



Changes in Quality Characteristics of Strawberry Tree (*Arbutus unedo* L.) Fruit Packed with Perforated Polyethylene Terephthalate During Cold Storage

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Received: 4 January 2024 / Accepted: 8 April 2024 / Published online: 15 May 2024

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Abstract

This study evaluated the effects of polyethylene terephthalate (PET) treatments with different numbers of holes on the preservation of strawberry tree (*Arbutus unedo* L.) fruit. For this purpose, PET treatments with different holes were evaluated for respiration rate, weight loss, color (L^* , a^* , and b^*) changes, soluble solids content (SSC), titratable acidity (TA), vitamin C, total phenolics, total flavonoids, antioxidant activity (DPPH and FRAP), and individual phenolics. Analysis has shown that weight loss increases as storage time increases. It has been determined that PET 2 treatment is more effective than PET 1 in terms of weight loss and respiratory rate. During the storage, TA and SSC were lower in PET 2, while the vitamin C content was higher. It was determined that PET treatments had different effects on color parameters, total phenolics, and total flavonoids. Particularly in the PET 2, total flavonoids and DPPH activity was significantly higher during storage. It has been determined that catechin is the major content in strawberry tree fruit, which increases in both treatments with the progress of cold storage. When the 10 individual phenolics examined were evaluated, storage time and PET treatments had different effects. The significant effect of PET treatments continued only for epicatechin and protocatechuic acid during storage. As a result, it has been shown that PET 2 treatment is more effective in preserving fruit quality and nutritional values.

Keywords Antioxidant · Catechin · HPLC · Total phenolics · Weight loss

Introduction

It is known that the homeland of *Arbutus unedo* L., popularly known as the strawberry tree, includes the borders of Anatolia, Lebanon, Greece, Ireland, and Southern Europe

(Anşin and Özkan 1993; Molina et al. 2011; Sakaldaş 2012). Strawberry tree, which can grow in Turkey's coastal areas and maquis regions, continues to exist in the Black Sea, Mediterranean, Marmara, and Aegean regions (Pekdemir 2010; Beyhan et al. 2020). In addition to fresh consumption, the fruit are also used dried (Bajoub et al. 2023), as marmalade, puree (Carvalho 2010), jelly, jam, pastry (Şeker et al. 2004), ice cream (Şanlıdere et al. 2018), wine, and liqueur (Oliveira et al. 2011). Strawberry tree fruit is rich in essential minerals such as potassium, magnesium, copper and manganese, as well as vitamin C, carotenoids and flavonoids (Şeker and Toplu 2010; Ruiz-Rodríguez et al. 2011; Morales et al. 2013; Ramires et al. 2024). This high nutritional content of the fruit brings it into the category of valuable fruits for health (Ruiz-Rodríguez et al. 2011; Bebek Markovinović et al. 2022). The quality and shelf life of fresh produce is affected by several factors, including storage conditions, packaging, pre-harvest and post-harvest treatments (Saracoglu et al. 2017; Ozturk et al. 2019; Ates et al. 2022). Proper storage

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is essential to maintain the quality and expand the market volume of fresh produce, including strawberry tree fruit. Cold storage is widely used to extend the shelf life of fresh produce. However, various quality-affecting changes can occur in fresh produce during cold storage, such as weight loss, changes in color, texture and taste, and increased susceptibility to rotting and spoilage (Fadda et al. 2015; Guerreiro et al. 2018).

Packaging is a key factor in maintaining the quality of fresh produce during storage. Packaging materials are used to control the storage environment, reduce weight loss and respiration rate, reduce moisture loss that can lead to changes in texture and taste, protect biochemical contents and increase the percentage of marketable products (Kaur et al. 2013; Azene et al. 2014). Various packaging materials are used for fresh produce, including polyethylene terephthalate (PET) and perforated PET (Nath et al. 2012; Maryam et al. 2021). Perforated PET has small holes to allow gas exchange between the packaged product and the environment. PET is also effective in reducing moisture loss and maintaining the quality of fresh produce during storage (Pérez et al. 2021). However, in the literature research, there are limited studies on the storage of strawberry tree fruit and no studies on preserving with PET treatments.

The main study question was: What is the effect of non-perforated PET or perforated PET with a different number of holes than standard perforated packages in preventing losses in strawberry tree fruit during cold storage? This research assumed that PETs with different numbers of holes would have a significant effect during cold storage. The study's main purpose is to determine the changes in some physical and bioactive contents of strawberry tree fruit preserved using different polyethylene terephthalates.

