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Physiological effects of MAP and calcium chloride treatments on biochemical metabolites and quality stability by reducing respiration rate in sweet cherry fruit during storage

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Abstract

Background Sweet cherry (*Prunus avium* L.) is an important fruit species with its high nutritional value and fresh consumption rate. However, it is very sensitive to mechanical damage, decay, water loss and microbial spoilage in the post-harvest period. Therefore, new preservation methods are needed to extend shelf life and preserve quality. In this study, the effects of postharvest 4% calcium chloride (CaCl_2) and modified atmosphere packaging (MAP) treatments on quality traits (weight loss, decay rate, SSC, titratable acidity, pH, respiration rate) and biochemical compounds (organic acids, vitamin C and phenolic compounds) of sweet cherry fruit were investigated.

Results During the storage period, the weight loss reached 7.14%, the lowest loss was observed in the CaCl_2 +MAP group. The highest decay rate was measured in the control group with 11.30%, and the CaCl_2 +MAP combination was the method that limited decay the most. SSC increased over time, but the lowest levels remained in the CaCl_2 +MAP group. The titratable acidity value decreased from 1.12% to 0.82%, CaCl_2 +MAP application was the method that best preserved acidity. The pH value increased by 25.2%, the lowest pH was again determined in the CaCl_2 +MAP group. The respiration rate decreased by 77%, the lowest respiration rate was determined in the CaCl_2 +MAP application. Organic acid and vitamin C contents decreased over time, and the application that best limited these losses was CaCl_2 +MAP. Significant decreases were also observed in phenolic compounds, and the combined application preserved phenolic stability at the highest level.

Conclusion CaCl_2 and MAP applications were effective in preserving the quality characteristics and bioactive compounds of sweet cherry fruit, especially the combined application (CaCl_2 +MAP) gave the most successful results. Therefore, in order to prevent post-harvest quality losses and extend shelf life, it is recommended that CaCl_2 and MAP applications be evaluated together by the producer and the supply chain.

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Keywords *Prunus avium*, Postharvest, CaCl_2 , Modified atmosphere packaging, Weight loss

Background

Sweet cherry attracts attention with its high soluble sugar content (13–25%), low acidity, rich vitamin, mineral and antioxidant profile [1]. Its brightly colored peel, aromatic flavor and high nutritional value make sweet cherry economically valuable for both consumers and producers [2]. However, due to its thin skin structure and soft fruit flesh, cherries are highly susceptible to quality losses such as mechanical damage, microbial spoilage, water loss, and stem browning in the postharvest period [3]. Especially high respiration rate and fungal infections significantly limit the shelf life of the fruit under room conditions, and pathogens such as *Botrytis cinerea* and *Penicillium expansum* pose serious threats to both quality and human health [4, 5]. Although cold storage, hydrocooling and various chemical/physical applications have been traditionally developed to prevent these postharvest losses, these methods often show limited success and, in some cases, carry the risk of leaving residues on the product or creating physiological stress [4]. Therefore, the need for alternative maintaining techniques to extend the shelf life of sweet cherry and prevent quality losses is increasing. Two important approaches that have emerged in recent years are CaCl_2 applications and MAP methods. CaCl_2 reduces quality losses by supporting fruit tissue physiologically and biochemically, and MAP slows down the spoilage process by regulating the atmospheric conditions around the fruit [6]. Calcium plays a critical role in ensuring the stability of cell walls that maintain the structural integrity of fruit tissue. CaCl_2 applications increase cell wall hardness and delay fruit softening, especially by interacting with pectin chains [7]. In addition, Ca^{2+} ions have been shown to protect fruit tissue against oxidative stress by stimulating antioxidant enzyme activities such as SOD, CAT and APX [8]. This effect ensures not only physical quality but also the preservation of bioactive substances such as phenolic compounds, vitamin C and organic acids [9]. It is thought that CaCl_2 applications, which give successful results in fruits such as strawberry, lychee, mango and blueberry, may also have similar effects on sweet cherry fruit [10]. On the other hand, MAP applications slow down fruit respiration and delay ripening processes by adjusting the gas compositions (O_2 , CO_2 and N_2) in the package at certain rates [11]. This contributes to preventing both physical losses (weight loss, discoloration, decay) and microbial spoilage [12]. MAP applied to fruits such as apricot, pomegranate and strawberry have come to the forefront as an effective method in terms of protecting fruit tissue, limiting enzyme activity and ensuring the stability of antioxidant compounds [13]. In particular, the

suppression of phenolic oxidation under low O_2 and high CO_2 conditions is an important factor in preventing color and aroma losses [14]. However, since quality losses due to anaerobic conditions may occur in long-term MAP applications, it is recommended to prefer advanced systems such as microporous packaging [15]. The studies evaluating the effects of CaCl_2 and MAP applications, either together or separately, to prevent postharvest quality losses in sweet cherry fruit are limited. In particular, comprehensive studies examining the effects of these two methods on quality parameters such as weight loss, decay rate, soluble solids content (SSC), acidity, pH and respiration rate, as well as vitamin C, organic acids and phenolic compound levels are largely lacking. This situation reveals the need for more scientific data to solve the problems that cause economic and quality losses for both the producer and the consumer. The aim of this study is to determine the effects of CaCl_2 and MAP methods applied after harvest on sweet cherry fruit on quality characteristics and biochemical compounds.

Materials and methods

In this study, the effects of postharvest CaCl_2 and MAP applications on quality traits and biochemical compounds in “0900 Ziraat” sweet cherry (*Prunus avium*) cultivar grafted onto MaxMa 14 rootstock were investigated. The fruit used in the study were harvested from trees planted at 4×4 m intervals and treated with the Step Leader training system in a grower’s orchards in Bolu, Türkiye on June 8, 2024, during the harvest maturity period. Fruits were harvested when they were bright red and had a SSC value of 13%. The harvested fruits were transported to the laboratory of the Department of Horticulture, Faculty of Agriculture, Bolu University. Firstly, the fruits were pre-cooled for 24 h in a cold storage at 5 °C and 85 ± 5% relative humidity. After the pre-cooling process, the fruits were selected in equal sizes; diseased, pest-infested and mechanically damaged fruits were sorted. The selected fruits were divided into 4 groups. The first group was the control group where no application was made, the second group fruits were treated with only MAP and the other two groups were treated with 4% calcium chloride. The fruits were immersed in 4% CaCl_2 solution for 15 min. After CaCl_2 application, fruits were dried at room temperature, MAP was applied to one group of calcium chloride-applied fruits and placed in perforated plastic boxes and stored in a cold room at 0 ± 0.5 °C and 90 ± 5% relative humidity. The modified atmosphere packaging is polyethylene (PE) and has an O_2 permeability of 150–300 cc m² day, CO_2 permeability of 300–800 cc m² day, and a water vapor transmission rate

(WVTR) of 0.5–1.5 g m² day. Separate storage was carried out for each analysis day, and during this process, the MAPs were not opened until they were removed from the cold storage. The following measurements and analyses were performed on the harvested and stored fruits at 8-day intervals for 24 days. The study was designed with three replications. In each measurement period, 20 fruits were used for each replication, 60 fruits for one application were used. In one measurement period, 60 × 4 = 240 fruits were used for 4 applications. Three packages were used for one application, one package for each repetition. 4 (applications) × 3 = 12 packages were used at one measurement time and 12 × 3 = 36 packages were used at the end of 3 times (8, 16 and 24) in total.

