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Postharvest application of *Aloe vera* gel and thymol enhances shelf-life of duke cherries via altering physiochemical parameters

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Abstract

Background Duke cherry is a non-climacteric fruit but deteriorates quickly during storage due to thin pericarp and succulent fruit tissue. The application of edible coatings, essential oils, or their combination is an appropriate technique to maintain the quality characteristics and reduce the deterioration of fruits during storage. This research assessed the effect of *Aloe vera* gel (AVG), thymol, and their combined use on the physicochemical and qualitative properties of duke cherries kept at 5 °C and 80% relative humidity for 28 d.

Results Compared to the uncoated fruits, duke cherries coated with a combination of AVG and thymol, showed more values of firmness (12.76–100.32%), total phenol (9.99–45.09%), antioxidant activity (7.90–84.56%), and sensory scores(50.15–100.00%), as well as the activity of guaiacol peroxidase (GPX) (12.03–185.11%) and catalase (CAT) (10.20–243.66%) enzymes during cold storage. Moreover, duke cherries coated with a combination of AVG and thymol had remarkably lower values of weight loss (32.57–42.67%), respiration rate (34.96–49.78%), stem browning (24.50–50.53%), spoilage percentage (84.55–100%), anthocyanin (14.21–23.16%), and total soluble solids/titratable acidity (TSS/TA) (35.64–50.15%), as well as hydrogen peroxide (H_2O_2) (16.66–32.35%) and malondialdehyde (MDA) (15.23–31.05%).

Conclusion The application of AVG and thymol, particularly their combination, can have a high practical potential to extend shelf-life and preserve the quality of duke cherries during cold storage. This treatment has various advantages including natural, edible, cost-effective, and efficient.

Keywords Antioxidant, Edible coating, Essential oil, H_2O_2, MDA, Respiration

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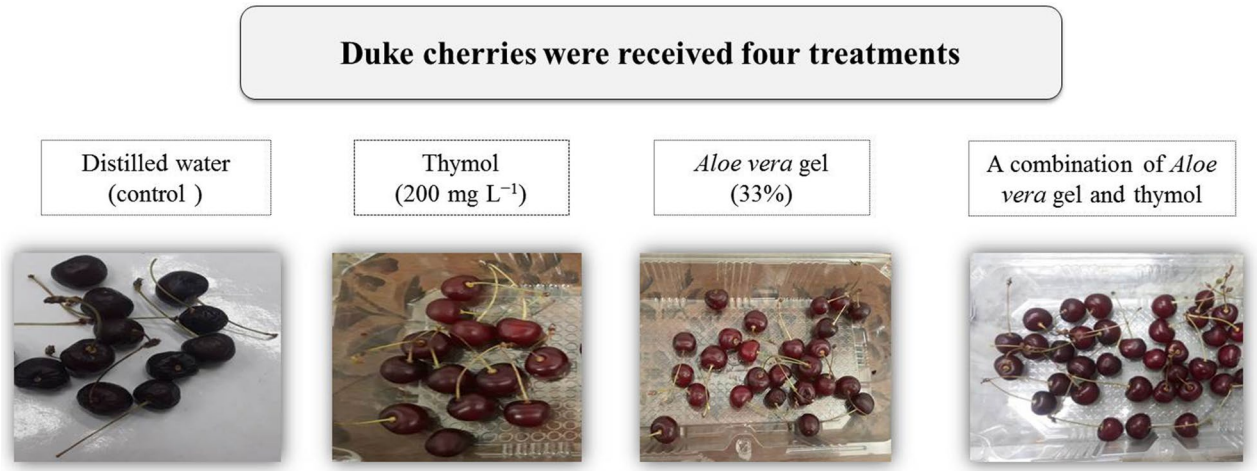
Graphical Abstract



Introduction

Sweet and sour cherry, the two main species of cherries, originated from Iran, Iraq, and Syria [1]. Duke cherry (*P.*×*gondouinii* Rehd.) is a natural hybrid between sweet and sour cherries and has trees and fruits that are intermediate between these two cherry species [2]. Duke cherry, as a popular fruit, is commercially important in temperate zones; and recent awareness of its health benefits has helped increase consumption [3]. Duke cherry has been widely cultivated in Iran, the most important duke cherry-producing countries are Turkey, Russia, Ukraine, Poland, and Iran [4].

Sweet and sour cherry are non-climacteric fruits; however, they deteriorate quickly during storage due to thin pericarp and succulent tissue that is greatly susceptible to weight loss, softening, surface pitting, color changes, and loss of acidity [5, 6]. According to Benichou et al. [7], approximately one-third of fresh fruits are lost before they reach the consumer. The shelf life of cherries is 7–10 d under ambient and 14–21 d under storage at − 1 to 0 °C with RH in the range of 90 to 95% [8, 9]. A main approach to increase the storage life and maintain the quality of fresh fruits is the use of synthetic chemicals. However, in most countries, the use of post-harvest chemicals to extend the storage life of fruits is restricted. Another effective method to extend the storage life of fruits is storage at low temperatures, although this approach is not sufficient to maintain fruit quality during the post-harvest period [10]. Thus, alternative, safe, and cost-effective strategies, combined with low temperatures are needed.

The use of edible coatings is accepted as a very suitable option for extending the storage life of fruits [11]. Natural coatings are composed of waxes, carbohydrates, proteins, and their composites, which create a semi-permeable coating on fruits [12]. Among edible coatings, *Aloe vera* gel has been widely applied to preserve the quality characteristics and prolong the storage life of several fresh fruits, including strawberry [12], apple [13], cornelian cherry [14], cherry laurel [15], jujube [16], plums [17], sapodilla [18], and apricots [19]. *Aloe vera* coating is an effective barrier against moisture and gas, which reduces weight loss, color changes, softening of tissues, oxidative browning, the proliferation of microorganisms, respiration rate, and production of ethylene in fruits [12, 20]. Furthermore, it is odorless, invisible, edible, safe for human health, and eco-friendly [21]. However, *Aloe vera* gel has low barrier efficacy due to its low lipid content [22]. It is possible to enhance the barrier efficacy of *Aloe vera* gel by adding lipids such as essential oils to the coating [12, 23]. Thymol is one of the most important essential oils of thyme (*Thymus vulgaris* L. thymoliferum) and is well known for its antimicrobial, and antioxidant properties. Furthermore, plant essential oils have shown the excessive potential to prolong the storage life of several fruits [24]. However, the use of essential oils to extend the shelf-life of fruits is often limited by their application costs and other disadvantages, such as pungent aroma and potential toxicity. An alternative to decrease the dose of essential oils while retaining their efficiency can be their combination in the formulation of edible coatings [24].

There are reports concerning the effect of edible coating and essential oils on the storage life of sweet and sour cherries [5, 25, 26]. In addition, a mixture of AVG and essential oils is widely studied as edible coatings for various fruits [12, 17]. However, as far as we know, no work has been reported on the effect of *Aloe vera* gel and thymol on the storage life of 'Majari' duke cherries during cold storage. Thus, the main purpose of the present research was to evaluate the effects of *Aloe vera* gel, thymol, and their combined use on quality characteristics, storage life extension, physicochemical characteristics, antioxidant enzyme activity as well as H_2O_2, and MDA levels of '*Majari*' duke cherries during 28 d of storage at 5 °C and 80% relative humidity. '*Majari*' is the most important commercial variety in Lorestan province with high performance and quality.

Materials and methods

Plant material and treatments

Duke cherries cv. '*Majari*' were picked at the commercial maturation time (25 July 2021) from a commercial 6-year-old orchard at Borujerd, Lorestan province, Iran. The trees were grafted on 'Mazzard' rootstocks. On the same day of harvesting, the fruit was transferred by a refrigerated vehicle (3–6 °C and 80% relative humidity) to the postharvest laboratory at Arak University. Duke cherries with similar size, shape, color, and healthy green stems were selected; whereas those with visible defects (bruised, diseased, injured, and sunburned fruits) were thrown away. The healthy fruits were divided into four groups of 300 fruits for the subsequent treatments in three replicates (each replicate included 100 individual fruits).

