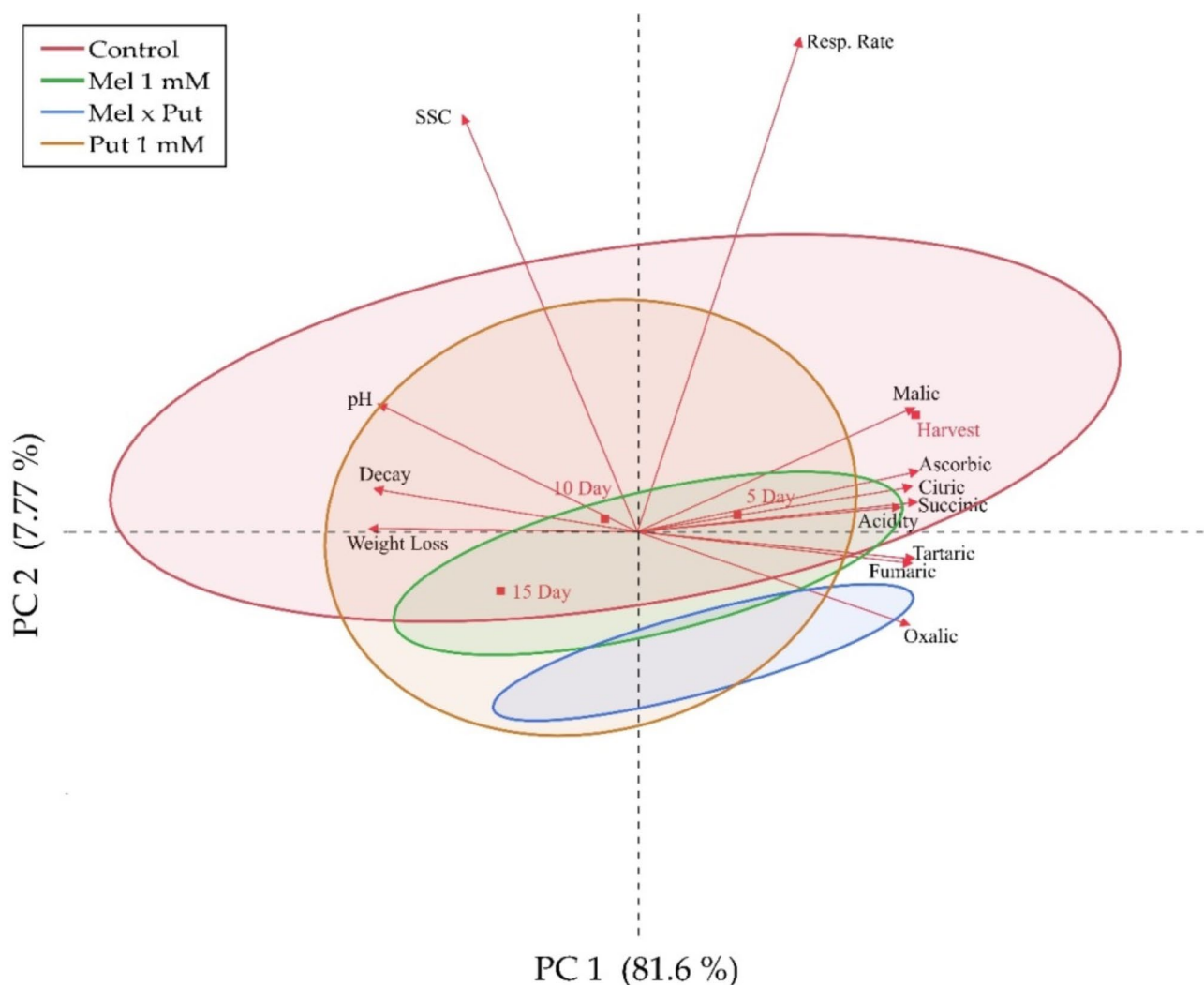


Fig. 2 Determination of the relationship between quality traits and organic acids according to applications using PCA