Weight loss and decay rate

The weight of the fruits was measured using a high-precision digital balance (accuracy ± 0.01 g; Radvag PS 4500/C/1, Poland). The mass reduction during the cold storage period was calculated by considering the difference in weight before and after storage in each replicate group (20 fruits) and expressed as a percentage. Fruit rot was determined by visual inspection during both storage and shelf life. Sweet cherry fruits with mycelium development on their surface were evaluated as rotten with the naked eye and the rotten fruit rate was presented as a percentage according to the total number of samples. These evaluations were made three times in each repetition.

Soluble solids content, titratable acidity, pH and vitamin C

For SSC, pH, titratable acidity and ascorbic acid analyses, a total of 30 fruits were selected from each replicate group and divided into three subgroups of 10. After removing the seeds of each fruit, juice was obtained using an electric juicer (HR1855/70, Philips, Türkiye). SSC measurement was performed with a digital refractometer (PAL-1, McCormick Fruit Tech., Yakima, USA) and expressed as a percentage. pH value was measured with a digital pH meter (Metler Toledo). For acidity determination, 10 mL of fruit juice sample was diluted with an equal volume of pure water and titrated with 0.1 mol/L NaOH solution until pH 8.2 was reached and the results were calculated as %. For the determination of vitamin C, a sufficient amount of fruit juice sample was taken, and the total volume was completed to 5 mL with 0.5% oxalic acid. The ascorbic acid test strip from Merck (Catalog No: 116981, Germany) was immersed in the solution for 2 s, waited for 8 s, and then measured with the Merck RQflex plus 10 reflectometer. The results are expressed as mg 100 g⁻¹.

Respiration rate

To determine respiration percentage, 10 fruits removed from the cold were selected and placed in airtight 1.5-liter containers with lids. A sensor was inserted through the hole in the top of the container, the container was tightly covered with plastic wrap, and the CO₂ amount was measured after 2 h at room temperature. The Headspace Gas Analyzer GS3/L (Gasboard-3210Plus) device was used for measurements. Respiration rate was calculated by substituting the CO₂ and fruit weight values into the formula below and is expressed in mg CO₂ kg⁻¹ h⁻¹ units.

$$\text{Respiration Rate} = (V_j - V_f) \times \%CO_2 \times 10 / FW \times T$$

V_j: Jar volume, V_f: Fruit volume, FW: Fruit weight (kg), T: Time (hour).

Organic acids

15 g of fruit sample was mixed with 0.009 N H₂SO₄ at a ratio of 1:1 to make it homogeneous and kept on a shaker for one hour. The resulting mixture was centrifuged at 15,000 rpm for 15 min and the upper solution was collected. This solution was made suitable for HPLC analysis by passing through a 0.45 µm membrane filter and a SEP-PAK C18 cartridge, respectively. Organic acid analysis was performed using an Agilent HPLC 1100 series G 1322 A (Germany) instrument with an Aminex HPX-87 H column (300 mm × 7.8 mm, Bio-Rad Laboratories, USA) according to the method recommended by Bevilacqua and Califano [16]. The results are expressed as g kg⁻¹, but for fumaric acid as mg kg⁻¹.

Individual phenolic compounds

The analysis of phenolic compounds at individual level was carried out according to the method developed by Rodriguez-Delgado et al. [17]. 5 g of sweet cherry sample was mixed with an equal amount of distilled water and centrifuged at 15,000 rpm for 15 min. The obtained supernatant was first filtered through a coarse filter, then through a 0.45 µm membrane filter and injected into the HPLC device (Agilent HPLC 1100 series G 1322 A, Germany). The separation process was carried out with an ODS column (HiChrom, USA) with a DAD detector and a size of 250 × 4.6 mm, containing 4 µm particles. The results were expressed as mg 100 g⁻¹.

Statistical analysis

The data obtained from the study were evaluated statistically using variance analysis (ANOVA). The differences between applications were considered significant at *p* < 0.05 and compared using the Tukey test. All analyses were performed using SAS Version 9.1 (SAS Institute

Inc., Cary, NC, USA) statistical software. The Pearson correlation analysis and principal component analysis (PCA) were performed using Jmp Pro 17 software.

Results and discussion

Weight loss

Postharvest weight loss in sweet cherry fruits is a critical parameter that directly affects quality and marketability. The findings obtained in this study showed that weight loss increased significantly during the storage period. The loss was 2.31% on the 8th day of the cold storage, whereas this rate increased to 5.17% on the 16th day and 7.14% on the 24th day (Table S1 and Fig. 1). When evaluated according to the initial weight, a total loss of 7.14% occurred at the end of the cold storage period. This increase was found to be statistically significant ($p \leq 0.001$) and is attributed to the decrease in mass due to water loss and respiration in the fruit. The findings show that as the storage period progresses, the moisture in the fruit tissues decreases and the mass loss increases accordingly. This situation is consistent with the studies of Karagiannis et al. [18], in which it was stated that sweet cherry fruit is very sensitive to water loss due to its high water content and thin peel structure. The highest weight loss was observed in the control group with 3.84% on the 8th day of the cold storage. This was followed by CaCl_2 (4%) and MAP applications, respectively, while the lowest weight loss was obtained in the group applied with the CaCl_2 +MAP combination with only 0.86%. A similar trend continued on the 16th day, with the control group showing the highest weight loss of 6.96%, while CaCl_2 +MAP application showed the lowest weight loss of 3.52%. On day 24, weight loss reached its peak in the control group with 9.23% while this rate was reduced to 4.73% with CaCl_2 +MAP (Table S1). These data reveal that CaCl_2 +MAP application effectively limits water loss in fruit tissues and thus contributes significantly to maintaining fruit quality. MAP application slows down respiration by decreasing O_2 and increasing CO_2 in the packaging atmosphere, which reduces transpiration and water loss [19]. This mechanism has shown similar effects in other fruit species such as strawberry and pomegranate; MAP applications helped preserve freshness and market value by reducing weight loss in these fruits [13]. In terms of weight loss, this study found that only MAP was more effective than only CaCl_2 . By day 24, only MAP resulted in 25% less weight loss compared to the control group. This rate increased to 50% with CaCl_2 +MAP application combined with the calcium effect. CaCl_2 application prevents the passage of water to the external environment and limits weight loss by creating a semi-permeable barrier on the fruit surface [10]. In addition, the stabilization of the cell membrane and cell wall structure by Ca^{2+} ions support the preservation of intracellular

water and reduces losses due to evaporation [20]. These mechanisms are particularly effective for thin-skinned and high-water fruits such as cherries. When MAP and CaCl_2 applications are used together, metabolic activities are slowed down by regulating gas composition and water loss is reduced by protecting the cellular structure. This synergistic effect, as observed in our study, stands out as the most effective strategy in controlling weight loss [21].