The treatments included three types of solutions: 33% AVG (v/v)., 200 mg L^{-1} thymol (Sigma Chemical Co., St. Louis, MO, USA, Minimum 99.5%), and 33% AVG + 200 mg L^{-1} thymol. The mixture of AVG and thymol was prepared by dissolving thymol in water, with the use of Tween-80, followed by the addition of AVG under vigorous shaking. The concentrations of AVG and thymol were chosen based on the preliminary experiments done in the laboratory. Duke cherries were dipped for 2 min in the prepared solutions. Fruits immersed in distilled water were considered as controls. All samples were air-dried for 60 min before packaging in polyethylene terephthalate and kept at 5 °C and 80% relative humidity in permanent darkness for 28 d. On days 0, 7, 14, 21, and 28, five fruit from each replicate were analyzed for all parameters.

After washing the *Aloe vera* leaves with distilled water, the colorless gel of the middle parts of the leaves was removed and mixed in a blender. Finally, the resulting mixture was filtered to remove the fibers.

Weight loss

The weight loss of fruits was concluded using the equation $[(A–B)/A] \times 100$, where A was the weight of fruits following treatment (day 0), and B was the weight of fruits at 7-day intervals (days 7, 14, 21, and 28) during storage [24].

Respiration rate

Three fruit were placed in a 1-L gas tight plastic for 2 h. Afterward, 1 ml gas samples were removed from the main space with a syringe to determine CO_2 using a gas chromatograph (model: Agilent 7890A). The column, detector, and injector temperatures were 90, 100, and 120 °C, respectively. Helium was used as a carrier gas with a flow rate of 60 mL min^{-1}. The respiration rate was stated as mg CO_2 kg^{-1} h^{-1} [27].

Stem browning

The percentage of stem browning was calculated using the equation $[(A–B)/A] \times 100$, where A was fruits with visible symptoms of stem browning and B was the total number of fruits [26].

Spoilage percentage

The spoilage percentage was calculated using the equation $[(A–B)/A] \times 100$, where A was fruits with spoilage and B was the total number of fruits [27].

Firmness

A penetrometer (STEP SYSTEM, Germany) was used to measure the firmness of duke cherries. A plunger tip with an 8 mm diameter and 21 mm height was used to measure the fruit's firmness. Results were stated as kg cm^{-2} [27].

Total soluble solids (TSS) and titratable acidity (TA)

Using a digital refractometer (Atago, PAL-1, Japan) TSS concentration of duke cherries was measured, and results were stated as % (°Brix). A pH meter (Az 86502, Taiwan) was used to measure the pH of fruit juice. Afterward, TA was specified by titration with 0.1 N NaOH up to pH of 8.1, using 1 mL of diluted juice in 25 mL distilled water, and the results were stated as % malic acid. TSS/TA was calculated by dividing TSS by TA percent [27].

Anthocyanin

The total anthocyanin content in the juice of duke cherries was determined according to the pH differential method as reported by Kim et al. [28]. Absorbance was measured using a spectrophotometer (Cary Win UV 100; Varian, Sydney, Australia) at 520 and 700 nm and readings expressed as mg cyanidin-3-glycoside equivalent (CGE) per 100 g of fresh weight (FW).

Total phenol

The total phenolic content in the juice of duke cherries was obtained using the Folin–Ciocalteau method according to the approach presented by Singleton et al. [29]. Absorbance was determined using a spectrophotometer (Cary Win UV 100; Varian, Sydney, Australia) at 765 nm, and total phenolic contents were calculated by applying a calibration curve drawn for the gallic acid standard solution and exhibited as mg gallic acid equivalent (GAE) per 100 g of fresh weight (FW).

Total antioxidant activity

Antioxidant activity in the juice of duke cherries was obtained based on the radical scavenging ability in reacting with DPPH (2,2-diphenyl-1- picrylhydrazyl) according to the approach presented by Brand-Williams et al. [30] with some modifications. Briefly, 100 µL of the methanol extract was mixed adequately with a 1900 µL DPPH (Sigma Aldrich, USA) solution. After 30 min, the absorbance was read against a blank (methanol) at 517 nm using a spectrophotometer (Cary Win UV 100, Varian, Australia). Total antioxidant activity as inhibition percentage of free radical DPPH was calculated by the subsequent equation:

$$\text{Total antioxidant activity (\%)} = [(\text{blank absorbance} - \text{extract absorbance}/\text{blank absorbance})] \times 100.$$

Hydrogen peroxide (H_2O_2)

The H_2O_2 concentration of duke cherries was measured according to the approach presented by Irani et al. [31] method. H_2O_2 concentration was exhibited as µmol g^{-1} FW.

Malondialdehyde (MDA)

According to the method recommended by Heath and Packer [32], lipid peroxidation of the membrane was determined. Lipid peroxidation was measured in terms of MDA concentration and exhibited as µmol g^{-1} FW.

GPX and CAT activity

Briefly, 0.5 g of duke cherries was powder powdered in a mortar with liquid nitrogen. Afterward, 100 mg of the frozen fruit powder was homogenized in 1.0 mL of sodium phosphate buffer (0.5 M, pH 7.8), containing 1 mM EDTA and PVP-40 (2% w/v). Samples were centrifuged at 12,000 × *g* for 20 min at 4 °C, and the supernatant was used to determine the activity of GPX and CAT enzymes.

Following the oxidation of guaiacol by H_2O_2, GPX activity was measured at 470 nm [33]. By measuring the absorption drop of H_2O_2, the activity of CAT was determined at 240 nm [34]. The readings were shown as a unit of mg^{-1} protein.

Table 1 Interaction of time and coating on the weight loss, stem browning, and spoilage percentage of duke cherries during the storage

Parameter	Treatment	Day storage			
		7	14	21	28
Weight loss (%)	Control	1.82 ± 0.10 c	3.07 ± 0.10 f	5.71 ± 0.15 j	8.82 ± 0.16 m
	AVG	1.38 ± 0.14 b	2.17 ± 0.23 d	4.33 ± 0.11 h	6.40 ± 0.21 k
	Thymol	1.50 ± 0.17 b	2.56 ± 0.24 e	4.79 ± 0.18 i	6.94 ± 0.24 l
	AVG + Thymol	1.09 ± 0.11 a	1.76 ± 0.15 c	3.85 ± 0.21 g	5.62 ± 0.29 j
Stem browning (%)	Control	1.88 ± 0.18 c	3.01 ± 0.14 e	6.12 ± 0.15 hi	9.25 ± 0.39 l
	AVG	1.30 ± 0.10 ab	2.30 ± 0.21 d	5.17 ± 0.23 g	7.21 ± 0.28 j
	Thymol	1.63 ± 0.11 bc	2.70 ± 0.24 e	5.75 ± 0.36 h	8.31 ± 0.44 k
	AVG + Thymol	0.93 ± 0.16 a	1.85 ± 0.28 c	4.62 ± 0.44 f	6.20 ± 0.25 i
Spoilage percentage (%)	Control	0.00 ± 0.00 a	2.11 ± 0.10 d	3.24 ± 0.16 e	6.15 ± 0.23 f
	AVG	0.00 ± 0.00 a	0.00 ± 0.00 a	1.23 ± 0.14 c	1.93 ± 0.25 d
	Thymol	0.00 ± 0.00 a	0.00 ± 0.00 a	0.89 ± 0.15 b	1.39 ± 0.27 c
	AVG + Thymol	0.00 ± 0.00 a	0.00 ± 0.00 a	0.00 ± 0.00 a	0.95 ± 0.35 b

Mean values followed by the similar letters across coating treatment and storage time for each parameter are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test)