Materials and Methods

Plant Materials

The strawberry tree fruits used as research material were obtained from an orchard in Perşembe district of Ordu (Türkiye) province. The fruit were harvested on the commercial harvest date, taking into consideration the color and soluble solids content (approximately 18–19% soluble solids content [SSC]). As a harvest criterion, coloration was considered, and fruit with homogeneous colors were collected. The harvested fruit were immediately transported to the laboratory with a refrigerated vehicle, and the fruit were sorted to provide a uniform sample. Polyethylene terephthalate [(PET; 375 g capacity, width: 110 mm, length: 120 mm, height: 253 mm), self-lid, leak-proof transparent plastic container] was purchased from a wholesaler. Then, holes were opened in the desired number and size. The

experiment was designed with three replications for two different PET treatments (PET 1 and PET 2) with four and eight holes. PET 1 was prepared as control treatment (four holes per unit area and 192 mm² total area of holes), and PET 2 was prepared as eight holes per unit area and 384 mm² total area of holes. Approximately 250 g of fruit was used for each replicate. The fruit were stored at 4 °C for 15 days in the 90 ± 5 RH, and measurements were made at 5-day intervals.

Weight Loss

Random samples from the fruit transported to the laboratory were divided to represent each application. Then, the separated fruit were placed in PET 1 and PET 2 in three replicates. At the beginning of cold storage, initial weights (W_i) were measured using a balance (Radwag, Radom, Poland) with an accuracy of 0.01 g. Then, the weight loss (WF: Final weights) of PETs was determined in each analysis period (WF), which was carried out for 15 days at 5-day intervals. The weight loss (WL) occurring in the fruit was determined as a percentage with the equation given based on the weight at the beginning of each measurement period.

$$WL = [(W_i - WF) \times 100] / W_i$$

Fruit Color

Color was determined by measuring 30 fruit in each replicate using a colorimeter (Minolta, model CR-400, Tokyo, Japan) from three opposite equatorial surfaces of the fruit. Color measurements are determined in CIE (Commission Internationale de l'Eclairage) L*, a* and b* (McGuire 1992).

Respiration Rate

The respiration rate was determined on a total 75 fruit, 25 in each replicate. First, the weight and volume of the fruit to be measured for gas were determined. For each replicate, fruit were placed in a 2-L gas-tight container and left for 1 h. Then, the CO₂ concentration released from the fruits into the container was measured by a gas sensor (Vernier, Oregon, USA). Accordingly, based on the amount of CO₂ released, the respiration rate of the fruit was calculated as nmol CO₂ kg⁻¹ s⁻¹ (Ozturk et al. 2019).

Soluble Solids Content, Titratable Acidity, and Vitamin C

A portion of fruit samples for each replicate were crushed using a blender and filtered through cheesecloth. SSC measurement in the resulting fruit juice was carried out us-

ing a digital refractometer (PAL⁻¹, McCormick Fruit Tech. Yakima, USA) and expressed as a percentage.

For SSC (%) measurement, 10 mL of juice taken from the prepared fruit juice was diluted with 10 mL of pure water. A volume of 0.1 mol L⁻¹ (N) sodium hydroxide (NaOH) was added to the resulting solution until the pH value reached 8.1. Accordingly, titratable acidity (TA) was calculated as g malic acid 100 g⁻¹, based on the amount of NaOH consumed.

Vitamin C was determined in fruit juice using a vitamin C test kit (Catalog no: 116981, Merck, Germany) via a reflectometer (Merck RQflex plus 10, Germany). The data obtained is expressed as mg 100 g⁻¹ (Ozturk et al. 2019).

Total Phenolics, Total Flavonoids, and Antioxidant Activity

A total of 50 strawberry tree fruit were taken from each replication and washed with distilled water. After homogenization using a blender (Model No. Promix HR2653 Philips, Turkey), 30 mL of homogenate was transferred to a 50-mL falcon tube and stored at -20 °C until analysis. Prior to analysis, the frozen samples were dissolved at room temperature (21 °C), and pulp and juice were separated using a centrifuge at 12,000 × g at 4 °C for 35 min. The resulting filtrate was utilized to determine total phenolics, total flavonoids, and antioxidant activity.