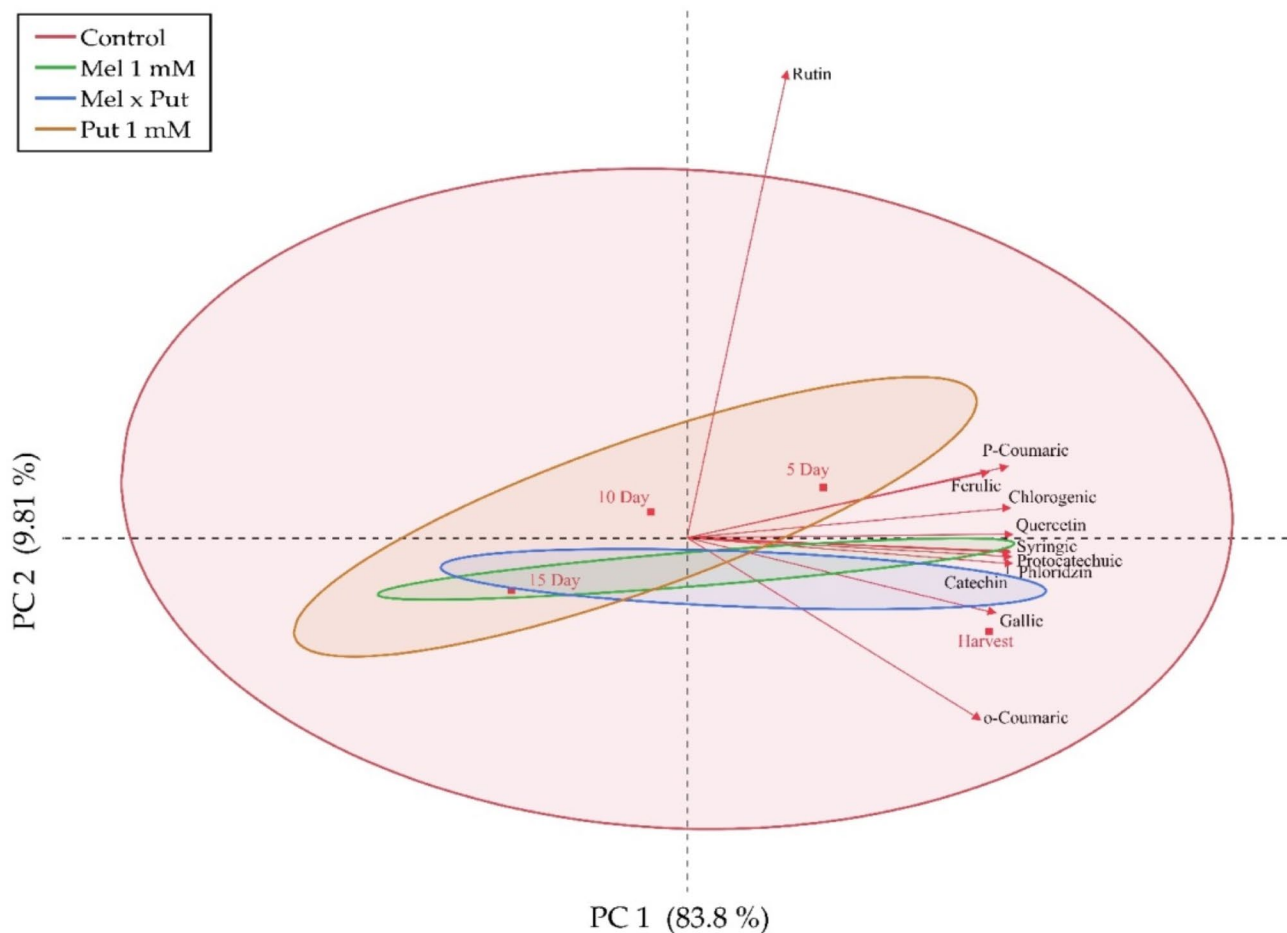


Fig. 3 Determination of the relationship between phenolic compounds according to applications using PCA

determined that rutin showed a different change from other phenolic compounds. Ferulic acid, p-coumaric acid, rutin and quercetin had a positive relationship in the PCA plane. Putrescine was seen to have a high intersection with 5 and 10 days of storage.

The criteria examined in the study, storage period and hapmap analysis of the applications are given in Figure. 4. When the storage period was evaluated in terms of application combinations, it was seen that the harvest time was separated from the applications and formed 2 groups. While the harvest time was found to have lower values (blue) in terms of weight loss, decay, pH and SSC, it was revealed that it had higher values (red) in terms of other properties except for rutin. Storage periods and melatonin, putrescine applications also formed two groups within themselves. In the 15 d storage period, applications other than the MelxPut combined application formed a separate group and were in the group with low values and more blue color. The combined application of melatonin and putrescine stored for 15 d was found in the group where all applications in the 5 d storage period were in red and green color intensity. It was

seen that weight loss, decay, pH and SSC formed a separate group in terms of the properties examined. It was seen that these properties generally had higher values (red) in the 15 d and 10 d storage periods. Although the features other than these formed a group within themselves, it was also determined that rutin was separated and formed a group on its own. It was observed that the respiration rate, acidity, organic acids and phenolic compounds had lower consequences (blue) in fifteen days of storage, excluding the combined application.