Values are means of three replicates ± SD

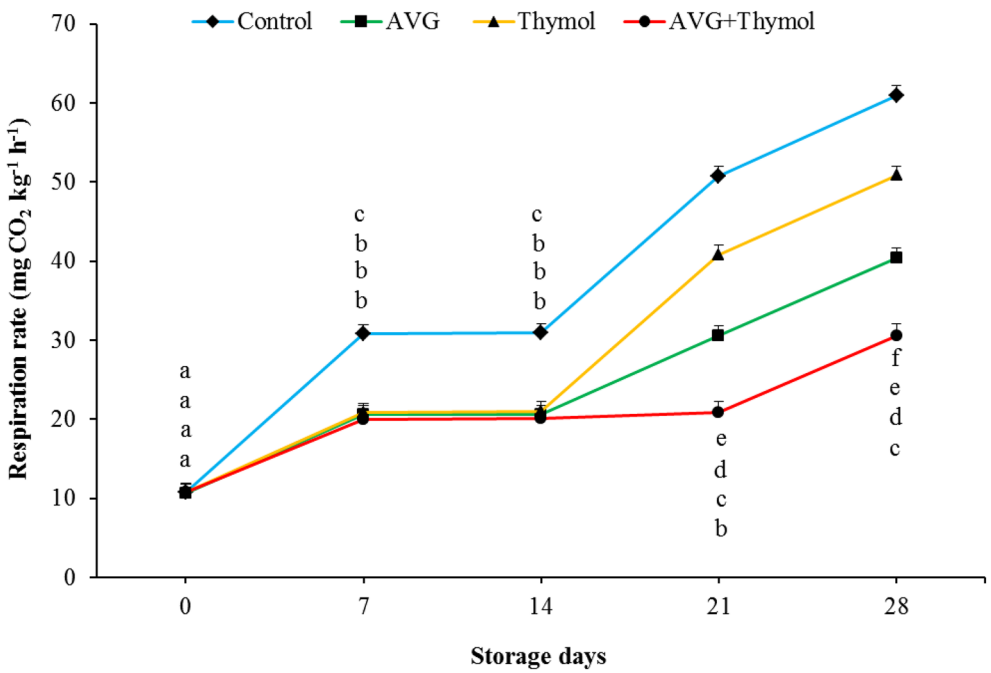


Fig. 1 Interaction of time and coating on the respiration rate of duke cherries during the storage. Mean values followed by the similar letters are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test). Vertical bars represent standard deviation (SD)

Sensory attributes evaluation

Throughout the post-harvest period, evaluation of kept duke cherries was done by five experts on a hedonic scale ranging from 0 to 5, where 1 = very bad, 2 = bad, 3 = medium, 4 = suitable, and 5 = excellent. Color, taste, appearance, and overall acceptability were evaluated by consumers, and average values were considered [27].

Storage life

The storage life was determined by recording the days that duke cherries remained in good condition without spoilage and deterioration. The end of storage life was when the deterioration of fruits exceeded 50% [24].

Statistical analysis

This experiment was carried out as a factorial in a completely randomized design (CRD) with two factors: the first factor was the storage period (0, 7, 14, 21, and 28 d) and the second factor was the edible coating (AVG, thymol, and their combination). Data were analyzed using the GLM procedure of SAS software (Version 9.1). Significant differences were evaluated at $P \leq 0.05$ using Duncan's multiple ranges. All the treatments were replicated three times.

Results and discussion

For all measured parameters, significant interactions were observed between storage time and edible coating; hence only mean comparisons related to interactions are shown.

Weight loss

Throughout cold storage, the weight loss of duke cherries increased for all treatments; however, coated fruits had lower weight loss than controls (Table 1). The effect of AVG + thymol was significantly greater than sole AVG or thymol coating. After 7, 14, 21, and 28 d of storage, duke cherries treated with AVG + thymol had 40.10%, 42.67%, 32.57%, and 36.28% lower weight loss than nontreated fruits, respectively (Table 1).

Cherries lose a lot of water during the storage period due to their low skin diffusion resistance and high surface-to-volume ratio [9, 35]. Water losses due to transpiration and respiration processes are the main reasons for weight loss in fruits [36]. Edible coatings create a transparent film around the fruit and seal small wounds; therefore, a decrease in weight loss of coated fruits during the storage period can be related to the effects of coatings on the reduction of transpiration and respiration rate, as

recognized in this study (Fig. 1). Delaying in weight loss of fruits with the application of AVG and essential oils have been reported for tomatoes [11], strawberries [12], and apricots [19], which is consistent with the results of the present work.

Respiration rate

Respiration rate is a good index of metabolic activity and can be used for evaluating the storage life of fruits [27]. The obtained results revealed that the respiration rate increased throughout the storage, although coated duke cherries with AVG, thymol, and their combination showed lower respiration rates than uncoated fruits (Fig. 1). At all sampling times, a combination of AVG and thymol was more effective than the application of AVG or thymol alone. Compared to the uncoated duke cherries, the respiration rate of fruits coated with AVG + thymol was 34.96%, 35.01%, 58.96%, and 49.78% lower after 7, 14, 21, and 28 d of storage, respectively (Fig. 1). Similar to our findings, it was found that coating sour cherries with AVG [5], strawberries with essential oils [37], and stone fruits with a combination of AVG and essential oils [22, 38] decreased respiration rate compared with uncoated fruits. The decrease in the respiration rate of fruits as the result of AVG application is likely to be because AVG, as a semi-permeable barrier, reduces the gas exchange resulting in decreases in O_2 and increases in CO_2 concentration [22, 38]. Since the lipid content of AVG is very low, the incorporation of a source of lipids such as essential oils into the coating could result in the formation of a coating with higher barrier efficacy [23], and in turn lower respiration rate, as documented in this research (Fig. 1).

Stem browning

Stem browning is a serious issue in the marketing of fresh cherries and affects buyer and consumer acceptance [39]. Our findings showed that stem browning rose during the storage period. However, duke cherries treated with AVG, thymol, and their combination, had lower stem browning than controls, where the effect of AVG + thymol was significantly greater than the other treatments except for AVG at day 7 (Table 1). In fruits treated with AVG + thymol, the stem browning was 50.53%, 38.53%, 24.50%, and 32.97% lower than nontreated ones after 7, 14, 21, and 28 d of storage, respectively (Table 1). The positive effects of edible coatings on reducing stem browning of sweet cherries during the storage period have been recognized [26, 40], which is consistent with the findings of the present research. Higher water loss is observed in the cherry pedicel than in the fruit [41], and desiccation is responsible for stem browning in the cherry [42]. The decrease in stem browning as the result of coating application can be due to decreased water loss from the stems [26]. There was no evidence regarding the influence of essential oils on the stem browning of cherries during the storage period.

Spoilage percentage

As shown in Table 1, the application of AVG and thymol was not only effective in controlling the spoilage of duke cherries but also in postponing the beginning of spoilage symptoms and deceleration the growth of microbes during cold storage. Nontreated control duke cherries exhibited spoilage after 14 d of storage, whereas spoilage was recognizable in coated fruits after 21 d (Table 1). The addition of thymol to AVG was more effective in controlling spoilage than AVG and thymol alone. By the

Table 2 Interaction of time and coating on the firmness and sensory attributes of duke cherries during the storage

Parameter	Treatment	Day storage				
		0	7	14	21	28
Firmness (kg cm^{-2})	Control	8.13 ± 0.08 a	7.05 ± 0.25 bc	5.94 ± 0.38 ef	4.57 ± 0.39 g	3.10 ± 0.33 h
	AVG	8.14 ± 0.09 a	7.68 ± 0.32 ab	6.90 ± 0.36 cd	6.13 ± 0.32 ef	5.70 ± 0.43 ef
	Thymol	8.14 ± 0.01 a	7.48 ± 0.38 abc	6.37 ± 0.47 de	5.50 ± 0.41 f	4.85 ± 0.60 g
	AVG + thymol	8.15 ± 0.00 a	7.95 ± 0.34 a	7.13 ± 0.51 bc	6.90 ± 0.45 cd	6.21 ± 0.54 e
Sensory attributes	Control	5.00 ± 0.00 a	4.66 ± 0.57 ab	4.33 ± 0.57 ab	3.33 ± 0.57 de	2.33 ± 0.57 f
	AVG	5.00 ± 0.00 a	5.00 ± 0.00 a	5.00 ± 0.00 a	4.66 ± 0.57 ab	3.66 ± 0.57 cd
	Thymol	5.00 ± 0.00 a	5.00 ± 0.00 a	4.66 ± 0.57 ab	4.00 ± 0.00 bc	3.00 ± 0.00 e
	AVG + thymol	5.00 ± 0.00 a	5.00 ± 0.00 a	5.00 ± 0.00 a	5.00 ± 0.00 a	4.66 ± 0.57 ab