Decay rate

It was observed that the decay rate in sweet cherry fruits increased statistically significantly as the storage period progressed ($p \leq 0.001$). The decay rate, which was 2.16% on the 8th day of cold storage, increased to 6.01% on the 16th day and 8.54% on the 24th day, reaching a total increase of approximately 8.5%. This increase clearly shows that microbial spoilage and pathogen development on the fruit intensifies over time. The highest decay rate was seen in the control group with 3.17% on the 8th day while the lowest value was measured in the CaCl_2 +MAP combination with 0.84%. This trend became more evident on the 16th day; the decay rate reached very high levels of 8.36% in the control group, and this rate decreased to 2.82% with the CaCl_2 +MAP application. On the 24th day, which is the last day of storage, the decay rate peaked at 11.30% in the control group, the only MAP application showed 5.87% lowest decay. The combination application had a lesser effect on the decay rate, reaching 7.88% at the end of day 24. (Table S1 and Fig. 1). These findings may be related to the fact that CaCl_2 application makes it difficult for microorganisms that cause rot to penetrate the cell wall. Calcium promotes pectate formation, strengthens intercellular connections and thus increases the resistance of the fruit to microbial infections [22]. In addition, Ca^{2+} ions suppress cell wall enzymes, making it difficult for pathogens to enter the fruit tissue [23]. The spread of pathogens such as *Botrytis cinerea* and *Penicillium expansum*, which are the most common decay agents in cherry fruit, can be limited by these mechanisms [5]. MAP significantly limits microbial growth with low O_2 and high CO_2 levels inside the packaging. In this way, decay rates are reduced [11]. This effect of MAP has also been supported by other fruits; it has been reported that it contributes to the reduction of decay rates in products such as pomegranate [24] and strawberry [25]. However, in long-term storage, CO_2 accumulation may have the opposite effect and cause an increase in decay rates [13]. Therefore, storage duration and gas rates should be carefully optimized in MAP applications. The combined application of CaCl_2 and MAP offers a very effective integrated strategy in terms of both preventing pathogen entry by strengthening the cellular structure and minimizing the decay rate by making the environmental gas

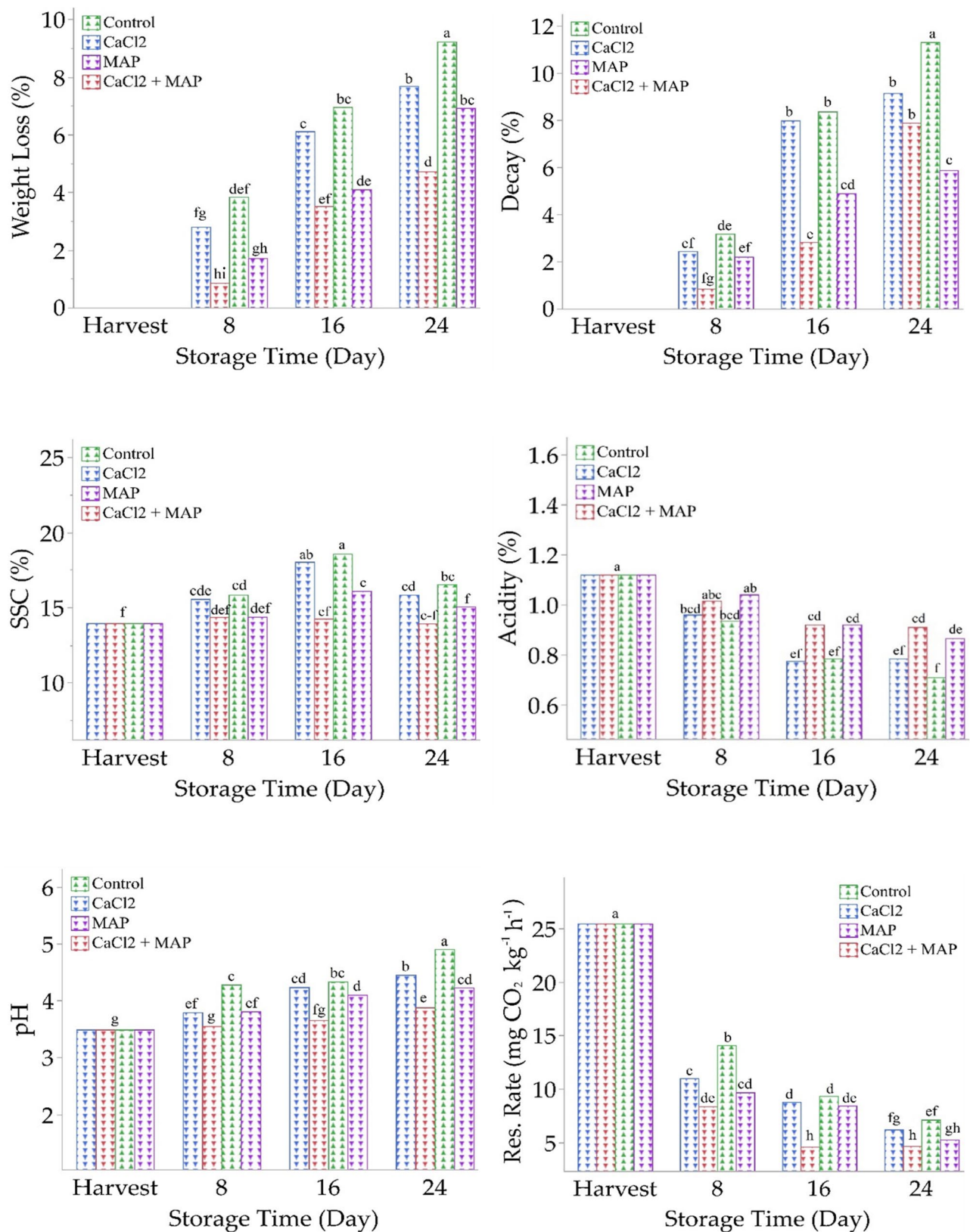


Fig. 1 Impact of postharvest calcium chloride and MAP applications on changes in fruit quality properties. Different letters on top of the storage periods indicate significant differences at $p \leq 0.05$

composition unfavorable for microbial growth [21]. The results of our study, in line with this literature, show that CaCl_2 +MAP application best controls the decay rate in sweet cherry fruit.

Soluble solids content

SSC in sweet cherry fruit is a critical quality parameter in terms of maturity and taste quality since it reflects the sum of sugar and other soluble components. In our study, the SSC value was determined as 13.95% on average at harvest. This value increased during the storage period; it reached 15.03% on the 8th day, the maximum value of 16.73% on the 16th day, and decreased slightly to 15.34% on the 24th day of the cold storage. The highest SSC value on the 16th day represents an increase of approximately 20% compared to the beginning of harvest ($p < 0.05$). This shows that sugar accumulation continues in fruit due to metabolic processes such as respiration activities and starch conversion to sugar that continue throughout storage. When evaluated on an application basis, the highest SSC value was measured in the control group with 15.85% on the 8th day while the lowest value was recorded in the CaCl_2 +MAP combination with 14.35%. On the 16th day; the control group had the highest SSC rate with 18.57% while the CaCl_2 +MAP application had the lowest SSC rate with 14.25%. On the 24th day, the control group still had a high value with 16.54% while the CaCl_2 +MAP group showed the lowest SSC level with 13.92% (Table S1). These findings suggest that CaCl_2 and MAP application delays quality loss by slowing down the ripening process and sugar accumulation of the fruit. Compared with the literature, SSC is a key parameter determining the taste and nutritional value of the fruit, and sugar consumption together with postharvest respiration activity may cause a decrease in SSC [26]. CaCl_2 application reduces the respiration rate by creating a protective barrier on the fruit surface and cell wall stabilization, thus preventing a rapid decrease in SSC. In addition, protection of the cell wall stabilizes sugar transport and metabolism. It is reported that MAP applications also slow down sugar metabolism by reducing the respiration rate, thus limiting SSC loss [27]. For example, the studies on strawberry fruit have reported that SSC values are preserved for a longer time by applying MAP [21]. Similar effects are expected to be observed in sweet cherry, and thus the taste quality of the fruit remains more stable throughout the storage period. Again, in this study, positive effects of only MAP use were observed and SSC was measured as 15.05% on the 24th day (Table S1). However, both MAP and CaCl_2 combined applications were more effective in this study. In previous studies, the combined use of CaCl_2 and MAP applications during the storage process both delays ripening and slows down sugar

accumulation, and preserves the sensory quality of the fruit by reducing the loss of SSC caused by respiration [28].