The spectrophotometric measurements for total phenolics, total flavonoids, and antioxidant activity were performed using a UV-Vis spectrophotometer (Shimadzu, Kyoto, Japan). Total phenolics were quantified based on the method by Singleton and Rossi (1965) and expressed as mg GAE (gallic acid equivalent) g⁻¹ fresh weight (fw). Total flavonoids were measured following the method by Chang et al. (2002) and expressed as mg quercetin equivalent (QE) g⁻¹ fw. Antioxidant activity of strawberry tree fruit was determined using two different procedures: 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) (Aglar et al. 2017) and ferric ion (Fe+3) reducing antioxidant power (FRAP) (Benzie and Strain 1996). The results were expressed in mmol Trolox equivalent (TE) kg⁻¹ fw.

Individual Phenolic Compounds

In this study, the content of various compounds, including catechin, chlorogenic acid, ferulic acid, caffeic acid, protocatechuic acid, rutin, 4-aminobenzoic acid, 4-hydroxybenzoic acid, and p-coumaric acid, was determined. Chromatographic separation was achieved using an ultra-high-performance liquid chromatography (UHPLC) system equipped with a diode array detector (DAD-3000, USA), following the method described by Ozturk et al. (2015).

The samples were prepared by mixing them with distilled water in a 1:1 ratio, followed by centrifugation at 15,000 × g for 15 min. The resulting supernatant was filtered through 0.45 μm Millipore filters and then injected into the UHPLC system. Separation of the analytes was carried out using a 250 × 3.0 mm, 5 μm Hypersil GD phenyl column (Thermo Scientific, USA) maintained at 30 °C. The elution solvents consisted of aqueous 2.5% formic acid (solvent A) and 100% methanol (solvent B). Detection of the compounds was performed at 274 nm, and the total run time was 60 min. Each injection volume was 20 μL, and the mobile phase flow rate was set at 1 mL min⁻¹. The results obtained from the analyses were expressed in terms of mg kg⁻¹.

Statistical Analysis

Data normality was assessed using the Kolmogorov–Smirnov test, and homogeneity of variance was examined using the Levene test. For data that met these assumptions, variance analysis was conducted by calculating descriptive statistics. The significance level between treatments was determined through two-group (stat, basic statistic, two-sample t) comparison tests ($p < 0.05$). Statistical analyses were carried out using Minitab® 17 Statistical software (Minitab Inc., State College, PA, USA).

Results

In the study, the effects of PET treatments with different holes on respiratory rate and weight loss were examined. The results revealed significant differences between PET treatments regarding weight loss and respiration rate during cold storage ($p < 0.05$). As the cold storage period progressed, the difference in weight loss between PET treatments increased. The highest weight loss was determined in PET 1 (6.94%) on the 15th day, and the lowest was determined in PET 2 (0.71%) on the 5th day. Additionally, the respiration rate was lower in PET 1 treatment than in PET 2 during cold storage. The difference between treatments was measured at the highest level in the first measurement (5th day) and the lowest in the last measurement (15th day). In both treatments, respiration rates decreased during cold storage (Fig. 1). This shows that both treatments are effective in increasing the durability of fruit. In terms of weight loss, it has been observed that PET 2 is more effective than PET 1 treatment. This suggests that although PET 1, which has a smaller opening, ensures that the fruit are less exposed to external influences, it lags behind PET 2 in preventing physical losses.

The effects of different PET treatments on the color changes of strawberry tree fruit during cold storage were