Mean values followed by the similar letters across coating treatment and storage time for each parameter are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test)

Values are means of three replicates ± SD

Table 3 Interaction of time and coating on the TSS, TA, and TSS/TA of duke cherries during the storage

Parameter	Treatment	Day storage				
		0	7	14	21	28
TSS (%)	Control	16.95 ± 0.15 a	17.31 ± 0.22 a-e	17.73 ± 0.18 efg	18.21 ± 0.33 hi	18.70 ± 0.26 j
	AVG	16.95 ± 0.11 a	17.05 ± 0.12 abc	17.25 ± 0.31 a–d	17.59 ± 0.19 d–g	17.86 ± 0.19 gh
	Thymol	16.96 ± 0.10 a	17.19 ± 0.20 a-d	17.41 ± 0.21 b–f	17.91 ± 0.14 gh	18.43 ± 0.26 ij
	AVG + thymol	16.95 ± 0.16 a	16.99 ± 0.20 ab	17.15 ± 0.46 abc	17.44 ± 0.16 d-g	17.75 ± 0.25 fg
TA (%)	Control	2.55 ± 0.15 a	2.33 ± 0.15 abc	2.07 ± 0.13 cde	1.42 ± 0.27 g	1.01 ± 0.20 h
	AVG	2.56 ± 0.15 a	2.49 ± 0.21 ab	2.37 ± 0.12 abc	1.99 ± 0.21 de	1.86 ± 0.17 ef
	Thymol	2.53 ± 0.18 a	2.43 ± 0.12 ab	2.22 ± 0.11 bcd	1.67 ± 0.22 fg	1.42 ± 0.15g
	AVG + thymol	2.55 ± 0.14 a	2.51 ± 0.13 ab	2.42 ± 0.12 ab	2.07 ± 0.17 cde	1.86 ± 0.23 ef
TSS/TA	Control	6.65 ± 0.12 a	7.42 ± 0.26 ab	8.56 ± 0.52 ab	13.10 ± 0.16 d	19.08 ± 0.79 e
	AVG	6.60 ± 0.16 a	6.87 ± 0.58 a	7.27 ± 0.13 a	8.81 ± 0.20 abc	9.58 ± 0.47 bc
	Thymol	8.06 ± 0.49 ab	7.06 ± 0.16 a	7.83 ± 0.20 ab	10.71 ± 0.38 c	12.92 ± 0.34 d
	AVG + thymol	6.63 ± 0.11 a	6.77 ± 0.12 a	7.08 ± 0.13 a	8.43 ± 0.15 ab	9.51 ± 0.18 bc

Mean values followed by the similar letters across coating treatment and storage time for each parameter are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test)

Values are means of three replicates ± SD

end of storage, duke cherries treated with AVG, thymol, and AVG + thymol had 68.61%, 77.39%, and 84.55% lower spoilage than uncoated fruits, respectively (Table 1).

The results of the current study agree with those obtained by Hassan et al. [12] on strawberries, Martínez-Romero et al. [40] on cherries, and Rasouli et al. [43] on oranges, reporting that microbial infection of AVG-coated fruits was lower than controls during storage. The inhibitory effects of AVG on the growth of postharvest pathogens can be due to the presence of Aloe-emodin, aloenin, and other active compounds in the coating [40]. Similarly, decreases in postharvest decay with the application of thymol have been described for figs [27], avocados [44], and kiwifruits [45]. Thymol

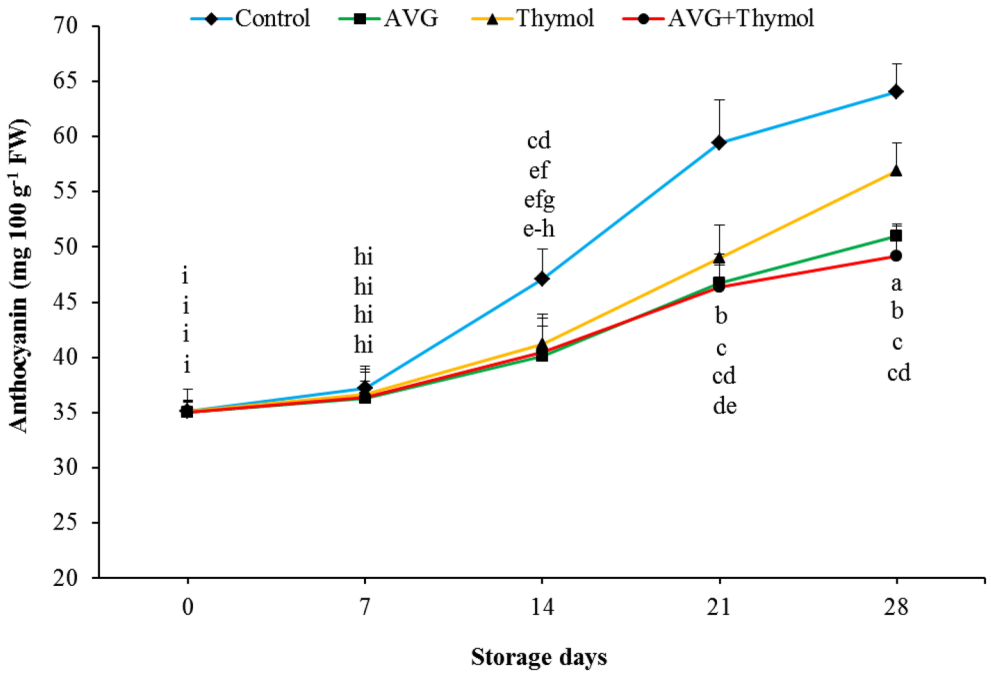


Fig. 2 Interaction of time and coating on the anthocyanin content of duke cherries during the storage. Mean values followed by the similar letters are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test). Vertical bars represent standard deviation (SD)

inhibits the pathogen's growth and spore germination by affecting the pathogen's cellular metabolism and the active sites of enzymes [46]. The obtained results in this study (Table 1) also confirm previous reports that combining essential oils and AVG presents a much better effect on controlling microbial infection than essential oils or AVG alone [12, 44].

Fruit firmness

Fruit firmness is one of the most important quality features of cherries. The firmness of duke cherries decreased with an extension in the storage period, with greater levels in coated compared with uncoated fruits (Table 2). At all sampling times, the highest firmness was related to AVG + thymol, although there were no statistically significant differences among edible coatings at day 7, and between AVG + thymol and AVG at days 14 and 28. On days 7, 14, 21, and 28, duke cherries coated with AVG + thymol had 12.76%, 20.03%, 50.98%, and 100.32% higher firmness than uncoated fruits, respectively (Table 2).

Consistent with our findings, a decrease in the firmness of cherries during the storage period has been reported [38, 40, 47], which could be associated with the activity of cell wall degrading enzymes, dehydration due to water loss, and pathogen infection [27]. Furthermore, similar findings on the positive effects of AVG, essential oils, and their combination on fruit firmness have been obtained for tomatoes [11], strawberries [12], plums [17], and peaches [38]. The decrease in the softening of fruits as the result of coating application can be due to the role of coatings in reducing respiration rates, enzyme activities, metabolic activity, and the ripening process [48].

TSS, TA, and TSS/TA

With an extension in the storage period, the levels of TSS and TSS/TA increased while TA decreased (Table 3), although these changes in TSS, TA, and TSS/TA were less noticeable in duke cherries coated with AVG, thymol, and particularly AVG + thymol. No statistically significant differences were found in TSS values of coated and uncoated fruits at day 7, among edible coatings at days 14 and 21, and between AVG + thymol and AVG at day 28 (Table 3). Moreover, coated and uncoated duke cherries did not show significant differences in TSS/TA values on days 7 and 21, but after that, fruits under AVG + thymol treatment showed the lowest TSS/TA value (9.51), although no significant difference was found between AVG + thymol and AVG regarding this trait (Table 3). On the other hand, the decline in TA was lower in duke cherries treated with coatings; the highest advantage was for AVG + thymol. Nevertheless, there were no statistically significant differences between coated and uncoated fruits on day 7, among edible coatings on day 14, and between AVG + thymol and AVG on days 21 and 28 (Table 3).

