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Synergistic effects of modified atmosphere packaging and cinnamaldehyde on bioactive compounds, aerobic mesophilic and psychrophilic bacteria of pomegranate arils during cold storage

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Abstract

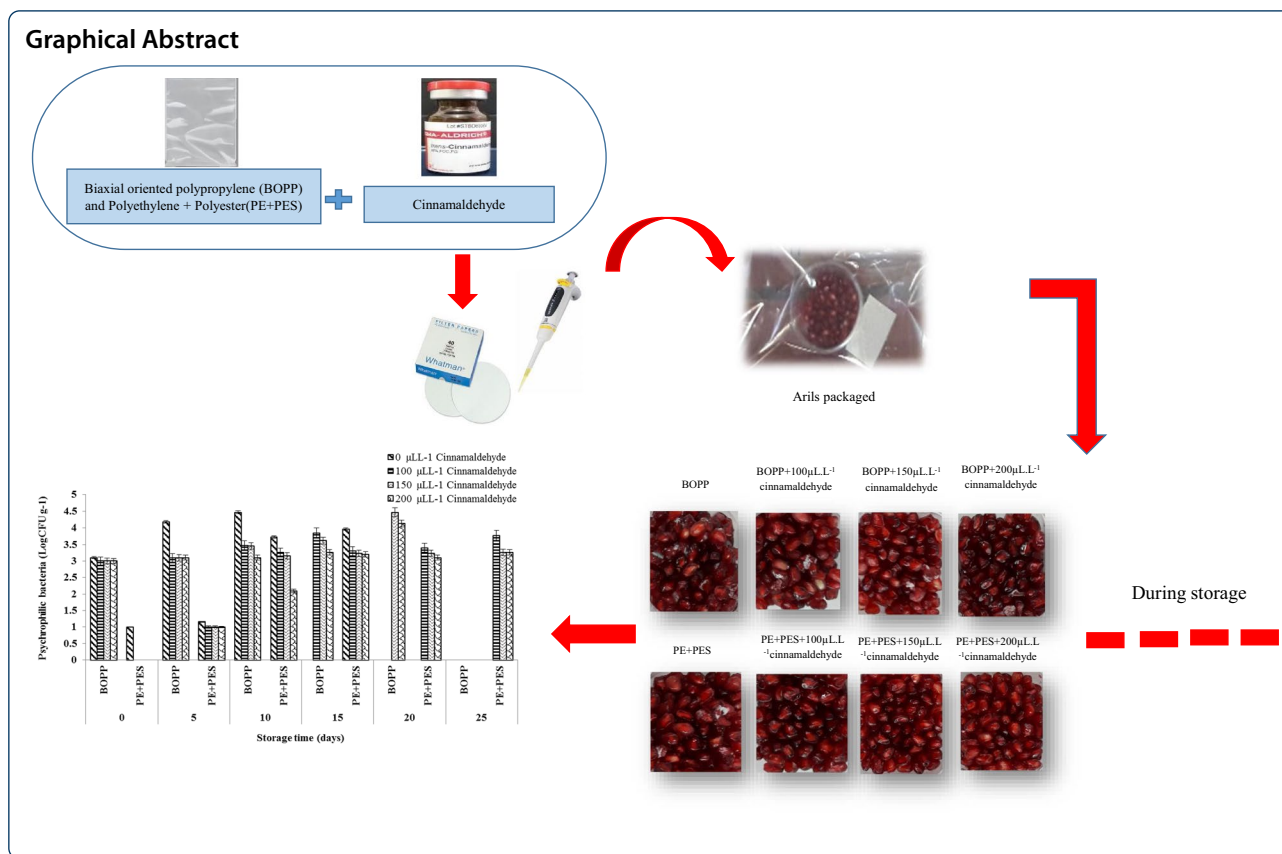
Background: Ready-to-eat pomegranate arils are very perishable. In this research, the effect of packaging with two kinds of films fumigated with cinnamaldehyde (0, 100, 150, and 200 $\mu\text{L L}^{-1}$), evaluated on bioactive compounds and microbial contamination of pomegranate arils during cold storage.

Results: Polyethylene + polyester (PE + PES) film containing cinnamaldehyde, preserved lightness (L^*), and chroma index (C^*) as compared with biaxial-oriented polypropylene (BOPP) film containing cinnamaldehyde. Anthocyanin content and phenolic compounds decreased during storage. PE + PES film containing cinnamaldehyde caused a significant delay in decreasing the trend of total antioxidant activity (TAA) during storage. The lowest number of aerobic mesophilic bacteria and psychrophilic bacteria were related to PE + PES film containing cinnamaldehyde.

Conclusions: Packaging with PE + PES film containing 200 $\mu\text{L L}^{-1}$ cinnamaldehyde was the best treatment for preservation of bioactive compounds and extending the shelf life of pomegranate arils up to 25 days. This new packaging technique is promising for the preservation of pomegranate ready-to-use arils.

Keywords: Antioxidant activity, Ascorbic acid, Biaxial-oriented polypropylene, Polyethylene, Polyester, Psychrophilic bacteria

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Introduction

Pomegranate is a nutritious fruit that is a favorite of many people worldwide. Recently, pomegranate arils are supplied instead of whole fruit due to fruit peeling problems. Pomegranate juice contains a considerable amount of sugars, organic acids, phenolic compounds, anthocyanins, amino acids, ascorbic acid (AA), and minerals [1]. Due to new findings of the effect of pomegranate arils on human health, it has received great popularity and its global production is increasing [2].