Titrateable acidity

Titrateable acidity in sweet cherry fruit is an important quality parameter in terms of fruit taste, aroma and microbial resistance. Titrateable acidity value was measured as 1.12% on average at the beginning of storage. A significant decrease was observed in TA values throughout the storage process; 0.99% on the 8th day, 0.85% on the 16th day and 0.82% on the 24th day of the cold storage, resulting in a total decrease of approximately 26.8% ($p < 0.001$). This decrease can be explained by the use of organic acids as an energy source in respiration and is a result of the natural metabolic activities of the fruit. When examined in terms of applications, the highest acidity value was determined in the MAP group with 1.04% on the 8th day, and the lowest was determined in the control group with 0.94%. On the 16th day, MAP (0.92%) and CaCl_2 +MAP (0.92%) applications provided the highest values by preserving titrateable acidity better while titrateable decreased to 0.79% in the control group. On the 24th day of the cold storage, CaCl_2 +MAP application provided the highest titrateable acidity value with 0.91% while the control group gave the lowest value with 0.71%. These results show that the combination of MAP and especially CaCl_2 +MAP slows down the acid loss of the fruit and thus preserves quality better compared to the control group (Table S1). Titrateable acidity is the main chemical parameter determining the sourness character of the fruit and tends to decrease over time with the depletion of organic acids during the respiration process [1]. Application of CaCl_2 limits the oxidation of organic acids through cell wall stabilization, thus reducing acid loss [6]. Similarly, MAP application slows down metabolic activities, delays acidity deterioration and contributes to the preservation of major organic acids such as malic acid [29]. The preservation of titrateable acidity during storage is critical for the stability of the fruit flavor profile and is also supported by the energy metabolism-slowing effect of CaCl_2 [30]. However, previous studies have reported that the effect of MAP application on titrateable acidity in different fruit species may vary depending on the variety and storage period [13].

pH

The significant increase in pH value was observed in sweet cherry fruit during storage. The average pH, which was 3.49 at harvest, increased to 3.86 on the 8th day of the cold storage, 4.08 on the 16th day and 4.37 on the 24th day, with a total increase of approximately 25.2% ($p < 0.01$). This increase is a natural result of the decrease in titrateable acidity in the fruit and shows that the acidic

structure is weakened. The highest pH value was measured in the control group with 4.28 on the 8th day while the lowest pH was determined in the CaCl_2 +MAP application with 3.55. On the 16th day, the control group had the highest pH value (4.34) while the CaCl_2 +MAP application had the lowest value (3.66). On the 24th day of the cold storage, the pH in the control group reached the highest level with 4.91, and the CaCl_2 +MAP application had the lowest pH value with 3.88, and therefore the group that best preserved its acidic structure (Table S1). These findings show that the combined application of CaCl_2 and MAP provides advantages in terms of taste and microbial stability by preserving the acidic structure in the fruit. While only MAP application was more effective than other applications in the first days of preservation, this effect decreased over time. However, only MAP applications were found to be more effective than only CaCl_2 application. In the literature, the pH value is inversely proportional to the acidity level of the fruit and is considered an indicator of spoilage and ripening processes [31]. It is reported that calcium applications slow down acid loss through cell wall stabilization, thus preventing excessive pH increases. Thanks to this mechanism, titratable acidity is preserved and fruit quality remains stable for a longer period. In addition, the consumption of organic acids decreases and the pH increase is limited by the decrease in the respiration rate in the MAP environment [25]. This contributes significantly to the preservation of both acidity and microbial stability in cherries. Since fruit pH is an indicator of ripeness, CaCl_2 applications delay ripening and slow down the pH increase, while MAP slows down the metabolism by reducing oxygen levels [32]. Thus, controlling the pH change increases the resistance of the fruit to microbial infections and extends its shelf life [33].

Respiration rate

The respiration rate in sweet cherry fruit provides critical information about the metabolic activity and ripening rate of the fruit. The respiration rate, which was an average of $25.43 \text{ mg CO}_2 \text{ kg}^{-1} \text{ hour}^{-1}$ at harvest, decreased significantly during the storage period, falling to 10.80 on 8th day, 7.81 on 16th day, and $5.84 \text{ mg CO}_2 \text{ kg}^{-1} \text{ hour}^{-1}$ on 24th day of the cold storage. This means a decrease of approximately 77% and clearly shows that fruit metabolism slows down during storage ($p < 0.001$). When we look at the application groups, the highest respiration rate was observed in the control group with $14.10 \text{ mg CO}_2 \text{ kg}^{-1} \text{ hour}^{-1}$ on the 8th day of cold storage while the lowest value was measured in the CaCl_2 +MAP combination with $8.40 \text{ mg CO}_2 \text{ kg}^{-1} \text{ hour}^{-1}$. On the 16th day, the control group maintained the highest respiration rate with $9.35 \text{ mg CO}_2 \text{ kg}^{-1} \text{ hour}^{-1}$ whereas the CaCl_2 +MAP application provided the lowest respiration rate with

$4.62 \text{ mg CO}_2 \text{ kg}^{-1} \text{ hour}^{-1}$. On the 24th day, the control group showed the highest respiration rate with $7.14 \text{ mg CO}_2 \text{ kg}^{-1} \text{ hour}^{-1}$, and this value decreased to $4.71 \text{ mg CO}_2 \text{ kg}^{-1} \text{ hour}^{-1}$ with CaCl_2 +MAP (Table S1 and Fig. 1). These results indicate that CaCl_2 +MAP application effectively suppresses fruit respiration, delaying ripening and thus preserving quality for a long time. The effect here is thought to be due to the high dose of MAP application. Because on day 24th, it was observed that only MAP application reduced the respiratory rate more than only CaCl_2 application (Table S1). Sweet cherry is a species of fruit that undergoes rapid quality loss after harvest due to high respiration rates [4]. Although CaCl_2 application does not have a direct effect on reducing the respiration rate, it contributes to indirect slowing down of metabolic rate through stabilization of cell wall and membrane structures. MAP suppresses respiration and slows down the aging process by decreasing oxygen level and increasing carbon dioxide level in the environment [34]. The combined use of these two applications reduces both energy consumption and weight loss in the fruit, thus preserving metabolic balance [35]. MAP applications, in particular, reduce the respiration rate and delay the ripening process by reducing O_2 concentration in sweet cherry. Similar effects have been reported in other fruits such as peach and pomegranate, resulting in the prevention of quality loss [36]. In addition, Ca^{2+} ions have been shown to limit oxygen transport by stabilizing plasma membranes and suppress ethylene production by regulating mitochondrial activities [37]. Thus, CaCl_2 indirectly slows down the biochemical aging of the fruit.