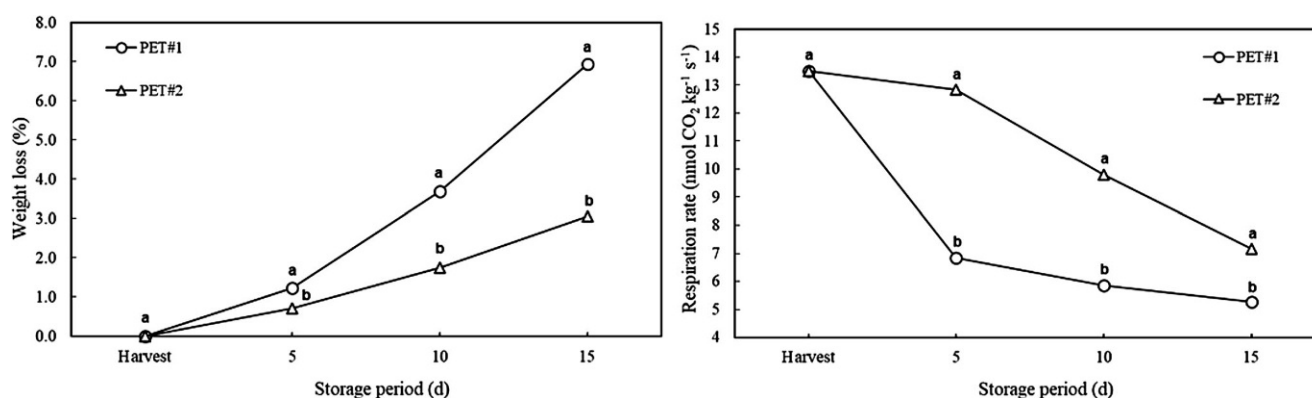


Fig. 1 Weight loss and respiration rate of strawberry tree fruit packed in different polyethylene terephthalate (PET) boxes during cold storage. The differences among the treatments indicated with the same letter vertically were not significant according to t-test at $P < 0.05$

examined. The results obtained from the study showed that the L^* value in strawberry tree fruits was at a similar level in all measurement periods ($p < 0.05$). A significant difference was detected in a^* and b^* values only in the 5th day. Additionally, on the 5th day, all color parameters were at their highest level for both treatments. In both treatments, L^* and b^* values decreased starting from the 5th day of cold storage. While the a^* value of fruit treated with PET 2 decreased during cold storage, on the contrary, it increased after the 10th day of PET 1 treated fruit (Fig. 2).

In addition, some differences were detected in SSC and TA in strawberry tree fruit stored with different PET treatments. During the cold storage of strawberry tree fruit, the highest SSC was 11.00% (PET 1, 15th day), and the lowest was 9.20% (at harvest). Analyses showed that SSC increased as cold storage time progressed. In the analyses, it was determined that the SSC content showed a significant difference between the treatments only on the 15th day, and it was at a similar level in the other measurement periods ($p < 0.05$). TA was significantly higher in the PET 1 treatment than the PET 2 on the 5th and 10th days of cold

Table 1 Individual phenolics compounds of strawberry tree fruit packed in different polyethylene terephthalate (PET) boxes during cold storage

Phenolic compounds (mg kg ⁻¹)	Treatment	Storage periods (days)			
		0	5	10	15
4-Aminobenzoic acid	PET 1	0.36 a	0.17 b	0.40 a	0.21 b
	PET 2	0.36 a	0.37 a	0.62 a	0.48 a
Caffeic acid	PET 1	0.26 a	0.20 a	0.23 a	0.18 b
	PET 2	0.26 a	0.15 b	0.29 a	0.31 a
Catechin	PET 1	14.76 a	15.43 a	15.52 b	17.22 a
	PET 2	14.75 a	14.76 b	16.03 a	17.13 a
Epicatechin	PET 1	3.56 a	6.35 a	4.76 a	5.88 a
	PET 2	3.56 a	5.23 b	4.09 b	4.03 b
Chlorogenic acid	PET 1	0.43 a	0.33 b	0.46 a	0.52 a
	PET 2	0.43 a	0.54 a	0.47 a	0.51 a
4-Hydroxybenzoic acid	PET 1	1.45 a	1.44 a	1.46 a	1.66 a
	PET 2	1.45 a	1.70 a	1.63 a	1.65 a
p-Coumaric acid	PET 1	2.09 a	2.20 a	2.34 a	2.31 a
	PET 2	2.09 a	2.13 a	2.39 a	2.33 a
Protocatechuic acid	PET 1	2.83 a	2.34 b	2.88 a	2.52 a
	PET 2	2.83 a	2.68 a	2.33 b	2.31 b
Trans-ferulic acid	PET 1	0.69 a	0.78 a	0.69 a	0.90 a
	PET 2	0.69 a	0.73 a	0.57 b	0.78 b
Rutin	PET 1	3.38 a	2.41 b	2.89 a	3.16 b
	PET 2	3.38 a	3.15 a	2.98 a	3.69 a

The differences among the treatments indicated with the same letter vertically were not significant according to t-test at $P < 0.05$