On the other hand, consumers' demand for fresh, healthy, and ready-to-eat crops resulted in increased industrial production of minimally processed fruits and vegetables. Minimally processing and packaging of fruit is an economical method to change fruit into 100% usable product. Minimally processing pomegranate is a beneficial technique to increase pomegranate consumption [3]. Ready-to-eat arils are sensitive to enzymatic browning, contamination by microorganisms, and loss of nutritional value [4]. Microorganisms limit post-harvest life. Microorganisms based on the temperature requirement for optimal growth include psychrophilic with an optimum growth temperature of 7 °C, mesophilic with an optimum growth temperature of 20–25 °C, and thermophilic with an optimum growth temperature above 30 °C

[5]. Mesophilic and psychrophilic bacteria are effective microorganisms after harvesting fresh-cut crops. Packaging protects arils against mechanical injury, microbial spoilage, and also improves organoleptic quality and marketability. Polymer films by creating a modified atmosphere, increase the shelf life of products. Atmospheric composition inside the packaging is influenced by the polymer film permeability and respiration of the product.

The most important films used in the packaging of fresh products are polyethylene (PE), polypropylene (PP), and polyvinyl chloride (PVC) [6]. It has been reported that PE and cellophane have a significant influence on the flavor and visual quality of blood orange fruit [7]. Packaging of fresh-cut apple cv. Fuji in PVC and nanofilm preserved the amount of soluble solids content (SSC) and titratable acidity (TA), reduced decay, and inhibited polyphenol oxidase (PPO) and peroxidase (POD) enzymes activity [8]. Pomegranate arils cv. Mridula packed in PP film had a lower rate of respiration, changing of color, and total sugars as compared with low-density PE film during cold storage [9].

Packaging of pomegranate arils is also effective for protecting against external pollution. Nevertheless, high humidity in the pack may increase fungal contamination

and moisture accumulation on the surface film may have an adverse effect on the permeability properties of the film and may create an undesirable atmosphere. On the other hand, the tendency to use “green” processed products forces post-harvest physiologists to find Generally Recognized as Safe (GRAS) products. So, the preparation of natural antimicrobial compounds from plant extracts has increased recently in order to improve the quality and safety of agricultural and industrial products [10].

Cinnamon in Asian countries is used traditionally and has biological activities such as antifungal, anti-insects, and antioxidant properties. Cinnamaldehyde is an aldehyde aromatic compound and the main part of cinnamon peel extract (about 65%). Also, it has an antimicrobial effect against many organisms and does not require direct contact for antimicrobial activity [11]. It has been classified as a GRAS compound by USA’s Food and Drug Administration (FDA) and has been confirmed to use in food products [12].

Effects of biocontrol of cinnamon extract on inhibition of post-harvest spoilage fungi have been proven [13]. Phenolic compounds are the main antioxidants in pomegranate, which determine its antioxidant activity. Essential oil components have the ability to increase the level of antioxidants (polyphenols, flavonoids, anthocyanins) in plant tissues which increases the oxygen uptake and hydroxyl radical scavenging capacity of tissues [14]. In this research, we hypothesized that the integration of cinnamaldehyde as an antimicrobial agent with modified atmosphere packaging (MAP) may prevent the growth of microorganisms synergistically and increase the shelf life of pomegranate cv. Rabbab which is one of the commercially important Iranian pomegranate cultivars. This cultivar is late ripening, medium to large size with thick red rind and red arils. In this cultivar, the size of arils is small and the fruit calyx length is short. Fruit has a thick peel. This cultivar is suitable for long time cold storage and export [15]. The purpose of this research was to investigate the effects of passive MAP in combination with cinnamaldehyde fumigation on bioactive compounds and the shelf life of pomegranate arils.

Materials and methods

Chemicals

Cinnamaldehyde (purity $\geq 99\%$, CAS number 104-55-2) was purchased from Sigma-Aldrich Company.

Fruit material, treatment, and storage condition

Pomegranate cv. ‘Rabbab’ were harvested at the commercial maturity stage and transported to the laboratory by ventilated car (~ 100 km). The fruits were selected based on uniformity in size, shape, and color before treatment. Then, they were disinfected with 1% sodium hypochlorite

for 5 min and washed with distilled water. Pomegranate arils were separated from the peel by hand and blended. In each test unit, 50 g of arils was considered. Different concentrations of cinnamaldehyde including 0, 100, 150, and 200 $\mu\text{L L}^{-1}$ were used as fumigation on a sterile gauze [16]. Arils packaged with two kinds of BOPP films with 150×250 mm dimensions, 40 μm thickness and PE + PES with 150×250 mm dimensions, 90 μm thickness (Table 1). Then samples were stored at 5 ± 1 °C and $92 \pm 3\%$ RH for 25 days. Sampling and measurement of parameters were performed every 5 days.

Measurement of color characteristics

The external color was determined by measuring L^* (lightness), a^* (greenness to redness), and b^* (blueness to yellowness (with Minolta Chroma-meter (CR-400, Japan) and converted to chroma (C^*) which represents the intensity or saturation of the color and Hue angle (h°) which indicates the type of color by Eqs. (1) and (2), respectively [17]:

$$C^* = \left[(a^*)^2 + (b^*)^2 \right]^{1/2}, \quad (1)$$

$$h^* = \tan^{-1} (b^*/a^*). \quad (2)$$

SSC, pH, and TA

SSC was measured with a digital refractometer (MA871, Hungary) at 20 ± 1 °C which was calibrated with distilled water, and results were expressed as percent (%). pH of the fruit juice was measured using a digital pH meter (3510, England). For measurement of TA, 3 mL of fruit juice was titrated with NaOH (0.1 N) to pH 8.2 [18]. The results were stated as a percentage of citric acid using Eq. (3):

$$\%TA(\text{wt/vol}) = (N \times V_1 \times \text{Eq.wt}/V_2 \times 1000) \times 100, \quad (3)$$

where V_1 and V_2 are used NaOH volume and sample volume, respectively, based on the weight equivalent of predominant acid (citric acid: $\text{C}_6\text{H}_8\text{O}_7 = 192.124$ g mol $^{-1}$).