The correlation relationships of the examined traits are given in Fig. 5. It was determined that weight loss, decay rate, SSC and pH values were positively correlated with each other. It was also found that SSC had a positive correlation (0.34) with rutin at $p \leq 0.05$ level and these traits had a negative correlation with the others. It was determined that acidity value had a negative correlation with weight loss (-0.82), SSC (-0.62), decay (-0.82) and pH (-0.79) and had a positive relationship with other traits. It was observed that phenolic compounds (except rutin) and organic acids had a high positive correlation with each other ($p \leq 0.001$) and had a negative relationship

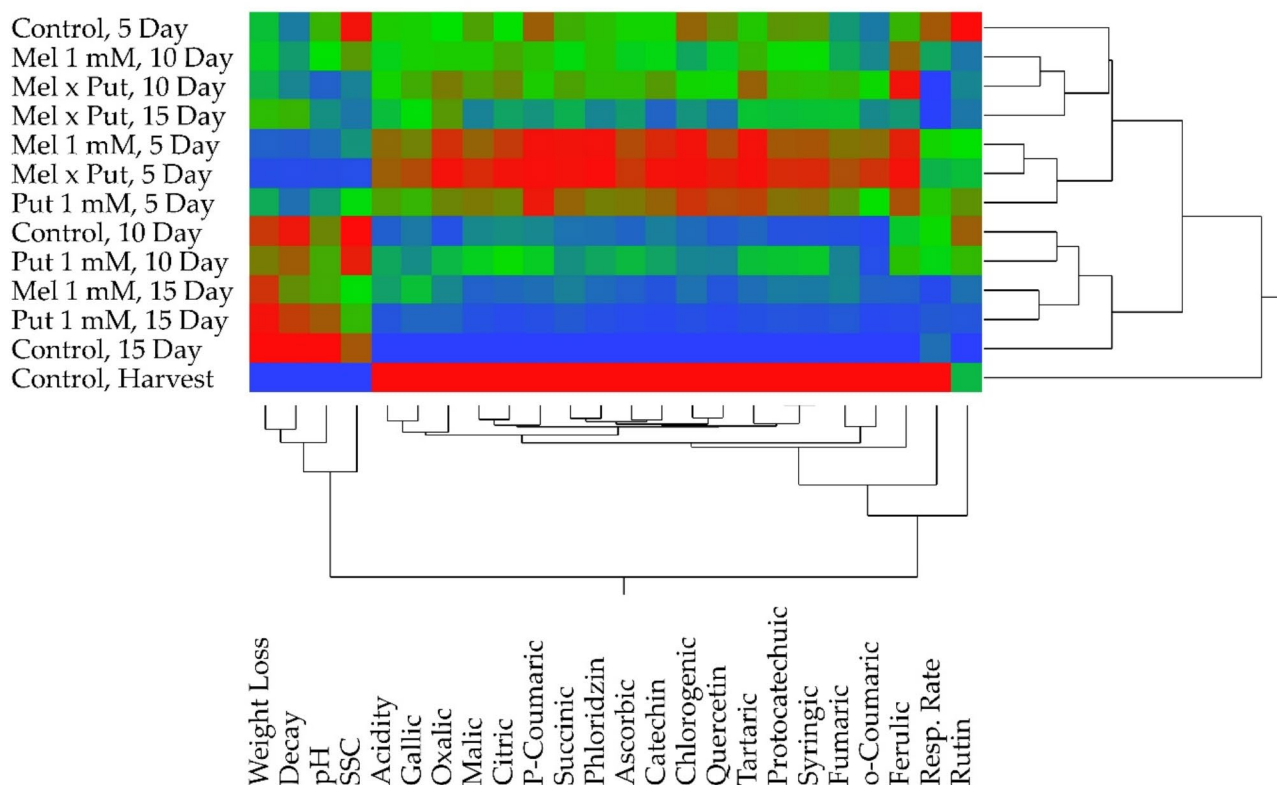


Fig. 4 Identification of the relationship between quality characteristics and biochemical contents according to applications by heatmap analysis. The color scale color from blue to red indicates the minimum to maximum values for each attribute

with weight loss, decay and pH. For example, a very significant positive correlation was determined between protocatechuic acid and syringic acid (1.0), between malic acid and ascorbic acid (0.97), between protocatechuic acid and succinic acid (0.96), between fumaric acid and tartaric acid (0.91), between citric acid and catechin (0.95), between catechin and syringic acid (0.93), between chlorogenic acid and quercetin (0.98). In addition, a positive correlation was established between acidity and respiration rate and organic acids and phenolic compounds. Rutin showed itself as the trait that exhibited a different attitude among all traits. It was determined that rutin had an opposite relationship with weight loss, decay and pH. On the contrary, rutin was found to have a high positive correlation with respiration rate, while it was found to have a positive correlation with SSC, malic acid, p-coumaric acid, chlorogenic acid, and ferulic acid at the level of $p \leq 0.05$.