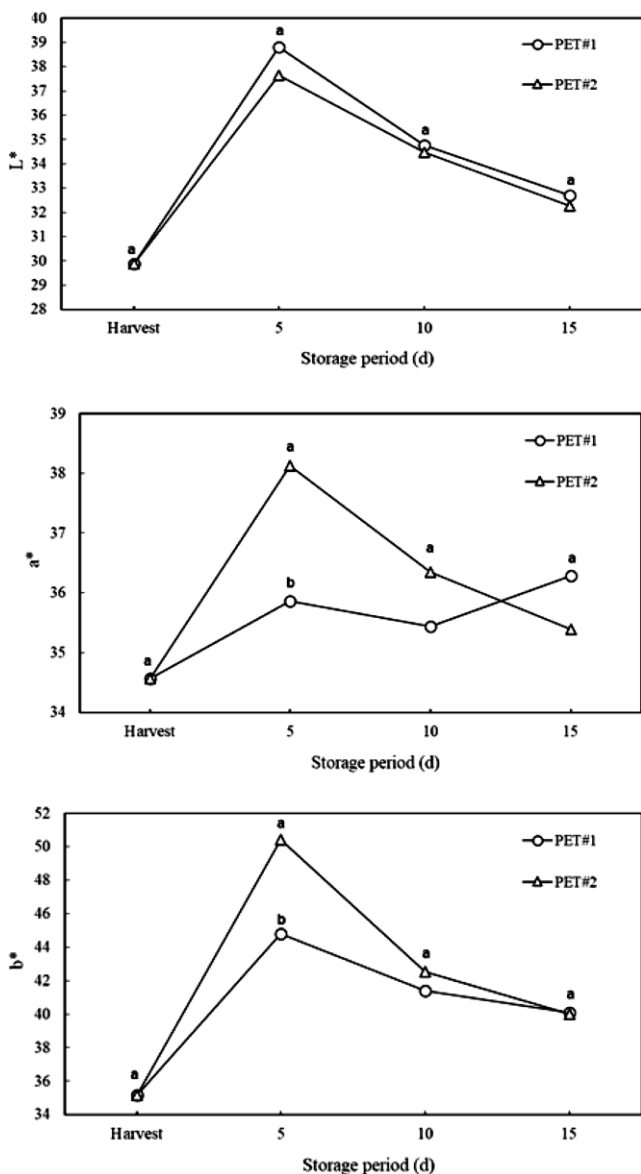


Fig. 2 Color characteristics (L*, a* and b*) of strawberry tree fruit packed in different polyethylene terephthalate (PET) boxes during cold storage. The differences among the treatments indicated with the same letter vertically were not significant according to t-test at $P < 0.05$

storage. However, on the 15th day of cold storage, it was determined that both treatments had similar TA ($p < 0.05$). A decrease in the vitamin C content of strawberry tree fruit was detected in both treatments due to the prolongation of the cold storage period. Vitamin C content, which was at a similar level on the 5th day of cold storage, showed significant differences between treatments in other measurement periods ($p < 0.05$). As a matter of fact, the study concluded that PET 2 treatment was more effective in preserving vitamin C content than PET 1 (Fig. 3).

As a matter of fact, in this study, some significant differences emerged in bioactive ingredients during cold storage.