Table 1 Permeability properties of two types of film polymers

Polymer	Oxygen transmission (g m $^{-2}$ day $^{-1}$ bar $^{-1}$)	Carbon dioxide transmission (g m $^{-2}$ day $^{-1}$ bar $^{-1}$)	Water vapor transmission (g m $^{-2}$ day $^{-1}$ bar $^{-1}$)
BOPP	500	350	900
PE + PES	60–70	45–50	45

BOPP biaxial-oriented polypropylene, PE + PES polyethylene + polyester

Total phenols content (TPC) evaluation

TPC was measured by Folin–Ciocalteu reagent [19]. Briefly, 60 μL of extract was mixed with 900 μL of 2% sodium carbonate. After 3 min incubation at room temperature, 180 μL of 50% Folin–Ciocalteu reagent was added, and the mixture was incubated for 30 min at the same condition. The absorbance was recorded at 750 nm using a microplate spectrophotometer (Epoch Biotech, Germany). The concentration of TPC was expressed as gallic acid equivalent (GAE) per L of fruit extract.

Total anthocyanins content (TAC) evaluation

Anthocyanin concentration was determined based on pH differential method as described by Lako et al. [20] with some modifications. 0.05 mL of aril juice of each treatment was mixed with potassium chloride buffer with pH 1.0 (0.025 M) and sodium acetate buffer with pH 4.5 (0.4 M), separately. The absorbance of the resulting mixtures was measured at 510 and 700 nm by a microplate spectrophotometer (Epoch Biotech, Germany). The results were expressed as mg cyanidin-3-glucoside per L of juice. To determine TAC, the absorbance (A) was first calculated by Eq. (4) [20]:

$$A = (A_{510} - A_{700})pH_{1.0} - (A_{510} - A_{700})pH_{4.5} \quad (4)$$

TAC based on the concentration of cyanidin-3-glucoside was calculated by Eq. (5) [20]:

$$\text{TAC}(\text{mg L}^{-1}) = A \times \text{MW} \times \text{DF} \times 1000/\epsilon, \quad (5)$$

where A is the absorbance, MW is the molecular weight of cyanidin-3-glucoside (449.2), DF is the dilution factor [5] and ϵ is the molar absorptive coefficient of cyanidin-3-glucoside (26,900).

Total antioxidant activity (TAA) evaluation

The antioxidant activity of each extract was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [21]. 100 μL of the extract was incorporated with 1 mL of DPPH (0.1 mM) and incubated at room temperature for 30 min. Then, the absorbance was measured using a microplate spectrophotometer (Epoch Biotech, Germany) at 517 nm. TAA was evaluated by Eq. (6):

$$\% \text{TAA} = \left[\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right] \times 100. \quad (6)$$

Ascorbic acid

10 mL of 1% metaphosphoric acid was blended with 100 μL of fruit extract, and then 1 mL of the mixture was added to 9 mL of 50 μM 2,6-dichlorophenol indophenols

and was vortexed for 5 s. The absorbance was measured using the spectrophotometer (Spectronic 20D, USA) at 515 nm [22].

Determination of microbial contamination

10 g of pomegranate arils was mixed with 90 ml of NaCl solution and homogenized by stomacher for 1 min. Dilutions (0.1, 0.01, 0.001) were prepared by NaCl solution. Microbial culture as pour plate was performed in plate count agar medium (PCA) for aerobic mesophilic and psychrophilic bacteria. All steps were performed under sterile conditions. The incubation condition for aerobic mesophilic bacteria was 37 ± 1 °C for 48 h, and 6.5 ± 1 °C during 5–7 days for psychrophilic bacteria [23]. The number of microbial colonies was calculated based on log of the number of colonies per gram of pomegranate aril.

Statistical analysis

All experiments were repeated in duplicate. The experimental data were analyzed according to a three-factor factorial design, including different polymer films, concentrations of essential oils, and storage period based on a completely randomized design (CRD) with three replicates. Data were subjected to the analysis of variance (ANOVA) using SAS software ver. 9.4 (Statistical Analysis System, SAS Institute Inc. 1985). The means were evaluated using Duncan's multiple range tests to analyze the difference between treatments and intervals at a 99% confidence level of each variable.

Results

Results of variance analysis showed the significant effect of treatments on L^* value, chroma index, and hue angle (except interaction effects of polymer film and storage time on chroma) at 1% level of probability. The amount of L^* and chroma index showed a decreasing trend during storage. The most amounts of L^* value (17.77) and chroma index (23.40) were found in arils packaged with PE + PES film containing 200 $\mu\text{L L}^{-1}$ cinnamaldehyde at the first day of storage with significant ($P \leq 0.01$) differences with the other treatments at the same time. The least amount of L^* value (3.96) and least amount of chroma index (3.93) were found in BOPP film without cinnamaldehyde on the tenth day after treatment; however, it was not significantly ($P \leq 0.01$) different with BOPP film containing 100 $\mu\text{L L}^{-1}$ cinnamaldehyde and PE + PES film without cinnamaldehyde at the same time. The most amount of hue angle (59.41) was recorded in BOPP film without cinnamaldehyde on the tenth day of storage with significant ($P \leq 0.01$) differences with the other treatments at the same time. The least amount of hue angle (27.10) was recorded in PE + PES film

containing 200 $\mu\text{L L}^{-1}$ cinnamaldehyde on the first day of storage, however, it was not significant ($P \leq 0.01$) different from the other treatments at the same time. The differences between the maximum and minimum amount of L^* value, chroma index, and hue angle treatments during storage were 13.81, 19.47, and 32.31, respectively (Tables 2, 3 and 4).

The quality standard for the entry of fruit into the market is the ratio of SSC to TA [24]. Results of variance analysis showed that the main effect, interaction effects of 2- and 3-fold treatments were significant on the amounts of SSC ($P \leq 0.01$). The changes in SSC showed increasing trend during storage. The most amount of SSC (18.5%) was recorded in arils packaged with BOPP film without cinnamaldehyde at the tenth day of storage with significant ($P < 0.01$) differences with the other treatments at the same time and the least amount (%16.58) was found in arils packaged with PE + PES film containing 200 $\mu\text{L L}^{-1}$ cinnamaldehyde on the first day after treatment with significant ($P < 0.01$) differences with the other treatments at the same time. The difference between the maximum and minimum amount of SSC was 1.92% during storage (Table 5).