Discussion

There is a direct relationship between the storage time of fruits and respiration rates [27]. In this study, the respiration rate increased during storage and the best results were obtained in the combined application of melatonin and putrescine in 15 d (Supplementary Table). The effect of the plant growth regulators used was clearly observed.

It is thought that putrescine and melatonin reduce physiological activities and reduce the respiration rate. It is known that putrescine is synthesized in the ACC synthesis pathway and directly affects ethylene synthesis and physiological events such as aging [28]. In this study, it is thought that the increase in the presence of putrescine in the environment causes these activities to decrease and, accordingly, the respiration rate in the environment decreases. Again, the reduction of respiration due to melatonin reducing cell damage [29] can also be shown as one of the reasons for the decrease in the respiration rate in this study. In the studies, it was stated that putrescine application reduced the respiration rate in pomegranates [30] and plums [31]. Similarly, melatonin has been reported to reduce respiratory rate in pears [32], sweet cherry [33] and strawberries [34]. In this research, in accordance with the studies, it was revealed that combined treatment of putrescine and melatonin reduced respiration rate more effectively. It has been observed that the storage period can be extended by reducing respiration in mulberry fruit with the combined use of putrescine and melatonin.

It is known that mulberry fruit lose weight during storage [4] and this causes marketing problems. Weight loss increased during storage in this study and was determined to be the least in the MelxPut combined

rate increased during storage and the best results were obtained in the MelxPut combined application at the end of storage (Supplementary Table). After five days of storage, the decay rate began to increase more, but it was observed that this rate was less with the applications. Both growth regulators were effective in reducing the decay rate and the combined application increased this effect even more. The positive effects of these regulators in this study can be shown as reducing fungal formation in fruits and preventing softening [41] and preventing aging [42]. Likewise, consistent with the study, 1.5 mM melatonin application reduced the decay rate by half in papaya fruit [43] and similarly, in bitter melon, the decay rate of control fruits was 3 times higher compared to putrescine [8]. It was stated that the amount of conjugated polyamine increased in apricots to which melatonin was applied and accordingly the browning rate in the fruits was less [42]. Although there is no study in which both growth regulators were given externally, Dong et al. [42] supports the results of this study. It was concluded that the combined application was more effective because melatonin delays aging and increases the effect of putrescine. In this application, it was observed that the amount of phenolic compounds was preserved more (Table 3). Thus, it is thought that it contributes to the decrease in the decay rate. Because it is known that phenolic compounds prevent the formation of harmful microorganisms and naturally reduce the decay rate in fruits [44]. According to this study, it is thought that the combination of putrescine and melatonin (1 mM) can increase the market value of mulberry by controlling the decay rate.

The amount of SSC initially increased and then decreased in this study. The pH increased and the acidity decreased during storage. The combined application of melatonin and putrescine has come to the fore in terms of preserving these features. In line with this study, Kibar et al. [38] reported that the pH amount in peach increased with putrescine application. Researchers determined that while the value of SSC increased during storage, the amount of acidity decreased and that melatonin application largely preserved these amounts [18]. In another study, 1 mM putrescine was used as in this study and it was reported that SSC was more preserved with 1 mM putrescine application, but acidity was not affected in plums [7]. It was observed that similar results were obtained with our study in terms of SSC. SSC appears to be protected by the applications, and this is thought to be related to decreased respiration. Likewise, substances such as carbohydrates are used during respiration [45] and their amount in fruits decreases. In this study, it was observed that SSC in fruit juice was preserved with the decrease in respiration and aging, and pH and acidity levels were also preserved with the decrease in spoilage.

As the storage period increased, the positive effects of putrescine and melatonin were reflected in these criteria and revealed their importance in terms of preserving fruit quality.