Organic acids

During storage, the significant decreases were observed in the contents of important biochemical compounds such as succinic, malic, citric, oxalic, fumaric acids and vitamin C in sweet cherry ($p \leq 0.001$). These decreases are due to the use of organic acids as energy sources in the respiration metabolism of the fruit and oxidative stress. Organic acids play a critical role in determining fruit flavor and maintaining pH balance, and their decrease negatively affects sensory quality. When comparing treatments, the combination of CaCl_2 and MAP was the most effective method for delaying organic acid and vitamin C losses (Table 1). This combined application slows down the metabolism of organic acids by reducing the respiration rate [38], reduces leaks by stabilizing cell membranes [9] and limits oxidative degradation. MAP contributes to the preservation of these compounds by suppressing respiration with a low oxygen environment [39]. We see this effect in the high preservation of all organic acid levels by only MAP applications. Similarly, only MAP applications appear to be more effective than only CaCl_2 applications (Table 1). Ascorbic acid (vitamin C), although

Table 1 Changes in organic acids (g kg⁻¹; for fumaric acid: Mg kg⁻¹) and vitamin C (mg 100 g⁻¹) during storage due to calcium chloride and MAP applications

Storage time		Succinic	Citric	Malic	Oxalic	Fumaric	Vitamin C
Harvest		11.08 ± 0.06a	2.52 ± 0.06a	3.35 ± 0.02a	1.33 ± 0.04a	3.47 ± 0.10a	11.05 ± 0.06a
Day 8		9.86 ± 0.08b	2.12 ± 0.07b	3.07 ± 0.04b	1.17 ± 0.04a	2.43 ± 0.16b	9.53 ± 0.17b
Day 16		9.09 ± 0.16c	1.71 ± 0.06c	2.93 ± 0.04c	0.96 ± 0.06b	1.75 ± 0.12c	8.39 ± 0.15c
Day 24		7.92 ± 0.13d	1.44 ± 0.08d	2.57 ± 0.06d	0.85 ± 0.06b	1.52 ± 0.12c	7.52 ± 0.18d
CaCl ₂ + MAP and Storage time interaction							
Harvest		11.08 ± 0.06a	2.52 ± 0.06a	3.35 ± 0.02a	1.33 ± 0.04a	3.47 ± 0.10a	11.05 ± 0.06a
Day 8	Control	9.58 ± 0.06d	1.89 ± 0.04d	2.94 ± 0.04c	1.04 ± 0.02ef	1.81 ± 0.05de	8.91 ± 0.09d
	CaCl ₂ (%4)	9.81 ± 0.04c	2.04 ± 0.03c	3.01 ± 0.03c	1.15 ± 0.02 cd	2.34 ± 0.08c	9.38 ± 0.10c
	MAP	9.86 ± 0.03c	2.24 ± 0.01b	3.13 ± 0.02b	1.21 ± 0.01bc	2.70 ± 0.09b	9.80 ± 0.03b
	CaCl ₂ + MAP	10.19 ± 0.06b	2.34 ± 0.04b	3.20 ± 0.03b	1.29 ± 0.03ab	2.88 ± 0.10b	10.05 ± 0.07b
Day 16	Control	8.46 ± 0.06 g	1.53 ± 0.04 g	2.76 ± 0.02d	0.69 ± 0.03 h	1.36 ± 0.05 g	7.80 ± 0.06 g
	CaCl ₂ (%4)	9.06 ± 0.08f	1.60 ± 0.02 fg	2.93 ± 0.01c	0.97 ± 0.02f	1.59 ± 0.06f	8.39 ± 0.04ef
	MAP	9.29 ± 0.04e	1.77 ± 0.02e	3.01 ± 0.01c	1.07 ± 0.03de	1.86 ± 0.03d	8.52 ± 0.06e
	CaCl ₂ + MAP	9.57 ± 0.06d	1.93 ± 0.03d	3.01 ± 0.03c	1.10 ± 0.03de	2.20 ± 0.04c	8.87 ± 0.13d
Day 24	Control	7.43 ± 0.10j	1.14 ± 0.04i	2.43 ± 0.07f	0.65 ± 0.04 h	1.09 ± 0.05 h	6.96 ± 0.17i
	CaCl ₂ (%4)	7.84 ± 0.03i	1.38 ± 0.01 h	2.46 ± 0.03f	0.81 ± 0.01 g	1.44 ± 0.04 fg	7.24 ± 0.06 h
	MAP	8.05 ± 0.02 h	1.58 ± 0.03 fg	2.59 ± 0.03e	0.86 ± 0.02 g	1.62 ± 0.05ef	7.72 ± 0.04 g
	CaCl ₂ + MAP	8.38 ± 0.07 g	1.66 ± 0.03f	2.80 ± 0.02d	1.09 ± 0.02de	1.95 ± 0.04d	8.15 ± 0.11f
ANOVA							
F (Storage time)		62***	27.84***	30.76***	9.44***	19.84***	45.99***
F (Storage time × CaCl ₂ + MAP)		311.27***	126.35***	83.51***	66.74***	105.67***	174.89***

Ns Not significant

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

significantly decreased during storage, was preserved at higher levels compared to other groups thanks to the CaCl₂+MAP application (Table 1). It is supported in the literature that Ca²⁺ ions prevent the oxidation of ascorbic acid by promoting antioxidant enzyme activation, and that MAP reduces oxidative stress with low O₂ and high CO₂ environment [40].

Individual phenolic compounds

As the storage period of sweet cherry fruit progressed, the significant decreases were observed in phenolic compounds such as gallic acid, quercetin, catechin, rutin, ferulic acid, chlorogenic acid, protocatechuic acid, caffeic acid and coumaric acid ($p \leq 0.001$). These decreases are due to the degradation of phenolics due to oxidative enzymes (PPO, POD) activity and oxidative stress under storage conditions. In particular, more than 50% reduction was achieved in some compounds such as o-coumaric acid. Among the applications, the combination of CaCl₂+MAP, where CaCl₂ and MAP were used together, stood out as the most effective method in preserving phenolic compounds. In this application, phenolic contents remained at higher levels compared to the control group and the degradation process slowed down significantly. Although CaCl₂ or only MAP applications also showed a protective effect, the stability of phenolics was increased thanks to the synergistic effect of the combined application (Tables 2 and 3). The preservation of

phenolic compounds is critical for maintaining the antioxidant capacity, color and functional quality of the fruit [41]. CaCl₂ prevents oxidative degradation of phenolics by stabilizing cell membranes [42], and MAP limits the activity of PPO and other oxidative enzymes by providing a low oxygen environment [33]. This synergistic protection is an effective strategy to extend the health benefits and shelf life of cherry fruit. In addition, it is reported that CaCl₂+MAP application limits the phenol oxidase-substrate interaction by forming a protective film on the fruit surface, thus increasing the stability of phenolic compounds [9]. In this way, combined application provides a significant advantage in preserving fruit quality by minimizing phenolic losses. This positive effect of combination applications appears to be particularly significant for only MAP applications. This is because it has been shown to have a more protective effect on all phenolic compounds compared to calcium applications (Tables 2 and 3).