TA is related to the concentration of fruits' dominant organic acids. The results of variance analysis showed that the main effect, interaction effects of 2- and 3-fold treatments on TA and pH were significant ($P \leq 0.01$). Changes in TA showed decreasing trend during storage. The most amount of TA (1.38%) and the least amount of pH (3.08) were found in arils packaged with

Table 2 Mean comparison interaction effects of the polymer film (BOPP: biaxial-oriented polypropylene and PE + PES: polyethylene + polyester), cinnamaldehyde and storage time on L^* value of pomegranate arils

Storage time	Polymer film	Cinnamaldehyde			
		0	100	150	200
0	BOPP	14.95fg	15.57e	15.97d	16.27c
	PE + PES	15.17f	16.53c	16.85b	17.77a
5	BOPP	8.20w	8.67uv	11.38k	13.1j
	PE + PES	8.47vw	14.08i	14.59h	14.75gh
10	BOPP	3.96z	6.83yz	9.71op	9.90o
	PE + PES	9.40z	10.22n	10.61m	11.04l
15	BOPP	–	4.60z	8.86tu	9.04st
	PE + PES	4.43z	9.19rs	9.36qr	9.55pq
20	BOPP	–	–	4.74z	5.10z
	PE + PES	–	7.14y	7.36xy	7.54x
25	BOPP	–	–	–	–
	PE + PES	–	5.55z	5.92z	6.13z

Means followed by similar letters are not significantly different according to Duncan's multiple range test ($P \leq 0.01$)

Table 3 Mean comparison interaction effects of the polymer film (BOPP: biaxial-oriented polypropylene and PE + PES: polyethylene + polyester), cinnamaldehyde and storage time on chroma index of pomegranate arils

Storage time	Polymer film	Cinnamaldehyde			
		0	100	150	200
0	BOPP	15.62e-h	16.35def	17.03cde	17.74bcd
	PE + PES	15.91gef	18.38bc	19.08b	23.40a
5	BOPP	9.14w-z	10.34u-x	14.02i-m	14.29h-l
	PE + PES	9.52w-z	14.58g-k	14.98f-j	15.31f-i
10	BOPP	3.93z	8.81z	12.88l-r	13.13k-q
	PE + PES	8.51z	13.3k-p	13.56j-o	13.80i-n
15	BOPP	–	7.25z	11.62q-v	11.83p-u
	PE + PES	5.80z	12.16o-t	12.40n-t	12.64m-s
20	BOPP	–	–	10.20v-y	10.50u-x
	PE + PES	–	10.89t-w	11.22s-v	11.46r-v
25	BOPP	–	–	–	–
	PE + PES	–	7.49z	7.93z	8.27z

Means followed by similar letters are not significantly different according to Duncan's multiple range test ($P \leq 0.01$)

PE + PES film containing 200 $\mu\text{L L}^{-1}$ cinnamaldehyde on the first day of storage with significant ($P \leq 0.01$) differences with the other treatments. The least amount of TA (1.06%) and the most amount of pH (3.41) were found in arils packaged in BOPP film without cinnamaldehyde on the tenth day of storage with significant ($P \leq 0.01$) differences with the other treatments at the

Table 4 Mean comparison interaction effects of the polymer film (BOPP: biaxial-oriented polypropylene and PE + PES: polyethylene + polyester), cinnamaldehyde and storage time on hue angle of pomegranate arils

Storage time	Polymer film	Cinnamaldehyde			
		0	100	150	200
0	BOPP	32.95xyz	32.19xyz	31.85xz	31.33xz
	PE + PES	32.61xyz	30.62xz	30.03xz	27.32z
5	BOPP	40.39lm	39.36 mn	34.87wxy	34.50xyz
	PE + PES	39.95lm	34.05xyz	33.61xyz	33.26xyz
10	BOPP	59.41a	47.72e	36.85rst	36.38stu
	PE + PES	46.49f	36.03tuv	35.50uvx	35.14vwx
15	BOPP	–	54.53b	38.81no	38.31op
	PE + PES	58.65a	38.01opq	37.61pqr	37.21qrs
20	BOPP	–	–	49.00d	50.91c
	PE + PES	–	41.85ij	41.31jk	40.81kl
25	BOPP	–	–	–	–
	PE + PES	–	44.93g	43.72h	42.69i

Table 5 Mean comparison interaction effects of the polymer film (BOPP: biaxial-oriented polypropylene and PE+PES: polyethylene + polyester), cinnamaldehyde and storage time on soluble solids content (%) of pomegranate arils

Storage time	Polymer film	Cinnamaldehyde			
		0	100	150	200
0	BOPP	16.92o	16.83p	16.80p	16.80p
	PE+PES	16.90o	16.73q	16.67r	16.58s
5	BOPP	17.30j	17.30j	17.05mn	17n
	PE+PES	17.30j	17n	17n	17n
10	BOPP	18.15a	17.67e	17.20k	17.17kl
	PE+PES	17.73d	17.10lm	17.10lm	17.10lm
15	BOPP	–	17.82c	17.30j	17.30j
	PE+PES	17.93b	17.20k	17.20k	17.20k
20	BOPP	–	–	17.60f	17.60g
	PE+PES	–	17.53i	17.40i	17.40i
25	BOPP	–	–	–	–
	PE+PES	–	17.50gh	17.50gh	17.45hi

Table 6 Mean comparison interaction effects of the polymer film (BOPP: biaxial-oriented polypropylene and PE+PES: polyethylene + polyester), cinnamaldehyde and storage time on titratable acidity (%) of pomegranate arils

Storage time	Polymer film	Cinnamaldehyde			
		0	100	150	200
0	BOPP	1.3d	1.30d	1.32c	1.32c
	PE+PES	1.32c	1.34b	1.34b	1.38a
5	BOPP	1.17j	1.17j	1.24f	1.27e
	PE+PES	1.17j	1.28e	1.28e	1.30d
10	BOPP	1.06p	1.10n	1.21h	1.21h
	PE+PES	1.08o	1.22g	1.23fg	1.23fg
15	BOPP	–	1.08o	1.17j	1.19i
	PE+PES	1.08o	1.19i	1.19i	1.21h
20	BOPP	–	–	1.21n	1.10n
	PE+PES	–	1.10l	1.14k	1.15j
25	BOPP	–	–	–	–
	PE+PES	–	1.11m	1.13l	1.13l

same time. During storage, the differences between the maximum and minimum amount of TA and pH were 0.32% and 0.33, respectively (Tables 6 and 7).