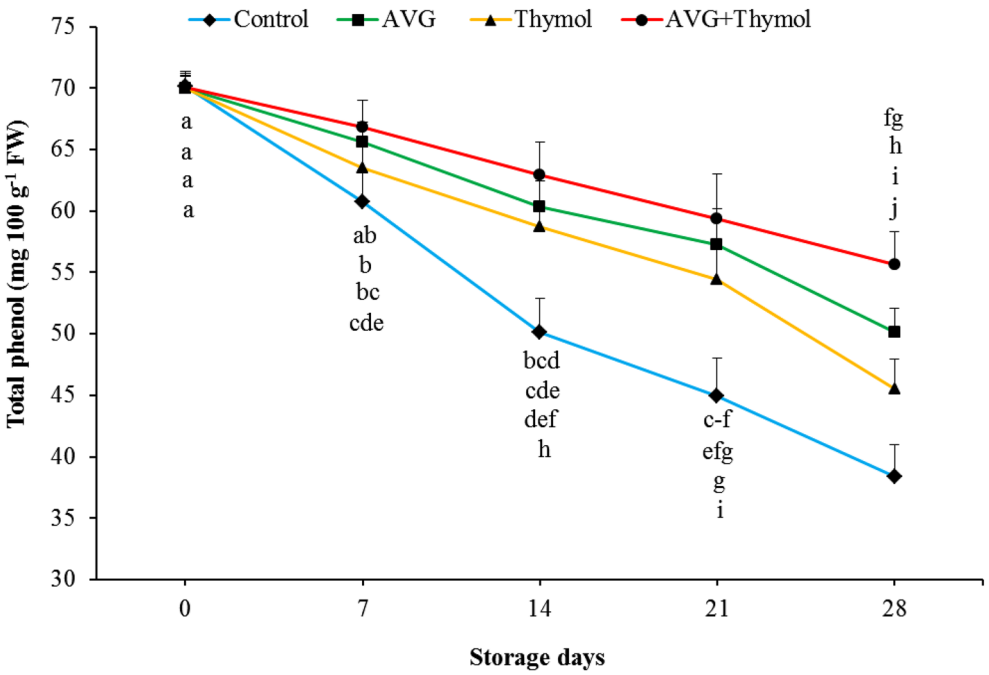


Fig. 3 Interaction of time and coating on the total phenolic content of duke cherries during the storage. Mean values followed by the similar letters are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test). Vertical bars represent standard deviation (SD)

The TSS/TA is an important parameter determining cherry acceptability by consumers. Similar to the results of this work, an increase in TSS and a decrease in TA of fruits during the storage period has been described for strawberries [12], mangos [49], and apricots [50] which could be due to the increase in respiration rate and consumption of organic acids, water losses, breakdown of glycoside into sub-units, and the catabolism of polysaccharides into simple sugars [24]. Furthermore, the positive effects of AVG, essential oils, and their combination on TSS, TA, and TSS/TA have been reported for tomatoes [11], strawberries [12], and plums [17], which is consistent with our findings. The lower changes in TSS and TA of coated fruits can be due to a decrease in respiration rate and water loss by coatings [27], as shown in the present research (Fig. 1 and Table 1).

Anthocyanin content

The anthocyanin content of duke cherries increased over the cold storage (Fig. 2). The increase in anthocyanin content was less in coated fruits than in those untreated. The lowest anthocyanin content was obtained for AVG + thymol; however, there were no statistically significant differences between coated and uncoated fruits on day 7, among edible coatings on day 14, and between AVG + thymol and AVG on days 21 and 28 (Fig. 2). In duke cherries coated with AVG + thymol, the anthocyanin content was 14.21%, 21.88%, and 23.16% lesser than uncoated fruits after 14, 21, and 28 d of cold storage, respectively (Fig. 2).

Our findings agree with those achieved by Shehata et al. [37] on strawberries, and Hu et al. [47] on sweet cherries, describing that the anthocyanin content of fruits increases during storage. This increase, especially in control, can be justified regarding the increases in respiration rate, sugar accumulation, and weight loss [24, 37]. Moreover, the results obtained in this work confirm previous research that the application of AVG, essential oils, and their combination decelerated anthocyanin synthesis in coated fruits during the storage period [12, 17]. The lower anthocyanin contents in coated fruits during the storage period may be due to the inhibitory effects of coatings on fruit ripening, which leads to a decrease in the activity of enzymes and biochemical reactions responsible for anthocyanin biosynthesis [26, 51].

Total phenol content

With increasing storage time, the total phenolic content of duke cherries decreased. The decline in total phenolic content was lesser in coated duke cherries than those uncoated (Fig. 3). At all sampling times, the highest total phenolic content was related to AVG + thymol, although there were no statistically significant differences among edible coatings at days 7 and 14, and between AVG + thymol and AVG at day 21 (Fig. 3). After 7, 14, 21, and 28

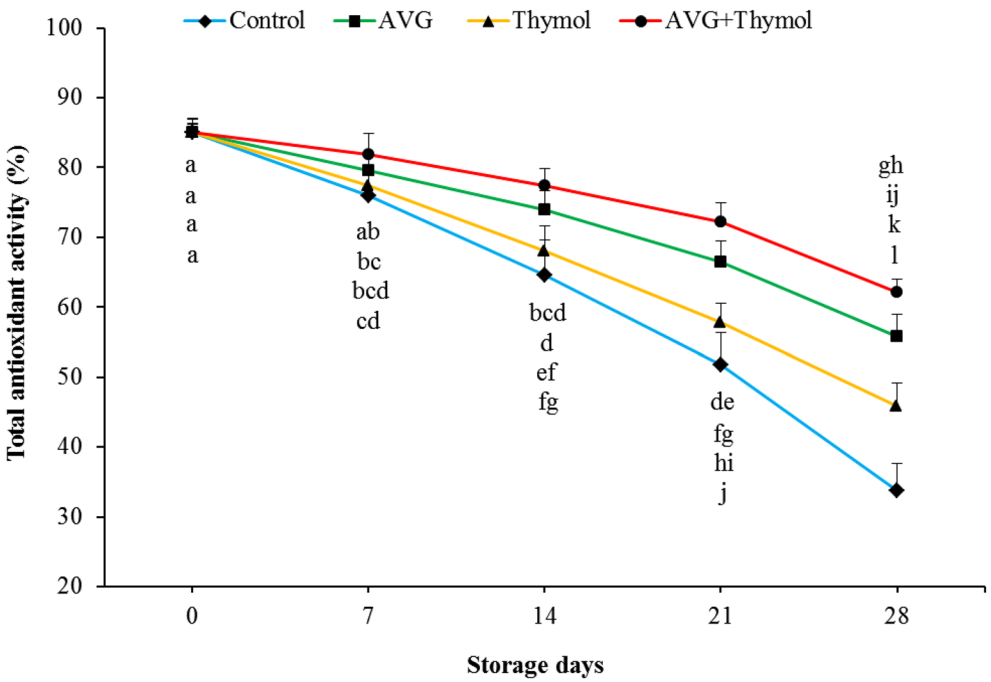


Fig. 4 Interaction of time and coating on the antioxidant activity of duke cherries during the storage. Mean values followed by the similar letters are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test). Vertical bars represent standard deviation (SD)