Correlation and principal component analyses

The relationships between fruit quality criteria and biochemical parameters were investigated in detail with correlation analyses (Fig. 2). The results showed that quality deterioration indicators such as weight loss, decay rate and respiration rate were negatively correlated with organic acids such as vitamin C, malic acid, citric acid and major phenolic compounds (e.g. gallic

Table 2 Changes in phenolic compounds (mg 100 g⁻¹) during storage due to calcium chloride and MAP applications

Storage time		Gallic	Quercetin	Catechin	Rutin	Ferulic
Harvest		121.60 ± 1.40a	13.29 ± 0.06a	5.07 ± 0.10a	10.47 ± 0.21ab	4.94 ± 0.05a
Day 8		107.12 ± 2.25b	11.80 ± 0.15b	4.48 ± 0.05b	11.32 ± 0.43a	4.60 ± 0.06b
Day 16		95.59 ± 1.84c	9.96 ± 0.22c	4.17 ± 0.07c	10.67 ± 0.27a	4.45 ± 0.06b
Day 24		82.59 ± 1.20d	9.02 ± 0.14d	4.02 ± 0.11c	9.80 ± 0.10b	3.78 ± 0.09c
CaCl ₂ + MAP and Storage time interaction						
Harvest		121.60 ± 1.40a	13.29 ± 0.06a	5.07 ± 0.10a	10.47 ± 0.21e	4.94 ± 0.05a
Day 8	Control	97.55 ± 0.75e	11.20 ± 0.08d	4.36 ± 0.03cde	13.20 ± 0.10a	4.35 ± 0.05d
	CaCl ₂ (%4)	107.66 ± 1.05c	11.78 ± 0.11c	4.41 ± 0.02 cd	11.30 ± 0.09c	4.55 ± 0.04c
	MAP	109.96 ± 1.00c	11.95 ± 0.04c	4.46 ± 0.04c	10.48 ± 0.05e	4.73 ± 0.06b
	CaCl ₂ + MAP	113.31 ± 0.87b	12.28 ± 0.04b	4.70 ± 0.07b	10.31 ± 0.05ef	4.76 ± 0.03b
Day 16	Control	89.17 ± 0.56 g	9.18 ± 0.08 g	3.95 ± 0.06f	11.70 ± 0.07b	4.27 ± 0.03d
	CaCl ₂ (%4)	93.51 ± 0.81f	9.68 ± 0.07f	4.05 ± 0.04f	10.92 ± 0.04d	4.34 ± 0.04d
	MAP	97.55 ± 1.25e	10.44 ± 0.09e	4.29 ± 0.04de	9.89 ± 0.08gh	4.52 ± 0.04c
	CaCl ₂ + MAP	102.14 ± 0.38d	10.55 ± 0.06e	4.40 ± 0.01 cd	10.18 ± 0.10efg	4.69 ± 0.03b
	Control	77.88 ± 0.94j	8.55 ± 0.03i	3.60 ± 0.06 g	9.43 ± 0.15i	3.51 ± 0.04 h
	CaCl ₂ (%4)	81.91 ± 0.75i	8.84 ± 0.05 h	3.92 ± 0.02f	9.79 ± 0.12 h	3.65 ± 0.04 g
Day 24	MAP	85.14 ± 0.74 h	9.10 ± 0.07 g	4.23 ± 0.02e	9.96 ± 0.06gh	3.85 ± 0.03f
	CaCl ₂ + MAP	85.45 ± 1.05 h	9.58 ± 0.05f	4.32 ± 0.05cde	10.04 ± 0.08fgh	4.11 ± 0.02e
ANOVA						
	F (Storage time)	48.71***	71.14***	14.83***	4.50*	32.66***
	F (Storage time × CaCl ₂ + MAP)	206.51***	500.36***	54.76***	97.23***	118.68***

Ns Not significant

Different letters in the same column indicates statistical differences at $p \leq 0.05$. *** indicates $p \leq 0.001$ **Table 3** Continuation of Table 3 (mg 100 g⁻¹)

Storage time		Chlorogenic	Protocatechuic	Caffeic	p-Coumaric	o-Coumaric
Harvest		1.98 ± 0.02a	1.75 ± 0.04a	8.87 ± 0.19a	5.94 ± 0.11a	8.58 ± 0.15a
Day 8		1.86 ± 0.04b	1.40 ± 0.03b	7.17 ± 0.13b	5.73 ± 0.07a	5.73 ± 0.38b
Day 16		1.55 ± 0.02c	1.11 ± 0.06c	5.68 ± 0.32c	5.06 ± 0.08b	4.45 ± 0.22c
Day 24		1.42 ± 0.03d	0.87 ± 0.05d	4.43 ± 0.25d	4.44 ± 0.11c	4.08 ± 0.17c
CaCl ₂ + MAP and Storage time interaction						
Harvest		1.98 ± 0.02a	1.75 ± 0.04a	8.87 ± 0.19a	5.94 ± 0.11a	8.58 ± 0.15a
Day 8	Control	1.80 ± 0.01c	1.33 ± 0.03 cd	6.75 ± 0.16 cd	5.48 ± 0.05c	4.60 ± 0.11efg
	CaCl ₂ (%4)	1.83 ± 0.03c	1.39 ± 0.03c	7.12 ± 0.13c	5.68 ± 0.05b	4.98 ± 0.10de
	MAP	1.89 ± 0.01b	1.39 ± 0.02c	7.12 ± 0.08c	5.82 ± 0.03ab	6.25 ± 0.09c
	CaCl ₂ + MAP	1.92 ± 0.01b	1.52 ± 0.03b	7.69 ± 0.11b	5.96 ± 0.03a	7.09 ± 0.13b
Day 16	Control	1.48 ± 0.02f	0.84 ± 0.02 h	4.28 ± 0.13hi	4.76 ± 0.02f	3.86 ± 0.10ij
	CaCl ₂ (%4)	1.53 ± 0.01ef	1.14 ± 0.03f	5.89 ± 0.19f	4.99 ± 0.06e	4.16 ± 0.12hi
	MAP	1.56 ± 0.01e	1.21 ± 0.03ef	6.14 ± 0.15ef	5.21 ± 0.04d	4.44 ± 0.16fgh
	CaCl ₂ + MAP	1.63 ± 0.03d	1.26 ± 0.03de	6.41 ± 0.15de	5.27 ± 0.06d	5.34 ± 0.17d
	Control	1.31 ± 0.01 h	0.71 ± 0.03i	3.62 ± 0.16j	4.06 ± 0.07i	3.65 ± 0.12j
	CaCl ₂ (%4)	1.37 ± 0.01 g	0.80 ± 0.02hi	4.06 ± 0.09ij	4.33 ± 0.07 h	3.71 ± 0.23j
Day 24	MAP	1.49 ± 0.01f	0.94 ± 0.02 g	4.76 ± 0.09 h	4.52 ± 0.03 g	4.24 ± 0.07ghi
	CaCl ₂ + MAP	1.52 ± 0.02ef	1.03 ± 0.07 g	5.29 ± 0.34 g	4.86 ± 0.06ef	4.71 ± 0.11ef
ANOVA						
	F (Storage time)	69.72***	33.48***	33.68***	44.35***	23.20***
	F (Storage time × CaCl ₂ + MAP)	165.33***	84.69***	87.61***	113.44***	117.89***