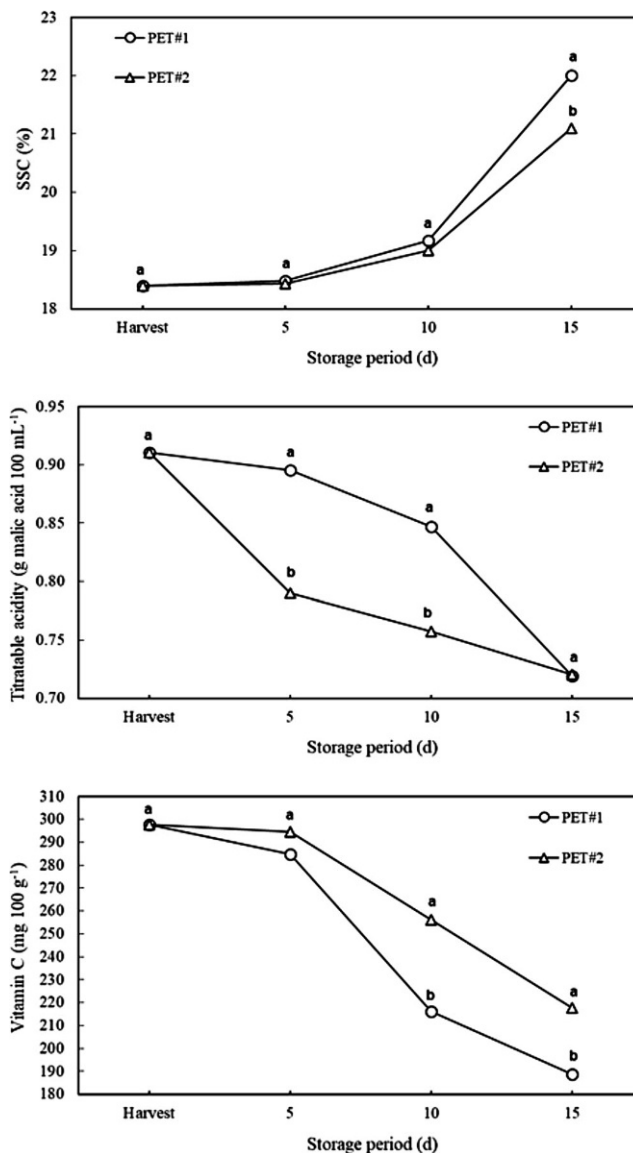


Fig. 3 Soluble solids content (SSC), titratable acidity and vitamin C of strawberry tree fruit packed in different polyethylene terephthalate (PET) boxes during cold storage. The differences among the treatments indicated with the same letter vertically were not significant according to t-test at $P < 0.05$

In the study, it was determined that the total phenolic content of fruit treated with PET 1 was preserved at a significant level on the 5th and 10th days of cold storage; on the contrary, it was found to be higher in fruit treated with PET 2 on the 15th day. Significant differences were detected between treatments in all measurement periods in terms of total flavonoids ($p < 0.05$). With the PET 2 treatment, the decrease in the total flavonoids in fruit was delayed compared to PET 1 during the cold storage (Fig. 4).

The antioxidant activity of fruit treated with PET decreased during cold storage. While DPPH activity was statistically significantly different between treatments in all

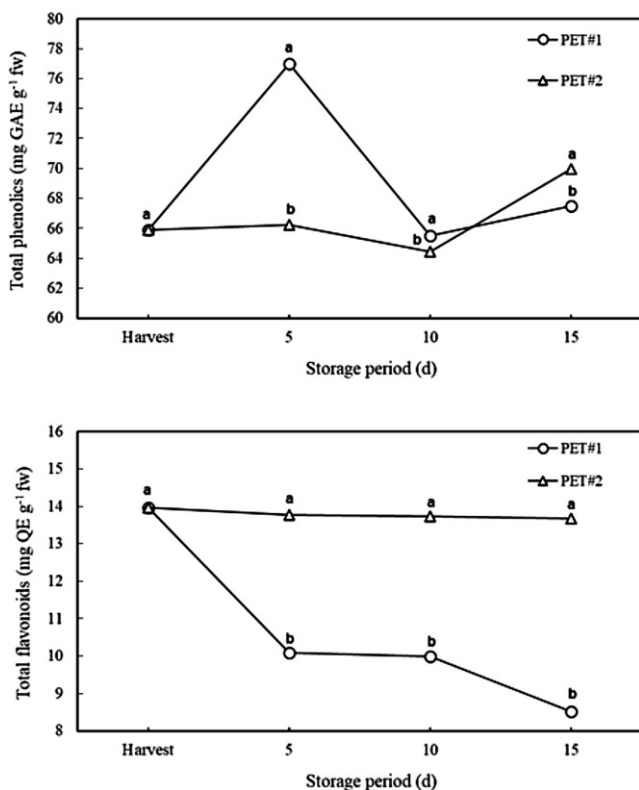


Fig. 4 Total phenolics and total flavonoids of strawberry tree fruit packed in different polyethylene terephthalate (*PET*) boxes during cold storage. The differences among the treatments indicated with the same letter vertically were not significant according to t-test at $P < 0.05$

measurement periods, FRAP content was found to be different only in the 5th day measurements ($p < 0.05$). In addition, the highest DPPH activities were measured in PET 2 during the cold storage. FRAP activity was higher in fruit treated with PET 2 until the 5th day of storage, while it was higher in PET 1 in subsequent measurements (Fig. 5).

In our study, the effects of different PET treatments on the individual phenolic contents of strawberry tree fruit were examined. The data obtained showed generally significant differences in individual phenolics during cold storage. However, in 4-hydroxybenzoic acid and p-coumaric acid, the difference between PET treatments was insignificant in all measurement periods. Additionally, catechin was detected as the major phenolic in the strawberry tree fruit examined. Epicatechin and rutin, respectively, followed this (Table 1).