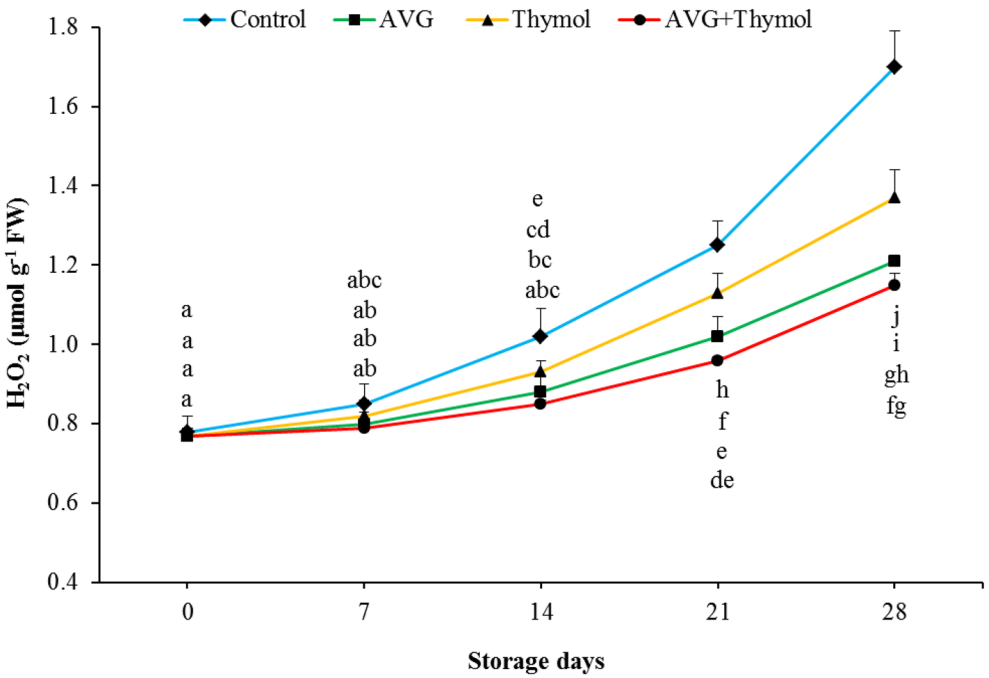


Fig. 5 The interaction effect of treatment and time on the H_2O_2 concentration of duke cherries during the storage. Mean values followed by the similar letters are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test). Vertical bars represent standard deviation (SD)

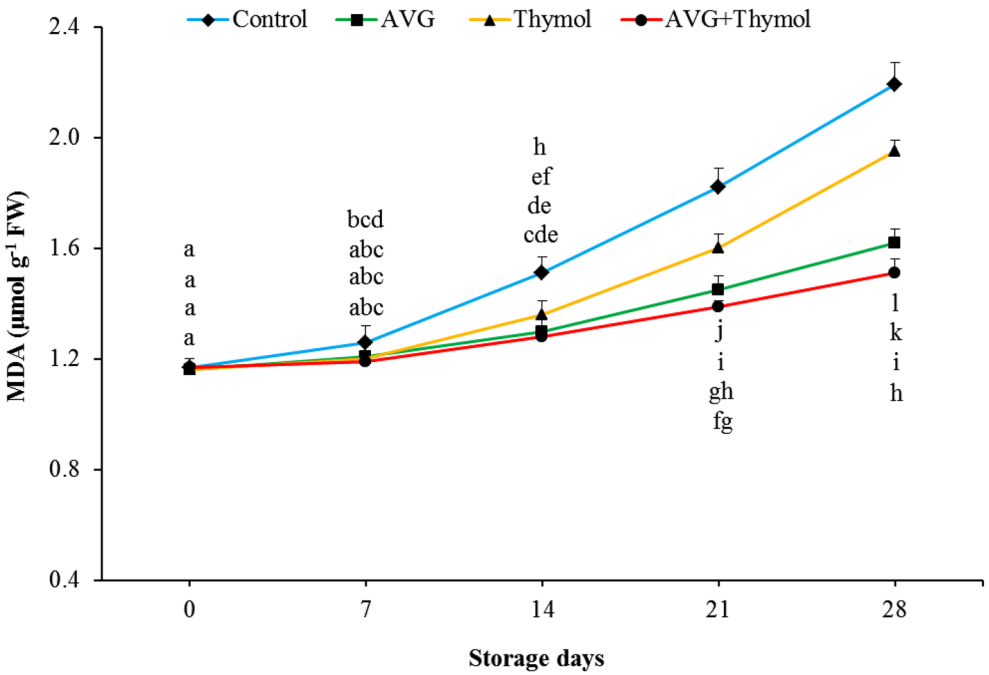


Fig. 6 The interaction effect of treatment and time on the MDA concentration of duke cherries during the storage. Mean values followed by the similar letters are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test). Vertical bars represent standard deviation (SD)

d of storage, duke cherries coated with AVG + thymol showed 9.99, 25.66, 32.20%, and 45.09% greater total phenolic content than uncoated ones, respectively (Fig. 3).

Phenolic compounds not only improve the quality and nutritional value of the cherries [47] but also play a major role in the defense mechanism against the invasion of plant pathogens [52]. Therefore, maintaining a high level of phenolic compounds in fruits during their storage life can be very beneficial. The findings of the current study are consistent with Petriccione et al. [53] who described that the total phenolic content of fruits was decreased during storage. On the contrary, the results of this study are dissimilar to reports that showed the total phenolic content of fruits increased during storage [12, 19, 54]. Furthermore, our findings are similar to Nourozi and Sayyari [19] for apricots and Hu et al. [38] for sweet cherries, who indicated total phenolic content of fruits treated with coatings, was greater than nontreated fruits during storage. The results of this work are also in agreement with those obtained by Gol et al. [51] who detected that total phenolic content was higher in coated fruit with a combination of chitosan and essential oils than in uncoated fruits. In contrast, Hassan et al. [12] reported that strawberries coated with AVG, lemongrass essential oil, and their combination had lower total phenolic content than uncoated fruits, which is contrary to the results of this research (Fig. 3).

Antioxidant activity

The antioxidant activity of duke cherries decreased with an extension in the storage time; however, coated fruits had greater antioxidant activity than uncoated fruits (Fig. 4). The combination of AVG and thymol was more effective than the application of AVG or thymol alone, although there were no statistically significant differences among edible coatings at day 7, and between AVG + thymol and AVG at day 14 (Fig. 4). On days 7, 14, 21, and 28, duke cherries coated with AVG + thymol had 7.90%, 20.03%, 39.57%, and 84.56% higher antioxidant activity than uncoated fruits, respectively (Fig. 4).

Decreases in the antioxidant activity of fruits during storage have been found in strawberry [12], table grape [24], and sweet cherry [53], which is consistent with the results of this work. Antioxidant activity can be associated with the contents of phenolic compounds and pigments such as anthocyanins. Degradation of anthocyanins and phenolic compounds results in a reduction of antioxidant activity [55]. In this regard, a continuous decrease in the level of anthocyanins and phenolic compounds of duke cherries during the storage period, as previously mentioned (Figs. 2 and 3) showed similar trends to the antioxidant activity of fruits (Fig. 4). Alternatively, the higher antioxidant activity with the application of AVG, essential oils, and their combination have been reported for strawberries [12], plum [17], and