Ns Not significant

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

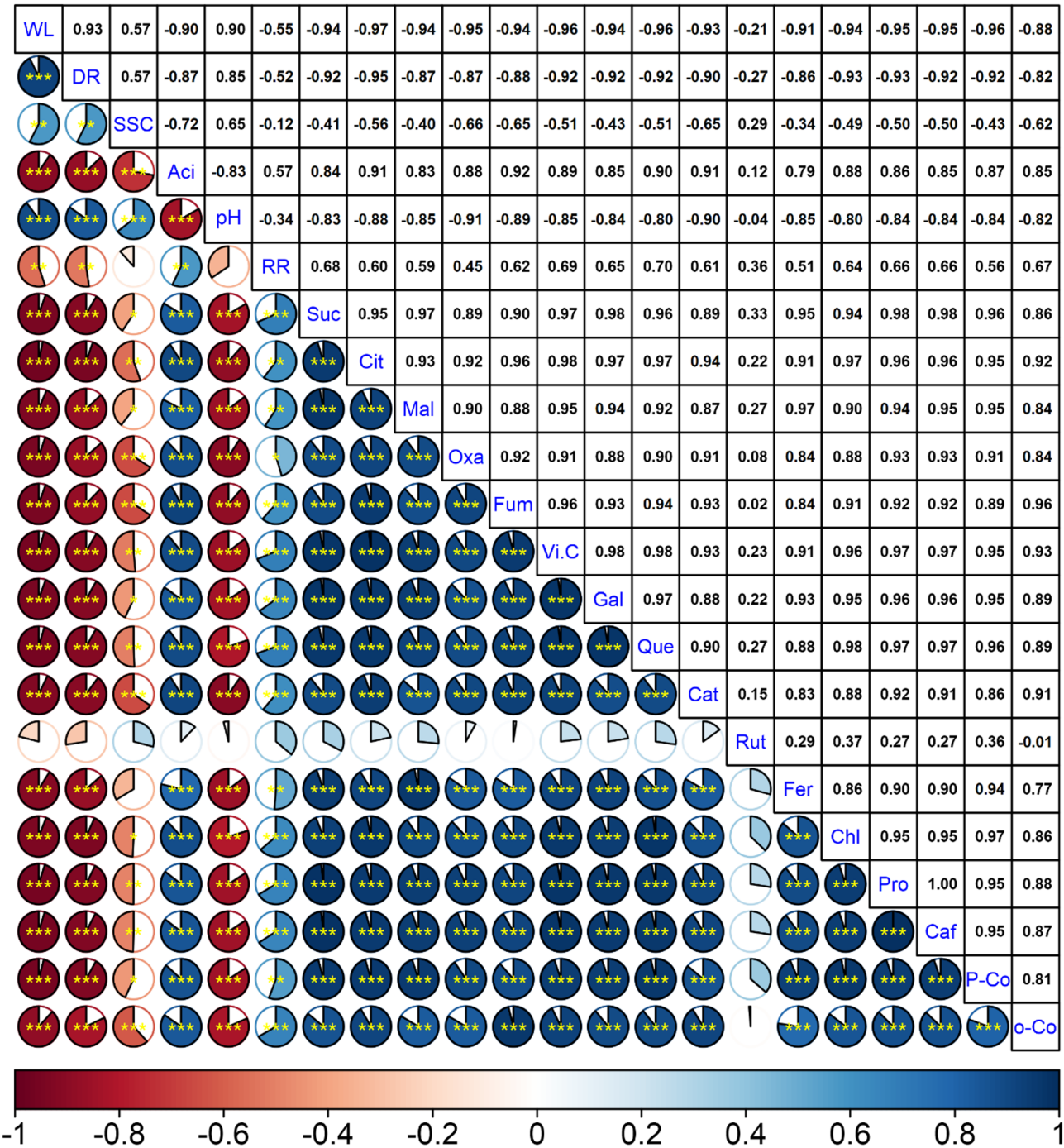


Fig. 2 Correlation between fruit biochemical properties and quality criteria. The color scale that disappears from dark to light shows the correlation values between -1 and $+1$. ***, and *** indicates significance at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively. WL: weight loss, DR: decay, SSC: soluble solid contents, Aci: acidity, RR: respiration rate, Suc: succinic acid, Cit: citric acid, Mal: malic acid, Oxa: oxalic acid, Fum: fumaric acid, Vi.C: vitamin C, Gal: gallic acid, Que: quersetin, Cat: catechin, Rut: rutin, Fer: ferulic acid, Chl: chlorogenic acid, Pro: protocatechuic acid, Caf: caffeic acid, p-Co: p-coumaric acid, o-Co: o-coumaric acid

acid, chlorogenic acid, rutin, ferulic acid). This situation shows that bioactive compounds decrease along with quality loss during storage period, that is, structures carrying nutritional value in the fruit are degraded by oxidative stress or respiration. At the same time, significant

positive correlations were detected between decay rate and respiration rate. This relationship is a direct indicator of the link between pathogen development and metabolic activity, suggesting that microbial spoilage is accelerated in samples with more active respiration. This finding is

also consistent with the studies conducted by Zhang et al. [4] and El Khetabi et al. [5] on pathogen susceptibility in cherry fruit.

The findings obtained with principal component analysis (PCA) also support these correlation relationships. In the analysis presented in Fig. 3, it was determined that the samples belonging to the CaCl_2 +MAP group were positioned in the same direction as positive compounds such as vitamin C, malic acid, and chlorogenic acid. This situation shows that the application in question maintains the metabolic balance in the fruit for a longer time. On the other hand, the control group samples clustered

in the opposite direction of these values, indicating that oxidative degradation and nutrient loss started earlier and progressed faster in untreated fruits. This separation of the PCA analysis proves that the combined application provides superiority not only in terms of physiological but also biochemical stability. In fact, a similar trend was observed in the PCA performed for phenolic compounds in Fig. 4. Here, the important antioxidant compounds such as rutin, ferulic acid, gallic acid, caffeic acid and protocatechuic acid and CaCl_2 +MAP samples were collected on the same axis, while the control group was significantly away from this axis. This distribution clearly