Discussion

The most basic principle of storage is to preserve product quantity and quality for a long time. Maintaining the amount of product is possible by reducing weight loss. The

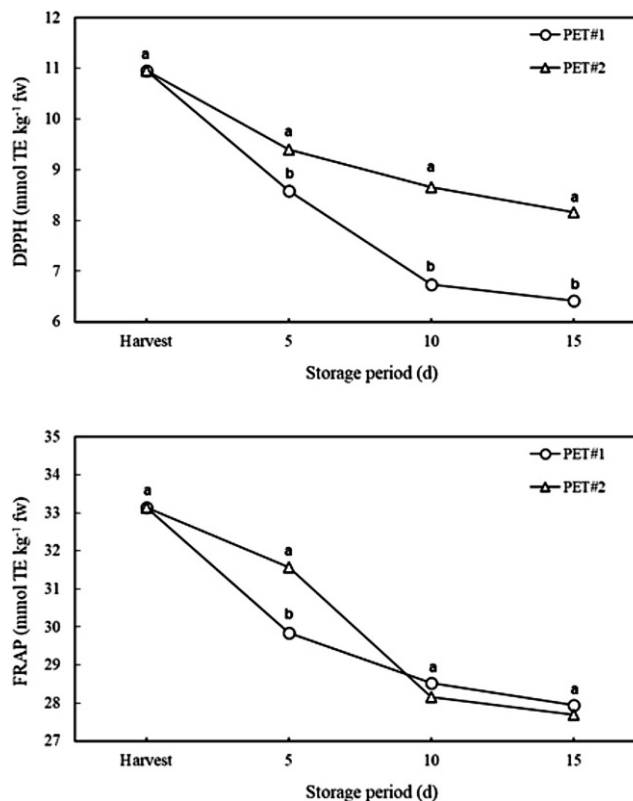


Fig. 5 Antioxidant activity according to 2,2-diphenyl-1-picryl-hydrazyl-hydrate (*DPPH*) and ferric ion reducing antioxidant power (*FRAP*) assays of strawberry tree fruit packed in different polyethylene terephthalate (*PET*) boxes during cold storage. The differences among the treatments indicated with the same letter vertically were not significant according to t-test at $P < 0.05$

long-term durability of stored products is also related to the respiration rate (Ates et al. 2022). In a study similar to ours, Rodov et al. (1997) reported that perforated polyethylene film was almost twice as effective in reducing weight loss as non-perforated film. Similarly, Widayanti et al. (2024) reported that holes were effective in reducing weight loss compared to control. Freitas and Mitcham (2013) reported that perforated packages effectively reduced weight loss but could not prevent the number of rotten fruits. In contrast, Ben-Yehoshua et al. (1998) reported that perforated packages delayed weight loss and fruit decay. Additionally, it has been reported that perforated packaging is more effective in extending the shelf life of pear (Nath et al. 2012) and ivy gourd (Shankarprasad and Hongal 2018).

Fruit color is one of the important factors affecting consumer preference and marketing of the fruit (Ozturk et al. 2022). However, sometimes fruit may not be delivered directly to the market. In this case, the stored fruit are expected to maintain their color as on the first day of harvest until they are presented to the market. For this purpose, it is desirable that the treatments made before storage preserve the color. In our study, it was determined that the

PET1 application, which has less clarity, was more effective in preserving fruit color. In contrast, Guerreiro et al. (2013) reported that the L^* value of strawberry tree fruit decreased due to the increase in storage temperature and that the effect of different film treatments on color was insignificant. Nath et al. (2012) emphasized the importance of the packaging material and reported that color change was more significant in perforated packages. Sanz et al. (1999) found that perforated packages were more effective in preserving color changes during storage, but the effect of hole sizes on color change was insignificant. In a study where pomegranate fruit were stored, it was reported that the L^* value of fruit in packages with a large number and diameter of perforate was generally high, and a^* value was low (Lufu et al. 2021). These results prove that the number and diameter of perforations in packaging design can affect color quality.

SSC and TA content in fruit is directly related to ripeness, taste, and flavor (Yarılgaç et al. 2019). The taste and flavor of the fruit, as well as its physical appeal, are attractive features for marketable products (Huang et al. 2021). However, since increasing SSC and decreasing TA amount will negatively affect the storage period, delaying the change in these values as much as possible is desirable under storage conditions. As a matter of fact, it was determined that the decrease in the amount of TA and vitamin C was also reduced in PET 1 treatment. Contrary to our research findings, Freitas and Mitcham (2013) found that perforated plastic bags did not affect SSC and TA. Mei (2010) stated that fruit in non-perforated packages had a higher SSC content compared to perforated packages, but this difference was insignificant. Similar to our findings, Guerreiro et al. (2013) reported that a slight increase in the amount of SSC was observed during storage and that the effect of film treatments was effective only in some measurement periods. However, Nath et al. (2012), in their study evaluating the effects of different packaging materials, stated that the SSC of fruit in non-perforated packages was lower, the TA was higher, and the vitamin C content was partially higher. Sanz et al. (1999) also stated that perforated packages were more effective in preserving the vitamin C content during storage and that fruit in packages with larger perforate diameters had higher vitamin C content on the last day of storage.