Results of variance analysis showed that the main effect, interaction effects of 2- and 3-fold treatments on total phenols were significant ($P \leq 0.01$). Changes in total phenols showed a decreasing trend during storage. The most content of total phenols (8332.83 mg GAE L⁻¹) was found in arils packaged in PE+PES film containing 200 $\mu\text{L L}^{-1}$ cinnamaldehyde at the first day of storage, however, it was not significantly ($P \leq 0.01$) different with

Table 7 Mean comparison interaction effects of the polymer film (BOPP: biaxial-oriented polypropylene and PE+PES: polyethylene + polyester), cinnamaldehyde and storage time on pH of pomegranate arils

Storage time	Polymer film	Cinnamaldehyde			
		0	100	150	200
0	BOPP	3.20qr	3.19rs	3.18s	3.18st
	PE+PES	3.19rs	3.16tu	3.15u	3.08v
5	BOPP	3.29hi	3.29hi	3.23no	3.22nop
	PE+PES	3.29hi	3.22opq	3.21pq	3.20qr
10	BOPP	3.41a	3.34bcd	3.26kl	3.25lm
	PE+PES	3.34bc	3.25lm	3.25lm	3.24mn
15	BOPP	–	3.35b	3.32ef	3.31fg
	PE+PES	3.36b	3.28ijk	3.27jk	3.27jkl
20	BOPP	–	–	3.33cde	3.32def
	PE+PES	–	3.28ij	3.28ij	3.28ij
25	BOPP	–	–	–	–
	PE+PES	–	3.30gh	3.30gh	3.30fgh

PE+PES film containing 100 and 150 $\mu\text{L L}^{-1}$ cinnamaldehyde at the same time. The least amount (4948.66 mg GAE L⁻¹) was found in BOPP film without cinnamaldehyde on the tenth day after treatment; however, it was not significantly ($P \leq 0.01$) different with BOPP film containing 100 $\mu\text{L L}^{-1}$ cinnamaldehyde at the same time. During storage, the difference between the maximum and minimum content of total phenols was 3384.17 mg GAE L⁻¹ (Table 8).

Results of variance analysis showed that the main effect, interaction effects of 2- and 3-fold treatments on content of anthocyanins were significant ($P \leq 0.01$). Changes in anthocyanins showed a decreasing trend during storage. The most content of anthocyanins (176.72 mg L⁻¹) was found in arils packaged in PE+PES film containing 200 $\mu\text{L L}^{-1}$ cinnamaldehyde at the first day of storage, however, it was not significantly ($P \leq 0.01$) different with PE+PES film containing 100 and 150 $\mu\text{L L}^{-1}$ cinnamaldehyde and BOPP film containing 150 and 200 $\mu\text{L L}^{-1}$ cinnamaldehyde at the same time. The least amount (118.93 mg L⁻¹) was found in BOPP film without cinnamaldehyde on the tenth day after treatment with significant ($P \leq 0.01$) differences with the other treatments at the same time. During storage, the difference between the maximum and minimum content of total anthocyanins was 57.79 mg L⁻¹ (Table 9).

Results of variance analysis showed that the main effect, interaction effects of 2- and 3-fold treatments were significant ($P \leq 0.01$) on TAA. Changes of TAA showed a decreasing trend during storage. The most amount of TAA (74.5%) was found in arils packaged in PE+PES film containing 200 $\mu\text{L L}^{-1}$ cinnamaldehyde at the first

Table 8 Mean comparison interaction effects of the polymer film (BOPP: biaxial-oriented polypropylene and PE + PES: polyethylene + polyester), cinnamaldehyde and storage time on total phenols content (mg GAE L⁻¹) of pomegranate arils

Storage time	Polymer film	Cinnamaldehyde			
		0	100	150	200
0	BOPP	7998.16qrs	8053.66qr	8102.16g	8135.66mn
	PE + PES	8049.33cd	8162bcd	8244bcd	8332.83abc
5	BOPP	5791uv	5924.66uv	7252.16ab	7391.16a
	PE + PES	5883uv	7597mn	7746.83gh	7901mno
10	BOPP	4948.66f	5378.66ef	6478.66de	6667.66v
	PE + PES	5310.66k	6796.66j	6989.83rst	7170.33j
15	BOPP	–	5066i	5418.83hi	5527.50uv
	PE + PES	5043.83pq	5987.66uv	6080lm	6252.33l
20	BOPP	–	–	5113.33tuv	5150.66tuv
	PE + PES	–	5551pq	5692.83op	5790uv
25	BOPP	–	–	–	–
	PE + PES	–	5175.83stu	5244rtu	5289.83rst

Table 9 Mean comparison interaction effects of the polymer film (BOPP: biaxial-oriented polypropylene and PE + PES: polyethylene + polyester), cinnamaldehyde and storage time on total anthocyanins content (mg L⁻¹) of pomegranate arils