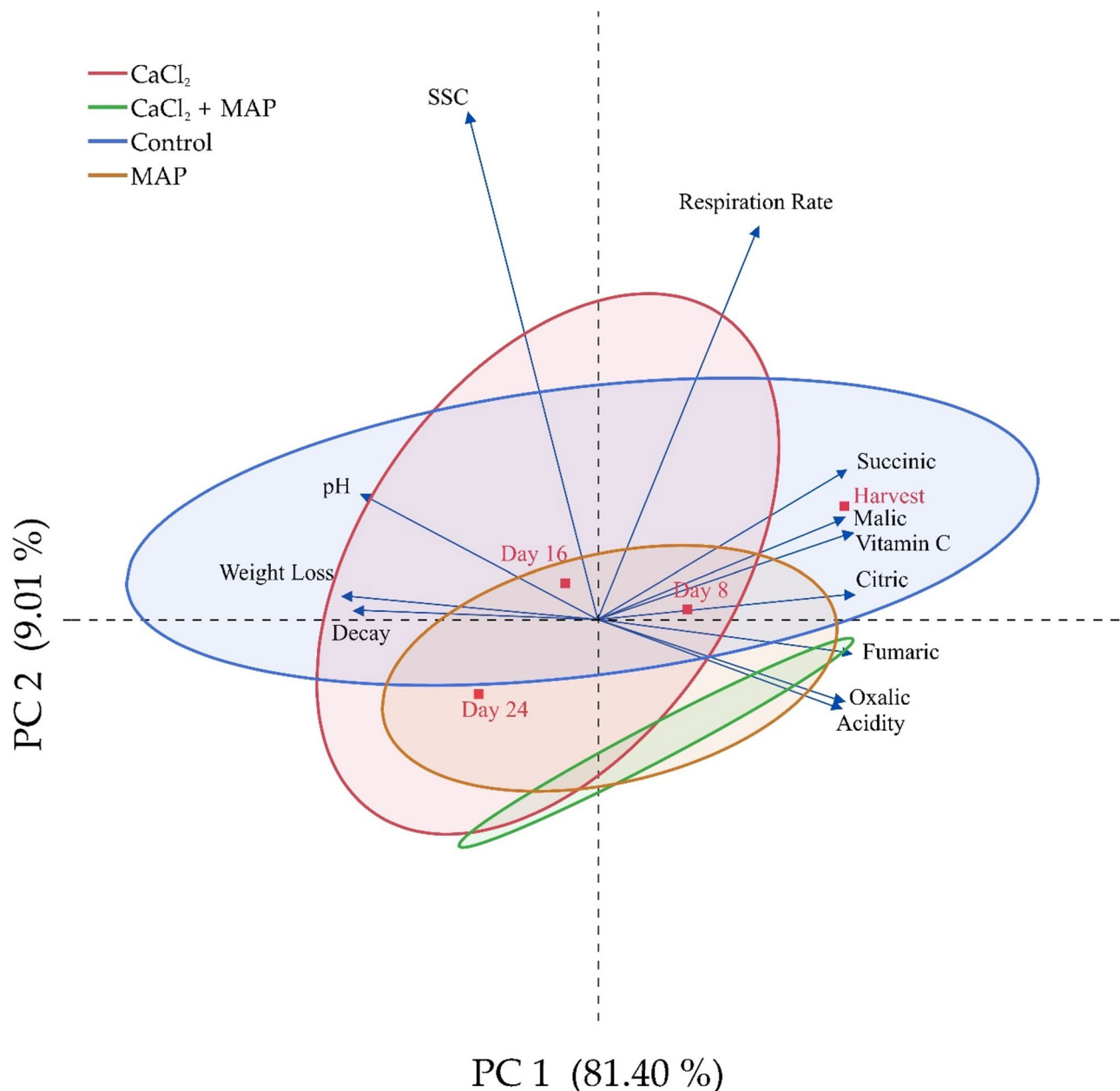


Fig. 3 Identification of the relationship between fruit biochemical properties of postharvest calcium chloride and MAP applications by PCA

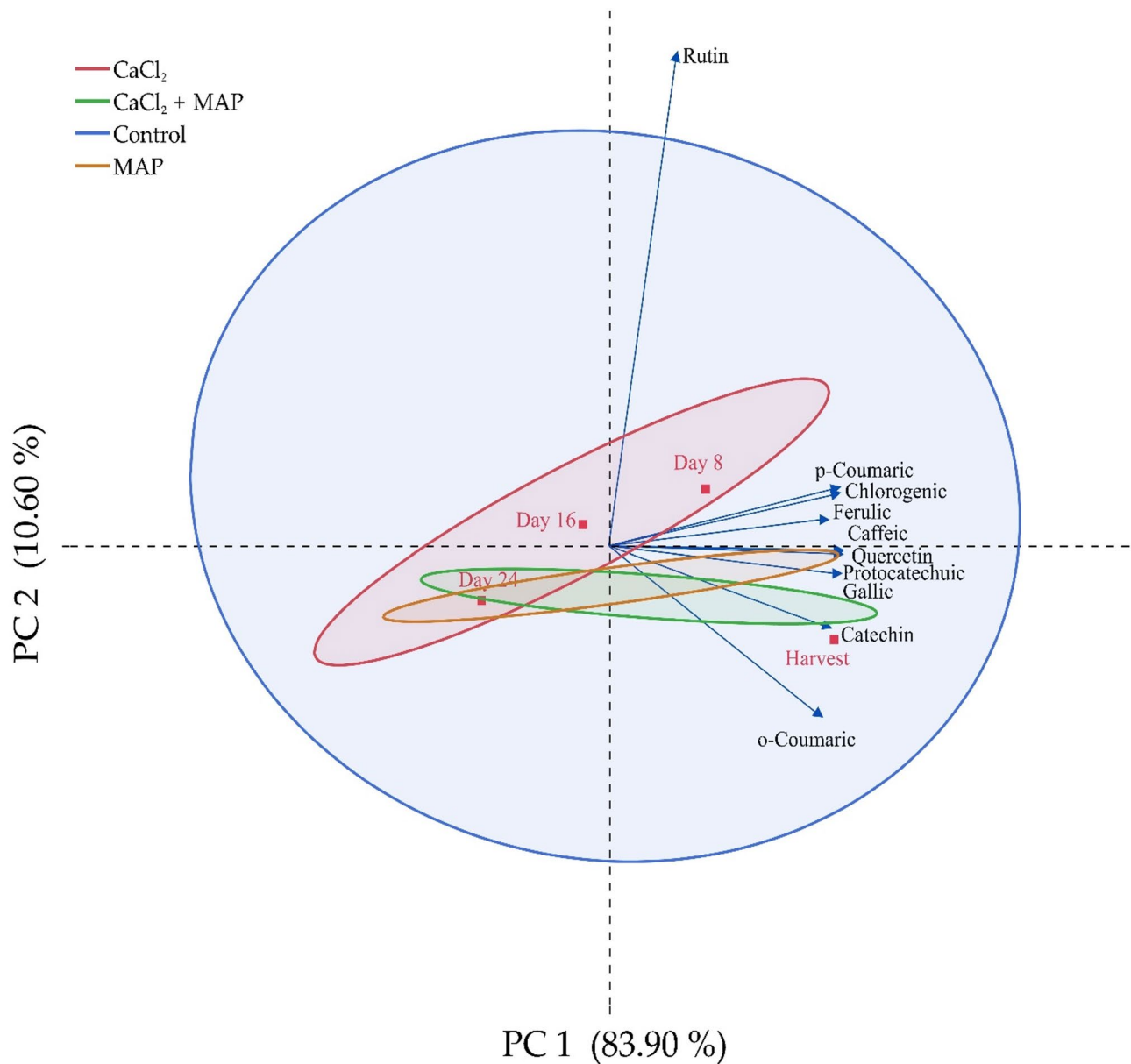


Fig. 4 Identification of the relationship between fruit phenolic compounds of postharvest calcium chloride and MAP applications by PCA

shows that phenolic compounds were lost the least in the CaCl₂+MAP group during storage. The findings of our study confirm that CaCl₂ application stabilizes the cell walls of fruit tissue, delays fruit softening by interacting with pectin structures, and also slows down oxidative deterioration by supporting antioxidant enzyme activities [8]. In this study, it was observed that MAP application limits the oxygen level by controlling the atmosphere inside the package, suppresses respiration by increasing the carbon dioxide level and reduces microbial activity, in line with the literature [11]. In particular, thanks to the suppression of phenolic oxidation under low O₂ and high CO₂ conditions, sensory properties such as color and aroma loss were also preserved.

Conclusion

In this study, the effects of 4% CaCl₂ and MAP methods applied after harvest on “0900 Ziraat” sweet cherry cultivar on fruit quality traits and biochemical compounds were evaluated. In all parameters examined, only MAP applications were found to be more protective than only CaCl₂ applications. However, the combined use of CaCl₂ and MAP applications ensures not only the physical quality of the sweet cherry fruit but also the preservation of nutrients and antioxidant compounds, thus extending the shelf life and keeping the nutritional value of the product reaching the consumer high. The combined application offers significant advantages in terms of post-harvest transportability and marketability of cherries. In

conclusion, this study shows that CaCl_2 and MAP methods have a strong and complementary effect in maintaining postharvest quality and biochemical balance in sweet cherry fruit. Both correlation and multivariate analyses revealed that this method is an effective alternative in reducing physiological deterioration and balancing fruit metabolism. Here, it is thought that the positive effect of only MAP applications increases with CaCl_2 . In this context, the generalizability of these findings can be increased by conducting comparative analyses on different CaCl_2 concentrations, packaging gas compositions and fruit varieties in future studies.

Abbreviations

h	Hour
min	Minute
pH	Power of hydrogen
SSC	Soluble solids content
TA	Titrateable acidity

Supplementary information

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Supplementary Material 1.

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Authors' contributions

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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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Competing interests

The authors declare no competing interests.

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