Flavonoids and phenolic metabolites have biological activities that contribute to human health with their antioxidant properties, especially their ability to neutralize free radicals (Tomás-Barberán et al. 2000; Jakobek 2015). Products experience stress during the production and storage of antioxidant compounds and enzymes, which sometimes leads to a decrease in their content. As a result, the products may be damaged (Pogorzelska-Nowicka et al. 2020). Therefore, it is essential to preserve the antioxidant activity in the products.

Considering the findings, it was seen that PET1 treatment was very effective especially in preserving the total flavonoids and DPPH antioxidant activity. Similarly to our research findings, Yuan et al. (2021) reported that products in small-diameter holes packages had higher total phenolic and antioxidant activity values in the initial measurement periods of storage, while fruit in packages with large-diameter holes had lower values at the end of storage. Bayogan and Photchanachai (2021) reported that the low number of holes in the packages effectively preserved the total phenolic content and antioxidant activity. Similarly, Nath et al. (2011) also stated that products in micro-perforated packages had higher antioxidant activity compared to products stored without packages. Nazoori et al. (2021) found in their study that the total phenolic content differed in fruit in packages with only 1% perforation size, the highest antioxidant activity was found in packages with 1.5% perforation size, and the highest flavonoids was found in fruit in packages with 1% perforation size. In our study, PET 2 treatment was found to affect total flavonoids and FRAP activity significantly. This can be explained by the relatively faster ripening of fruit treated with PET 2. Da-Silva and do Amarante (2020) reported that the gas composition can directly and indirectly affect the antioxidant activity of packaged products. Put another way, Opara et al. (2017) reported that bioactive change may occur due to ethylene accumulation, respiration rate and physico-chemical changes. On the contrary Khalil et al. (2024) reported that packaging with different openings did not have a significant effect on biochemical contents, and besides, the antioxidant level increased equivalent to storage.

Phenolic compounds are secondary plant metabolites associated with fruits' taste and color characteristics (Andlauer et al. 2000). It is known that some pre-harvest and post-harvest practices also have an effect on physical and bioactive compounds. While pre-harvest practices aim to increase bioactive compounds (Gomes et al. 2021), post-harvest practices aim to protect (Ozturk et al. 2019). Kuru Berk et al. (2023) stated that individual phenolics in strawberry fruit decreased during storage. Similarly, Ozturk et al. (2019) reported that individual phenolics decreased during storage, but packaging practices could delay this. Rivera-Pastrana et al. (2010) reported that low temperatures during storage were effective in preserving some bioactive ingredients (ferulic and caffeic acids) in papaya fruit. Again, Gonçalves et al. (2004) reported that low-temperature storage conditions were more effective in preserving the amount of rutin, catechin and epicatechin in cherry fruit. Su et al. (2019) stated that the amount of rutin decreased directly in apples during storage, but there were irregular decreases in caffeic acid, chlorogenic acid, catechin and epicatechin. In our study, it was observed that some individual contents were preserved due to the

effect of cold storage conditions, while there were irregular decreases in others.

Conclusions

As a result, some quality characteristics of PET treatments with different numbers of holes were determined during cold storage of strawberry tree fruit. Accordingly, it has been determined that the PET 2 treatment, which has more holes, is more effective in preserving strawberry tree fruits' quality and nutritional values. In this context, it is thought that this study will provide potential benefits to the packaging industry in selecting packages that can be used in the preservation processes of fresh products.

Funding No financial support was received for this study.

Author Contribution *BO*: Conceptualization, validation, formal analysis, investigation, resources, *UA*: Project administration, data collection, data analysis, writing and editing of manuscript, investigation, resources, *SU*: writing original draft preparation, writing review and editing, visualization, investigation, resources, *OK*: data curation, supervision, formal analysis, investigation, *MAO*: data collection, data analysis, *CAH*: data collection, data analysis.

Data availability Not applicable.

Conflict of interest B. Ozturk, C.A. Hekimoglu, M.A. Olcer, U. Ates, S. Uzun and O. Karakaya declare that they have no competing interests.

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