Storage time	Polymer film	Cinnamaldehyde			
		0	100	150	200
0	BOPP	171.81de	173.39bcd	174.41abc	174.94abc
	PE + PES	173.01cd	175.50ab	175.91a	176.72a
5	BOPP	145.57pq	147.62op	160.24i	162.65h
	PE + PES	146.60p	164.96g	168.15f	169.82ef
10	BOPP	118.93yz	131.67w	153.75l	156.08k
	PE + PES	129.16x	157.16jk	157.89jk	158.98ij
15	BOPP	–	123.02z	132.84vw	134.91uv
	PE + PES	120.81yz	149.42no	150.84mn	152.25lm
20	BOPP	–	–	124.59yz	126.31y
	PE + PES	–	140.06r	141.08r	143.54q
25	BOPP	–	–	–	–
	PE + PES	–	136.62tu	137.64st	138.97rs

Table 10 Mean comparison interaction effects of the polymer film (BOPP: biaxial-oriented polypropylene and PE + PES: polyethylene + polyester), cinnamaldehyde and storage time on total antioxidant activity (% DPPH) of pomegranate arils

Storage time	Polymer film	Cinnamaldehyde			
		0	100	150	200
0	BOPP	72.66 fg	72.83e	73.50d	74.00c
	PE + PES	72.83f	74.50c	74.50b	74.50a
5	BOPP	47.00w	51.00uv	68.50k	70.33j
	PE + PES	48.50vw	71.66i	71.83h	72.66gh
10	BOPP	32.83 g	41.33z	61.16op	63.16o
	PE + PES	40.66a	64.00n	66.16m	67.66l
15	BOPP	–	34.16ef	45.33tu	46.00st
	PE + PES	35.50f	54.50rs	56.50qr	58.83pq
20	BOPP	–	–	36.16e	37.00d
	PE + PES	–	42.16y	43.33xy	44.16x
25	BOPP	–	–	–	–
	PE + PES	–	38.00z	39.00z	40.00z

day of storage with significant ($P \leq 0.01$) differences with the other treatments at the same time. Also, the least amount of TAA (32.83%) was found in BOPP film without cinnamaldehyde on the tenth day of storage with significant ($P \leq 0.01$) differences with the other treatments at the same time. During storage, the difference between the maximum and minimum amount of TAA was 41.67% (Table 10).

AA is an important antioxidant involved in reducing the rate of senescence. Results of variance analysis showed that the main effect, interaction effects of 2- and 3-fold treatments on AA amount were significant

($P \leq 0.01$). Changes of AA showed an increasing trend up to 15 days of storage and then decreased to the end of storage. The most amount of AA (25.19 mg L⁻¹) was found in arils packaged in PE + PES film containing 200 μ L L⁻¹ cinnamaldehyde on the 15th day of storage with significant ($P \leq 0.01$) differences with the other treatments at the same time. Also, the least amount (5.68 mg L⁻¹) was found in BOPP film without cinnamaldehyde on the tenth day of storage, however, it was not significantly ($P \leq 0.01$) different with BOPP film containing 100 and 150 μ L L⁻¹ cinnamaldehyde and PE + PES film without cinnamaldehyde at the same time. During

Table 11 Mean comparison interaction effects of the polymer film (BOPP: biaxial-oriented polypropylene and PE+PES: polyethylene + polyester), cinnamaldehyde and storage time on ascorbic acid content (mg L^{-1}) of pomegranate arils

Storage time	Polymer film	Cinnamaldehyde			
		0	100	150	200
0	BOPP	13.34yz	13.73yz	14.29y	14.61x
	PE+PES	14.05y	15.03w	15.52v	15.91u
5	BOPP	16.47t	16.82s	17.74q	18.02p
	PE+PES	17.39r	18.23op	18.44o	18.97n
10	BOPP	5.68z	11.97z	13.10yz	19.88l
	PE+PES	12.46z	20.27k	20.55j	20.97i
15	BOPP	–	8.91z	11.62z	12.81z
	PE+PES	11.37z	23.57c	24.42b	25.19a
20	BOPP	–	–	10.04z	10.71z
	PE+PES	–	22.10f	22.55e	23.22d
25	BOPP	–	–	–	–
	PE+PES	–	19.46m	21.29h	21.68g

storage, the difference between the maximum and minimum amount of AA was 19.51 mg L^{-1} (Table 11).

Results of variance analysis showed that the main effect, interaction effects of 2- and 3-fold treatments on the number of aerobic mesophilic bacteria were significant ($P \leq 0.01$). Changes in colony forming units (CFU) of aerobic mesophilic bacteria showed an increasing trend during storage. During storage, the most CFU of aerobic mesophilic bacteria ($2.33 \text{ Log CFU g}^{-1}$) was found in arils packaged in PE+PES film without cinnamaldehyde at the fifteenth day of storage with significant ($P \leq 0.01$) differences with the other treatments at the same time. While the lowest number of aerobic mesophilic bacteria (1 Log CFU g^{-1}) were found in arils packaged with PE+PES film containing $200 \mu\text{L L}^{-1}$ cinnamaldehyde at the same time. During storage, the lowest number of aerobic mesophilic bacteria (0 Log CFU g^{-1}) was found in arils packaged in PE+PES film containing 150 and $200 \mu\text{L L}^{-1}$ cinnamaldehyde at the first day of storage with significant ($P \leq 0.01$) differences with the other treatments at the same time (Table 12).

Results of variance analysis showed that the main effect, interaction effects of twofold (cinnamaldehyde \times storage time and polymer film \times storage time) and threefold treatments on the number of psychrophilic bacteria were significant ($P \leq 0.01$). Changes in CFU of psychrophilic bacteria showed an increasing trend during storage. During storage, the most CFU of psychrophilic bacteria ($4.46 \text{ Log CFU g}^{-1}$) was found in arils packaged in BOPP film without cinnamaldehyde at the tenth day of storage with significant ($P \leq 0.01$)

Table 12 Mean comparison interaction effects of the polymer film (BOPP: biaxial-oriented polypropylene and PE+PES: polyethylene + polyester), cinnamaldehyde and storage time on aerobic mesophilic bacteria (Log CFU g^{-1}) of pomegranate arils

