

Postharvest quality responses of pomegranate fruit (cv. Shishe-Kab) to ethanol, sodium bicarbonate dips and modified atmosphere packaging

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: Pomegranate fruit is very popular due to its high commercial importance and health benefits. This experiment aimed to evaluate the sensory quality, color, and biochemical properties (TSS, TA, TSS/TA, anthocyanin content and total antioxidant capacity) of pomegranate fruit under post-harvest treatments, included ethanol (EtOH), sodium bicarbonate (SBC), and different packaging. Experimental treatments included: 10% (v/v) EtOH, 1% (w/v) SBC, and the type of packaging (passive-MAP and vacuum). Fruit were then stored at 5±1°C and 90% relative humidity for ten weeks. The peel and aril color evaluations indicate that EtOH treatment and vacuum packaging (VP) improved the quality of pomegranate color by increasing a^* and decreasing L^* . These treatments made the skin color and aril color lighter and redder in pomegranate. In addition, the treatments reduced decay and maintained total soluble solids (TSS), and titratable acidity (TA). Interestingly, EtOH treatment improved fruit nutritional quality as it increased total antioxidant capacity and anthocyanin content by 20% and 50%, respectively, compared to the control. The sensory analysis indicated that treated fruit with EtOH and VP scored higher in taste, color, texture, and appearance, and showed the best acceptability from the panelists' viewpoint. In conclusion, EtOH and VP significantly improved pomegranate fruit quality during cold storage since preserved sensorial quality and bioactive compounds and reduced decay.

1. Introduction

Pomegranate is mainly confined to the tropics and subtropics and grows well in arid and semi-arid climates. The edible portion of pomegranates (arils) is about 55 to 60% of the total fruit weight, and contains 80% juice and 20% seeds (Erkan and Kader, 2011). The fresh juice contains 85% water and 15% sugars, pectins, ascorbic acid, polyphenolic flavonoids, anthocyanins, and amino acids (Erkan and Kader, 2011). The amount of these compounds vary with pomegranate variety, maturity, and environmental and cultivation conditions. The statistics on acreage and production of pomegranate are not available with Food and

Agriculture Organization at the global level. However, the estimated global cultivated area of pomegranate is around three hundred thousand hectares, with the production of three million tones (Venkitasamy *et al.*, 2019).

Pomegranate is classified as a non-climacteric fruit due to low respiration and ethylene production rates after harvest (Kader *et al.*, 1984). Despite its non-climacteric nature, the fruit still undergoes both qualitative and quantitative losses during postharvest handling and storage, resulting in chilling injuries, husk scald, weight loss, and decay (Opara *et al.*, 2015). Postharvest losses of fresh horticultural products occur from harvest and continue during the handling and storage period, estimated more than 20% of production in developed countries and about 40-50% in developing countries (Watkins, 2020). To reduce quantitative and qualitative losses during the supply chain and increase food availability, postharvest decay control is one of the significant factors to be considered. Because most of the commercial pomegranate cultivars are susceptible to chilling injury when they are stored at low temperatures less than 5°C, commonly held at 5°C or higher temperature (Moradinezhad *et al.*, 2020). As a result, postharvest decay occurs by different fungi. Various fungicides have been used traditionally to control postharvest decay in fresh fruits. However, there is public concern about food safety regarding fungicides application in pre and postharvest stages (Tzatzarakis *et al.*, 2020).

Chemical treatments should be compounds with known and minimal toxicological effects on mammals and impact on the environment. As substances that will be in contact with fresh produce, they should be affirmed as generally recognized as safe (GRAS) by the United States Food and Drug. Ethanol, carbonates, and bicarbonates belong to the GRAS group (Palou, 2018). Previous reports (Teksur, 2015; Droby *et al.*, 2016; Dukare *et al.*, 2019) focused on using environmentally friendly, effective, and safe alternative control methods to fungicides to reduce postharvest decay of fresh fruits. Safe inorganic compounds have shown antimicrobial activity (Deliopoulos *et al.*, 2010). Successful control of postharvest decay in various fresh fruits was indicated using different safe chemical compounds applications such as ethanol (EtOH), sodium carbonate (SC), sodium bicarbonate (SBC), and ozone (Nunes, 2010). EtOH is a volatile organic compound with antimicrobial potential, widely used as a disinfectant. The positive effects of

EtOH application have been indicated in fresh fruits like, grapes, peaches, oranges, nectarines, strawberries, apples, and Chinese bayberries (Dao and Dantigny, 2011). SBC is a common food additive that has been used in the food industry as a safe and effective chemical for controlling fungi growth as a bio fungicide. It is cheap, readily available, and a low risk of injury to the fresh fruit (Vilaplana *et al.*, 2018).

The beneficial effects of modified atmosphere packaging (MAP) have been demonstrated on different fruits and vegetables. The results of various studies of MAP in different cultivars of pomegranate showed significant improvement in quality maintenance and extension of the storage life of fruit (Moradinezhad *et al.*, 2018; Sahel *et al.*, 2018; Venkataramudu *et al.*, 2018; Candir *et al.*, 2019; Moradinezhad *et al.*, 2019, 2020). MAP also reduces the growth of pathogens and consequently postharvest decay (Pareek *et al.*, 2015; Teksur, 2015; Rodriguez and Zoffoli, 2016; Ansarifard and Moradinezhad, 2021). Despite MAP has been widely applied to pomegranate; however, the literature review shows that no research focused on the effect of pre-storage EtOH or SBC dips and their combination with modified atmosphere packaging on postharvest decay control and quality attributes of the pomegranate fruit. Therefore, this study aimed to assess the efficacy of pre-storage EtOH or SBC dips and MAP on physiological responses and quality of pomegranate fruit cv. Shishe-Kab during postharvest storage.