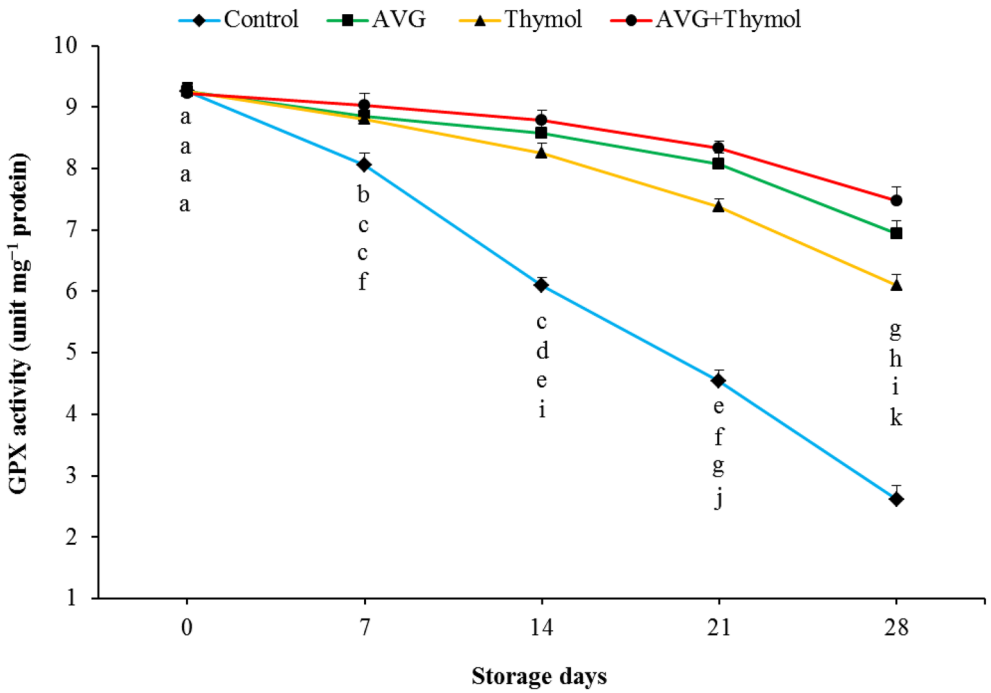


Fig. 7 The interaction effect of treatment and time on the GPX activity of duke cherries during the storage. Mean values followed by the similar letters are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test). Vertical bars represent standard deviation (SD)

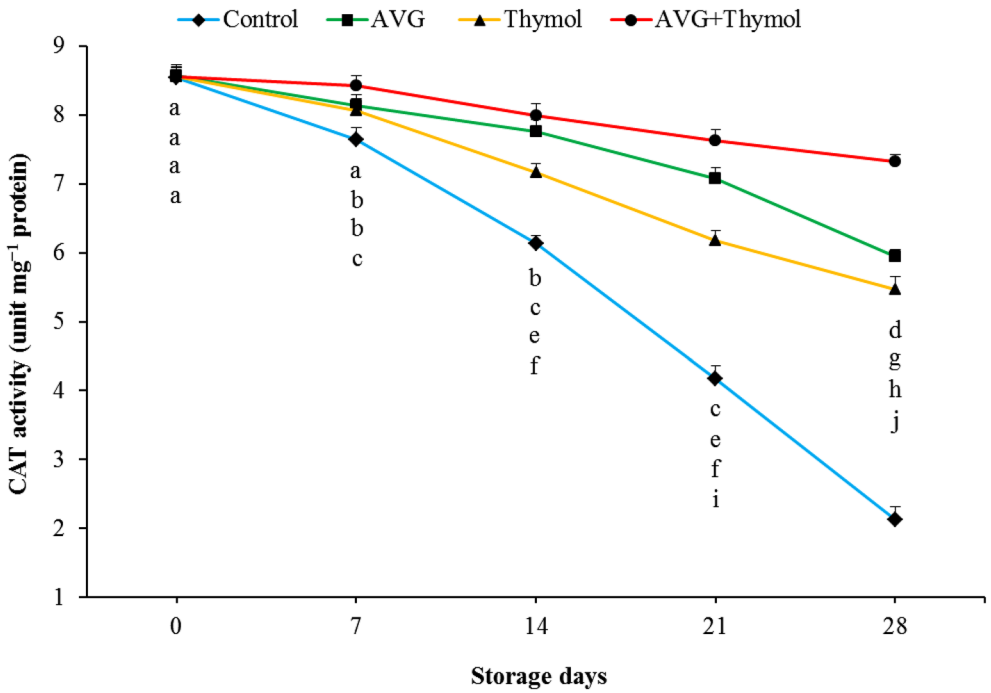


Fig. 8 The interaction effect of treatment and time on the CAT activity of duke cherries during the storage. Mean values followed by the similar letters are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test). Vertical bars represent standard deviation (SD)

avocados [44], which is in agreement with our findings. The positive impact of essential oils on the antioxidant activity of coated fruits during storage can be associated with the fact that most of them comprise relative amounts of antioxidant compounds [56]. In addition, one of the main constituents of AVG is aloe-emodin, which leads to increased antioxidant activity in coated fruits [57].

H_2O_2 and MDA concentration

H_2O_2 and MDA concentrations increased throughout the storage (Figs. 5 and 6), although this increase was less noticeable in coated duke cherries. The edible coatings significantly affected the H_2O_2 and MDA concentration of fruits on days 14, 21, and 28, whereas they had no significant effect on H_2O_2 and MDA concentration on day 7 (Figs. 5 and 6). The lowest H_2O_2 concentration was recorded in fruits coated with AVG + thymol; however, the effect of AVG + thymol was only statistically greater to the control treatment at day 14 and to the control and thymol treatments at days 21 and 28 (Fig. 5). In addition, duke cherries coated with AVG + thymol had the lowest MDA concentration, although there were no statistically significant differences among edible coatings at day 14, and between AVG + thymol and AVG at day 21 (Fig. 6).

The results of this research agree with reports that showed H_2O_2 and MDA concentrations of fruits increased during storage [24, 58]. Furthermore, our findings are consistent with ValizadehKaji et al. [24], who indicated H_2O_2 and MDA concentration of fruits treated with an edible coating, the essential oils, and their combination was significantly lower than nontreated controls during storage. The lower concentrations of H_2O_2 and MDA in duke cherries coated with AVG, thymol, and their combination, might be related to the high antioxidant activity [24] and the high activity of antioxidant enzymes [59], as documented in the present study (Figs. 4, 7 and 8).

GPX and CAT activity

Over the cold storage, the GPX and CAT activity of duke cherries decreased; however, coated fruits showed more GPX and CAT activity compared to uncoated ones, and the maximum values were related to AVG + thymol which was significantly greater than the other treatments (Figs. 7 and 8). GPX activity of fruits under AVG + thymol treatment was 12.03%, 43.93%, 83.88% and 185.11%