Storage time	Polymer film	Cinnamaldehyde			
		0	100	150	200
0	BOPP	1h	1h	1h	1h
	PE+PES	1h	1h	0i	0i
5	BOPP	1.1gh	1h	1h	1h
	PE+PES	1h	1h	1h	0i
10	BOPP	2.16ab	1.63c-f	1.61c-f	1.66c-f
	PE+PES	1.25fgh	1.1gh	1.1gh	1.1gh
15	BOPP	–	2.01abc	1.86bcd	1.83bcd
	PE+PES	2.33a	1.47d-g	1.35e-h	1.1gh
20	BOPP	–	–	1.99abc	1.91abc
	PE+PES	–	1.81bcd	1.78bcd	1.75b-e
25	BOPP	–	–	–	–
	PE+PES	–	1.98abc	1.95abc	1.81bcd

differences with the other treatments at the same time. While the lowest number of psychrophilic bacteria ($2.1 \text{ Log CFU g}^{-1}$) were found in arils packaged with PE+PES film containing $200 \mu\text{L L}^{-1}$ cinnamaldehyde at the same time. During storage, the lowest number of psychrophilic bacteria (0 Log CFU g^{-1}) was found in arils packaged in PE+PES film containing 100, 150, and $200 \mu\text{L L}^{-1}$ cinnamaldehyde at the first day of storage with significant ($P \leq 0.01$) differences with the other treatments at the same time (Table 13).

Table 13 Mean comparison interaction effects of the polymer film (BOPP: biaxial-oriented polypropylene and PE+PES: polyethylene + polyester), cinnamaldehyde and storage time on psychrophilic bacteria (Log CFU g^{-1}) of pomegranate arils

Storage time	Polymer film	Cinnamaldehyde			
		0	100	150	200
0	BOPP	3.1hi	3i	3i	3i
	PE+PES	1k	0l	0l	0l
5	BOPP	4.17ab	3.1hi	3.1hi	3.1hi
	PE+PES	1.16k	1k	1k	1k
10	BOPP	4.46a	3.47d-i	3.45d-i	3.1hi
	PE+PES	3.72b-g	3.25f-i	3.15hi	2.1j
15	BOPP	–	3.85b-e	3.61c-h	3.25f-i
	PE+PES	3.96a-d	3.3f-i	3.23f-i	3.2ghi
20	BOPP	–	–	4.46a	4.13abc
	PE+PES	–	3.4e-i	3.23f-i	3.1hi
25	BOPP	–	–	–	–
	PE+PES	–	3.77b-f	3.26f-i	3.26f-i

Discussion

PE + PES film-maintained L^* value during storage due to low permeability to O_2 . It has been shown that MAP reduces discoloration [25]. These effects could be related to the delayed biosynthesis of anthocyanins and carotenoids [26]. The beneficial effect of essential oil on the maintenance of L^* is in line with previous findings with Artess et al. Active components of menthol, thymol, and eugenol in a controlled atmosphere have resulted in a reduced color change of grapes and cherry [24]. Grape packaging with menthol and eugenol decreased changes of L^* and a^* values depending on the concentration of active components. Delay in senescence is a reason for decreasing color change and maintaining lightness due to the use of essential oils [16].

The chroma index indicates the intensity of the color or its saturation degree. Fruit with less chroma has less color clarity [27]. In accordance with our results, packaging conditions and storage time have a significant effect on the color purity of arils [28]. In this research, the chroma index decreased during storage time significantly which can be related to the senescence of fruit. Differences in the efficiency of films for protecting color index can be related to their ability in delaying senescence and decreasing the activity of anthocyanins' destructive enzymes [29]. Nevertheless, there are contradictory reports that MAP and storage time do not influence color parameters [30]. It seems that TAA of essential oils decreases the breakdown of pigments, fruit color change, and browning disorder [24].

Hue angle is an index of fruit color. Following our results, increasing hue angle during storage time was reported earlier [3], which indicates the destruction of anthocyanins during storage time [31].

PE films are not permeable to WV molecules and therefore create a micro atmosphere around the fruit that is saturated with moisture [32]. Percent of SSC depends on the content of soluble solids and fruit moisture. The higher amount of SSC in arils packaged with PE + PES film may be attributed to decreased respiration and lower WV transmission from package film. In accordance to our results, increase in the sugar content of pomegranate cv. 'wonderful' has reported under controlled atmosphere condition [33].

Reducing acid and increasing sugar that occurs simultaneously during storage time may be the result of changing acids into sugars or gluconeogenesis [34]. As the pomegranate is a non-climacteric fruit, increasing SSC is due to a decrease in weight loss over time and concentration of fruit juice [35].

PE + PES containing essential oil caused better preservation of organic acids may be due to creating an optimal atmosphere, reducing respiration, and preventing

the consumption of organic acids during metabolic processes as compared with BOPP film containing essential oil and films without essential oil. According to these findings, pH of arils in different packages enhanced during storage time which was the result of decline in TA [36]. In line with these results, preservation of acidity was observed in pomegranate cv. 'Mollar de Elche' during storage under modified atmosphere, which may be due to the changes in metabolic activity under abiotic stresses [37]. Films with low permeability lead to increase carbon dioxide and decreased pH [35]. It seems in packages containing high carbon dioxide, respiration and degradation of organic acids are reduced. Also, dissolution of carbon dioxide may produce carbonic acid (HCO_3) and H^+ which lead to a decrease in pH [38].

Film permeability needs to be balanced to create a favorable modified atmosphere, decrease fruit metabolism, respiration rate, and consumption of respiratory substrates, and slow down the ripening process after harvest. Our results are in line with those noted that essential oil reduces the consumption of organic acids by reducing oxidative processes such as respiration, ripening, and senescence [39].

Preservation of phenolic compounds in arils packaged with PE + PES film is may be due to the induction of non-biological stress by a high concentration of CO_2 [40]. Preservation of phenolic compounds in arils packaged with PE + PES film is may be due to the increase in phenylpropanoid pathway under low oxygen stress [41]. According to the results of Fawole and Opara [2], essential oils maintained higher levels of phenolic compounds than the control.