Organic acids, which are important for human health as well as the flavor and aroma of the fruit, decreased during storage. However, in 15 days, MelxPut combined was the application in which these acids were preserved the most (Table 2). While the amount of ascorbic acid increased in mandarin fruit with putrescine application during storage [46], it was measured at a lower level in pomegranate fruit [30]. In a study conducted on papaya fruit, it was stated that 1.5 mM melatonin application caused less loss in the amount of ascorbic acid compared to the control during storage [36]. Although there is a difference between studies in terms of ascorbic acid, it decreased in this study. Despite this, it was observed that this value was preserved by reducing the loss with the application of putrescine and melatonin. It is thought that the amount of organic acids is preserved by reducing the deterioration in fruits. On the other hand, in a study conducted on raspberries, it was stated that higher amounts of malic, fumaric, citric, oxalic and succinic acid were measured compared to the control after one month of storage with putrescine (1.0 mM) application [47]. It is thought that the difference with this study may be due to the longer storage period. Because it is known that the spoilage rates in fruits increase as the storage period increases. In this study, factors such as chemicals such as putrescine and melatonin delaying postharvest ripening and reducing respiration and ethylene synthesis prevent the consumption of organic acids. Therefore, it was concluded that the amount of organic acids was preserved in the mulberry fruits to which the treatment was applied in this study. At the end of 15 days of storage, it was observed that the amount of organic acid was preserved with putrescine and melatonin, allowing the market to offer more delicious and higher quality fruit.

Phenolic compounds are organic compounds that are important for health due to the combination of taste and color of fruits and their ability to prevent diseases. However, these compounds lose value in cold storage conditions and various studies are being carried out to preserve them. Again, in this study, phenolic compounds decreased during the study. However, it was observed that these compounds other than rutin were better preserved in MelxPut combined applications than control (Tables 2 and 3). In the study conducted, it is thought that the respiration rate is lower in this application during storage (Fig. 1) and this decrease causes an increase in the accumulation of phenolic compounds. Similarly, in a study conducted on blueberries, it was stated that the amount of anthocyanin and flavonoids increased in fruits treated with melatonin during storage and had higher

amounts than the control group [42]. Mishra et al. [7] reported that the amount of total phenolic compounds was preserved more in 1 mM putrescine application. During cold storage, total antioxidants are observed to be preserved by plant growth regulators, while individual phenolic compounds show variability as in this study. In studies conducted on figs, it was reported that gallic, chlorogenic, syringic and p-coumaric acid and rutin had higher values compared to the control during storage with melatonin application [48] and putrescine application [49]. Similar results were obtained in this study, and it is predicted that melatonin and putrescine slow down aging by inhibiting ethylene and respiration rate and as a result, slow down the degradation of accumulated phenolic compounds. Because it is known that melatonin increases antioxidant enzyme activities that delay aging and therefore increases the amount of phenolic compounds [50]. While there are studies on the individual use of melatonin and putrescine, studies on their combination are insufficient. However, in this study, it was seen that the combination application was more effective on specific phenolic compounds than individual uses. This study is important in terms of preserving phenolic compounds that positively affect the appearance and quality of fruits, especially by affecting their color.

Conclusion

Since mulberry fruit has a delicate fruit texture, storage life decreases when it is preserved while preserving fruit quality. Therefore, the effect of putrescine and melatonin, which protect fruit quality by reducing post-harvest losses during storage, was investigated in mulberry fruit.

In general, it was observed that melatonin was more effective than putrescine in mulberry storage. At the end of this study, it was finalized that the combined treatment melatonin and putrescine (1 mM) was more effective in terms of weight loss, respiration rate, reduction of decay rate and preservation of biochemical contents of the mulberry in 15 days of storage. Melatonin and putrescine can be used together to extend the storage period of mulberry and preserve their quality. Thus, the market period of mulberries will be extended and the rate of meeting with consumers will increase. In addition, this study is a pioneer in terms of the use of melatonin and putrescine combination. Studies on the combined use of these regulators in mulberry fruit should be studied in more detail by increasing the dose and storage period.

Abbreviations

h	Hour
pH	Power of hydrogen
SSC	Soluble solids content
min	Minute
HPLC	High performance liquid chromatography
Mel	Melatonin
Put	Putrescine

mg	Milligram
mL	Milliliter
g	Gram
PCA	Principal component analysis

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12870-025-06459-0>.

Supplementary Material 1

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Author contributions

SPE: Visualization, Conceptualization, Methodology, Data analyzing, Supervision, Writing - original draft, Review, and editing. AMÇ, SKB, MS: Investigations, Data collections, Visualization. MG and FŞ: Methodology, Supervision, Visualization, Writing - original draft, Review, and editing. MG: Methodology, Supervision.

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Data availability

Data will be available on request to corresponding authors.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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