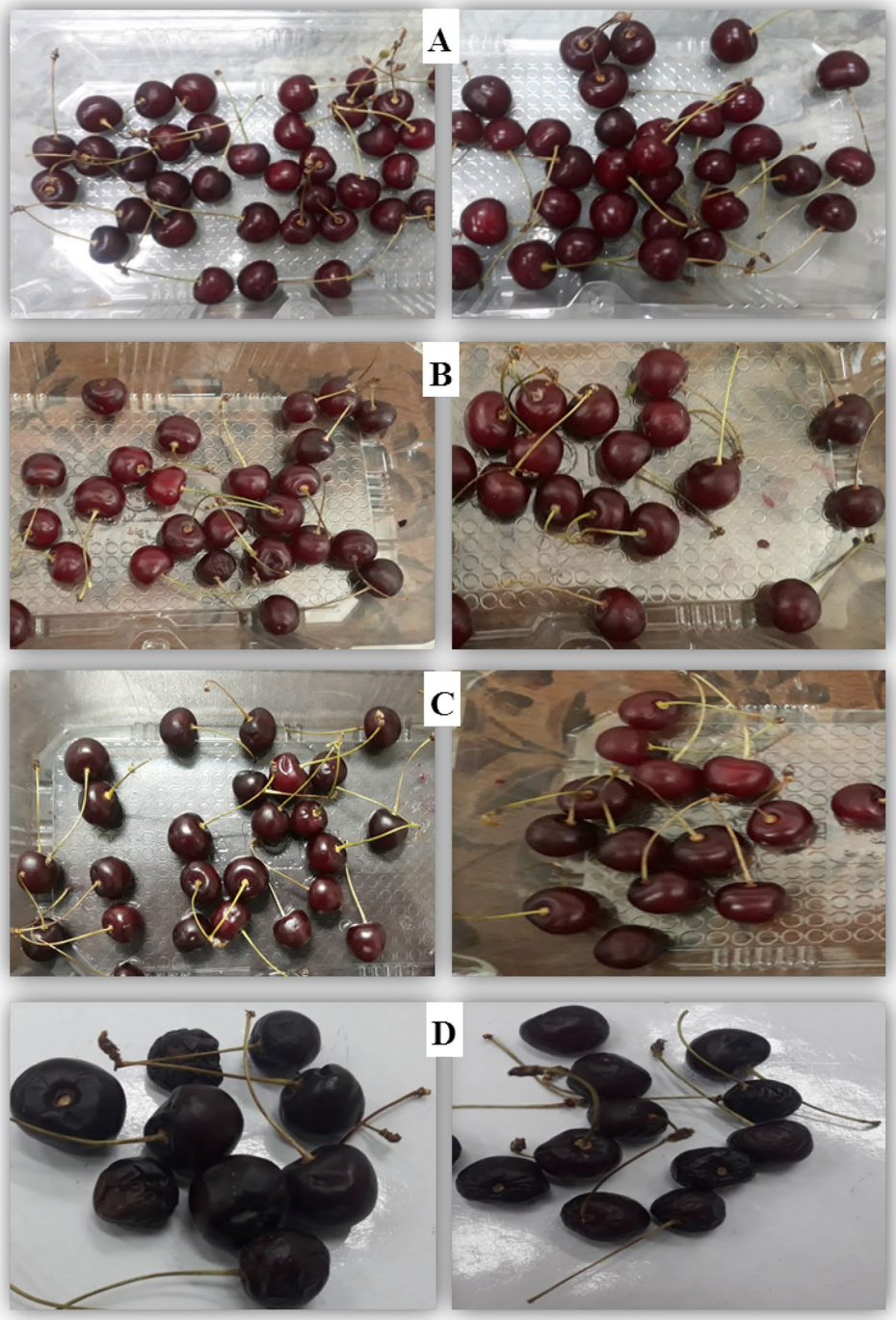


Fig. 9 Duke cherries after 28 days of storage at 5 °C. **A** the coated fruits with AVG + thymol, **B** the coated fruits with AVG, **C** the coated fruits with thymol, **D** uncoated control fruits

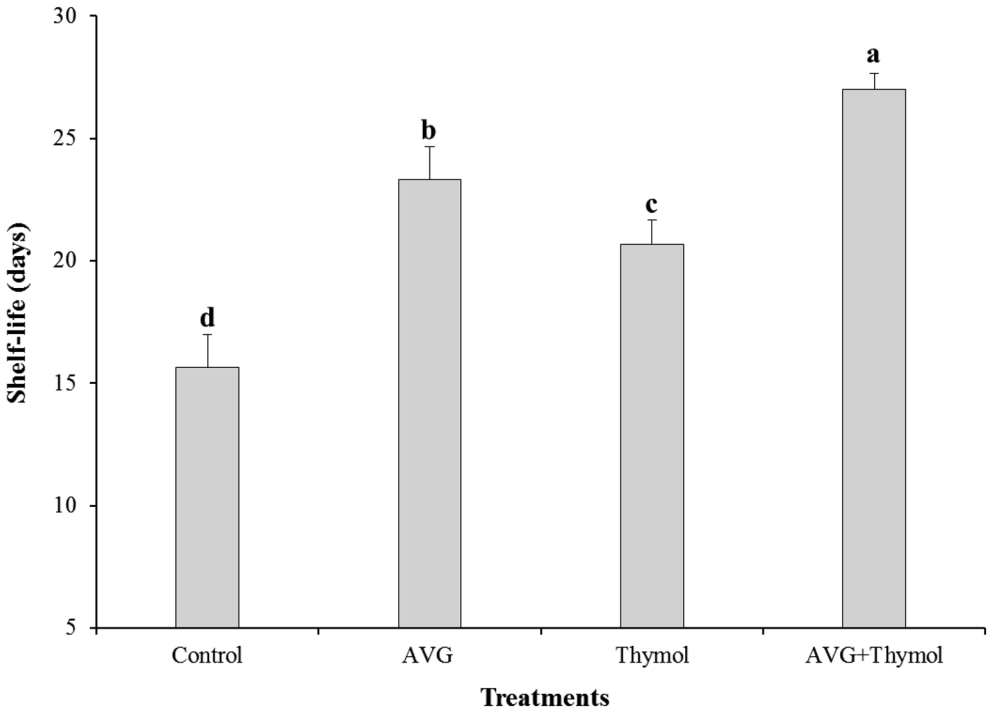


Fig. 10 Influence of the edible coatings on the shelf-life of duke cherries stored at 5 °C. Mean values followed by the similar letters are not significantly different from each other at $P \leq 0.05$ (Duncan's multiple range test). Vertical bars represent standard deviation (SD)

greater than controls after 7, 14, 21, and 28 d of the storage, respectively (Fig. 7). In addition, fruits coated with AVG + thymol had 10.20%, 30.34%, 82.97% and 143.66% higher CAT activity than uncoated ones at days 7, 14, 21, and 28, respectively (Fig. 8).

The results of the current study are consistent with those obtained by Shehata et al. [37] but contrary to the results of Ghorbani et al. [59], who stated that the antioxidant enzyme activity of fruits increased during storage. Moreover, the findings of this work support those achieved by ValizadehKaji et al. [24] and Bill et al. [44], who described that the activity of antioxidant enzymes in fruits coated with edible coatings, essential oils, and their combination was greater than in nontreated fruits during storage. The production of reactive oxygen species in fruits increases during storage [60]. Antioxidant enzymes play a role in keeping plant cells from oxidative damage by reactive oxygen species. Since the increase in the activity of antioxidant enzymes leads to an increase in the tissue's ability to remove H_2O_2 and MDA, the levels of H_2O_2 and MDA were lower in the duke cherries (Figs. 5 and 6).

Sensory attributes evaluation

During the cold storage period, the sensory attributes of duke cherries decreased; with better sensory scores in coated compared with uncoated fruits (Table 2; Fig. 9). The edible coatings had no statistically significant effect on the sensory attributes of duke cherries at days 7 and 14, but after that, coated fruits had significantly higher sensory scores than nontreated ones (Table 2). Application of AVG + thymol was more effective in maintaining sensory attributes of duke cherries than AVG and thymol alone, although AVG + thymol and AVG were not statistically different at day 21 (Table 2). By the end of storage, duke cherries coated with thymol, AVG, and AVG + thymol had 28.75%, 57.08%, and 100.00% higher sensory scores than nontreated fruits, respectively (Table 2). Similar findings on the positive effects of edible coatings, essential oils, and their combination on sensory attributes of fruits during storage have been described for tomato [11], fig [27], and stone fruits [38].

Storage life

The edible coatings had a statistically significant effect on the storage life of duke cherries (Fig. 10). Fruits coated with AVG + thymol showed the highest storage life (27.00 d), which was significantly greater than the other treatments, whereas uncoated fruits had the lowest storage life (15.66 d) (Fig. 10). The higher storage life with the application of edible coatings, essential oils, and their combination have been reported for strawberry [12],

sweet cherry [26], and fig [27], which is in agreement with our findings.

Conclusion

Duke cherries coated with AVG, thymol, and particularly AVG + thymol, showed lower values of weight loss, respiration rate, stem browning, fungal infection, and TSS/TA, as well as H_2O_2 and MDA, whereas they had higher values of firmness, anthocyanin, total phenol, antioxidant activity, antioxidant enzyme activity, and sensory scores during cold storage. Therefore, applying AVG, thymol, and particularly AVG + thymol, for prolonging the shelf storage of duke cherries has revealed great practical potential. This combined treatment has various advantages including natural, edible, and efficient. Despite considerable findings of the present research, further work is needed to be done on a combination of other coatings and essential oils to preserve the quality of duke cherries. In addition, combined treatments must be tested for infected fruits to provide an effective decay control measure to the organic duke cherry industry.

Abbreviations

TSS	Total soluble solids
TA	Titratable acidity
H_2O_2	Hydrogen peroxide
MDA	Malondialdehyde
GPX	Guaiacol peroxidase
CAT	Catalase

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Author contributions

BV designed the experiment. NF performed the experiments. BV and NF conducted the laboratory measurements. BV analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

All authors contributed in design and preparation of the research, and they have read the final version of the manuscript.

Consent for publication

We declare our agreement.

Competing interests

The authors declare that they have no competing interests.

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