Anthocyanins are polyphenolic compounds that exist in pomegranate peel and arils [36]. It seems that the permeability of polymer films to O_2 and CO_2 and also contents of these gases around packaged products can influence the amounts of anthocyanin, rate of synthesis, and its degradation. PE + PES film protected anthocyanins because of low permeability to O_2 and CO_2 that's corresponded with obtained results in pomegranate cv. Hicaznar [30]. According to results reported for plum and strawberry, more stability of anthocyanins in a controlled atmosphere was due to lack of oxidation [30, 36]. Nevertheless, excessive concentration of CO_2 in controlled atmosphere conditions prevents the activity of phenylalanine ammonia-lyase enzyme, anthocyanins synthesis and converts phenylalanine into cyanide acid and it also barricades disorder in the conversion of cyanide acid into hydroxyl phenolic compounds [42].

Anthocyanin content is influenced by pH, acidity, sugar, and other phenolic compounds so using the pH destroys anthocyanin [43]. In our findings, a decrease in anthocyanins is described by increased pH during

storage time. Essential oil reduces the reaction of anthocyanins with O_2 by saturating the inner space of the pack and then placing it on the arils. Anti-senescence properties of essential oil are effective in reducing the degradation of polyphenols and preserving the high contents of phenolic compounds. Most of the antioxidant capacity of pomegranate is related to phenol compounds. Therefore, if phenolic content decreases, the reduction of TAA will be predictable [44].

Preservation of TAA in arils packaged in PE + PES film is may be due to lower O_2 content, which increases antioxidant capacity by scavenging free radicals [45]. Addition of essential oils or their components to packages have a synergistic effect on TAA. These results are in line with the previous report on the use of essential oils on food products which increased antioxidant properties [46]. Considerable reduction of antioxidant activity in BOPP film may be due to high permeability to O_2 . It has been found that high concentrations of oxygen decrease the major antioxidant compounds of pomegranate arils such as anthocyanins and other phenolic compounds because of acceleration in oxidation [30] and increases the production of free radicals [45].

In this work, the use of PE + PES film resulted in a little change in the amount of AA during storage may be due to the low permeability to O_2 . Also, preventing the effects of film on the humidity inside the package is effective in keeping AA. The amount of AA is strongly influenced by the water loss, and in fact, the reduction of fruit juice causes the oxidation of AA [47]. Preserving organic acids in arils packaged with PE + PES films can be a possible explanation for maintaining AA as organic acid [48]. According to our results, the amount of AA in grapes treated with menthol and thymol [16] and strawberry treated with thymol [49] increased during storage. The reduction of AA as an antioxidant agent is due to its use as an electron donor to oxidants for neutralizing free radicals in the final days of storage [50]. Also, decreased AA is attributed to fruit respiration and chilling injury [49].

Polymer films prevent the growth of microorganisms by changing the ratio of respiratory gases and reducing respiration, delaying aging, reducing physiological abnormalities [51]. CO_2 has an inhibitory effect on the growth of Gram-negative and aerobic bacteria. Research shows that the antimicrobial potential of CO_2 against psychrophilic bacteria is due to its greater solubility at low temperatures and therefore can increase the shelf life of food at low temperatures [52]. CO_2 when dissolved in water, produces H_2CO_3 , which decreases pH of food products. Low pH inhibits the microorganism's growth by delay in the lag phase. Therefore, the levels of soluble CO_2 in the product are determinative of the inhibit microbial growth in a

modified atmosphere [36, 53]. PE + PES films reduced the microbial load compared to BOPP by preserving carbon dioxide. Essential oils have an inhibitory effect on the growth of microorganisms [54]. According to the results of this experiment, eugenol, thymol, and menthol in a controlled atmosphere in grapes and cherries reduced microbial growth significantly [24]. Cinnamaldehyde and carvacrol are effective in reducing the microflora of fruits and this effect is more in fruits that have lower pH. In general, at lower pH, essential oils and their components are more effective [55]. The effect of cinnamaldehyde in controlling microbial load to be due to the low pH of pomegranate aril. The inhibitory effects of cinnamaldehyde are probably due to the binding of its carbonyl group to proteins and the inhibition of the role of the aromatic L-amino acid decarboxylase. Also, the inhibitory effects of compounds with aldehyde structure are due to the reaction of the sulfhydryl group with microorganisms [55].

Conclusion

Packaging of pomegranate arils in PE + PES films containing $200 \mu\text{L L}^{-1}$ cinnamaldehyde could extend the shelf life of arils up to 25 days compared with BOPP films containing $200 \mu\text{L L}^{-1}$ cinnamaldehyde which extended arils shelf life up to 10 days. PE + PES films containing $200 \mu\text{L L}^{-1}$ cinnamaldehyde could preserve pomegranate arils by preventing the change of color, maintaining color indices and TA, SSC, anthocyanins, total phenols, TAA, AA and inhibition of microbial load. Packaging in a film with low permeability to O_2 and CO_2 creates a desirable atmosphere around the product and keeps the qualitative characteristics. Cinnamaldehyde due to its strong antioxidant properties, Prevents the oxidation of bioactive compounds. Also, due to their antimicrobial properties, they will inhibit microbial contamination and increase shelf life.

Abbreviations

PE + PES: Polyethylene + polyester (PE + PES); C*: Chroma; BOPP: Biaxial-oriented polypropylene; TAA: Total antioxidant activity; AA: Ascorbic acid; PE: Polyethylene; PP: Polypropylene; PVC: Polyvinyl chloride; SSC: Soluble solids content; TA: Titratable acidity; PPO: Polyphenol oxidase; POD: Peroxidase; GRAS: Generally recognized as safe; FDA: Food and Drug Administration; MAP: Modified atmosphere packaging; TPC: Total phenol content; TAC: Total anthocyanins content.

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Authors' contributions

AR: data curation, formal analysis, writing original draft; AR: supervision, lab equipment's, editing paper. Both authors read and approved the final manuscript.

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