2. Materials and Methods

Fruit preparation and treatments

About 200 fully mature pomegranate fruit cv. Shishe-Kab were harvested from a commercial orchard in South Khorasan province, Birjand, Iran, in October 2019. Pomegranate fruits were harvested and placed in carton boxes. A row of fruits was placed in each box so that the fruits would not be damaged. The fruits were transported to the Postharvest Lab of the University of Birjand, Iran, immediately after harvest. Uniform fruits (267-320 g) free of defects were selected and were then dipped in 200 ppm sodium hypochlorite solution for 1 min for surface disinfection. Before applying the treatments on fruits, five fruits were peeled and their juice was taken for initial analysis (Color properties, TSS, TA, TSS/TA, anthocyanin content and total

antioxidant capacity). Thereafter, fruits were dipped in chemical solutions including 10% (v/v) EtOH or 1% (w/v) SBC for 2 minutes. Control fruit were immersed in distilled water (20°C). All the chemicals used in this experiment were obtained from the Merck company (Germany). Fruit were then air-dried and placed into Low-Density Polyethylene (LDPE) bags (Carton Plast Co., Iran) (3 treatments × 2 packages × 3 replications) with 0.05 mm thickness (ten fruit per bag). Bags were sealed after removal of air by a vacuum pump to make vacuum packaging (VP) or sealed without removal of air (passive MAP) and then stored at 5±1°C and 85±5% relative humidity. Physico-chemical and sensory quality attributes of fruits were determined after 10 weeks of cold storage.

Fruit quality assessments

Color attributes. Aril and peel color of fruit were evaluated in all treatments. The color was determined in terms of L^* , a^* , and b^* values using a colorimeter (TES-135 A, country of manufacture Taiwan). Chroma and hue were obtained with the following equations (1 and 2):

$$\text{Chroma} = [a^{(0.5+)} + b^{(0.5)^2}] \quad (1)$$

$$\text{Hue angle (h)} = \tan^{-1} (b/a) \quad (2)$$

Measurements were made at three different points on the skin of each fruit. Three fruits in each replicate were used.

Determination of total soluble solids (TSS), titratable acidity (TA), and TSS/TA ratio. To prepare the juice, we first separated the pomegranate arils from fruit peel. Then pomegranate juice was prepared by hand pressure, and at the end it was filtered using a thin cloth. To prepare juice, three fruits selected from each replication. For higher accuracy in the experiment, the average of three samples was presented as one replication (juice was prepared separately from each fruit).

TSS in the extracted juice of each slice (one center section) was measured by a hand-held refractometer (RF 10, °Brix, 0-32%, Extech Co., USA). To measure titratable acidity, 5 ml of extracted fruit juice titrated with 0.1N sodium hydroxide. The TA was calculated as a percentage of citric acid. The TSS to TA ratio calculated by dividing TSS to TA in each replication of treatments.

Determination of anthocyanin content. The total anthocyanin content of juice determined by the pH-

differential method using two buffer systems comprised of potassium chloride (pH 1, 0.025 M) and sodium acetate (pH 4.5, 0.4 M). One ml of juice sample mixed with 10 mL of buffer, and the absorbance (A) measured at 510 and 700 nm using a spectrophotometer (Unico 2100, China) (Wagner, 1979).

Determination of total antioxidant capacity. To determine the total antioxidant activity, DPPH radical inhibitor activity method was used. The DPPH radical-scavenging activity of the samples was evaluated according to the method described by Turkmen *et al.* (2005) dissolved in distilled water at different concentrations. Pomegranate juice samples were mixed with 1 mL of a freshly made methanol solution of DPPH radical (100 µM). The contents were vigorously incorporated and incubated at room temperature in the dark for 20 min, and the absorbance was read at 517 nm. Methanol solutions of tested extracts and DPPH were used as blank and control measurements, respectively. All experiments were carried out three times on two separate occasions. The percentage of total antioxidant activity (TAA) of the tested extracts was calculated according to the following equation (3).

$$\% \text{ radical scavenging activity} = [(\text{absorption control} - \text{absorption sample}) / \text{absorption control}] \times 100 \quad (3)$$

Sensory quality and decay percentage. Sensory evaluation of samples was done by a panel of ten trained members, based on a 5-point hedonic scale at the end of the cold storage period. In all treatments, aril was first isolated from the peel and the panelist evaluated and scored three fruits from each treatment. Mean scores was considered as one replication. After tasting each sample by the evaluators, we asked them to drink some water. All the treatments were randomized at room temperature (22°C), and panelists rated the appearance, taste, color, texture, and acceptance of pomegranate arils on a five-point scale, 1= the extremely bad, and 5= extremely good (3≤ acceptable) as described by Moradinezhad *et al.* (2018).

The decay evaluated visually during the storage time. Fruit were examined daily and considered infected when a visible lesion was observed (such as surface mycelia, slimy patches, bruises, and blemishes). Results expressed as the percentage of infected fruits. In fact, upon observing the first effects of decay on the fruit, that fruit considered as a percentage of decay in the whole box. In other words, in a package of ten fruits, if one fruit has decay symp-

toms, 10% of decay is reported for that box.

Statistical analysis

The recorded data were subjected to a two-way analysis of variance (ANOVA) with two factors, pre-storage treatments and packaging methods in three replications using the GenStat program (version 12, 2010, VSN International, Ltd., UK). LSD test at 1% level of probability ($P \leq 0.01$) was used to compare means of different treatments.

3. Results

There were no significant interactive effects of chemical treatments \times packaging type on all evaluated traits (data not shown). Therefore, only the simple effects presented in the tables.

Aril and peel color attributes

The aril color analysis showed that the L^* , hue angle (h°), and chroma (C) values in all treatments

decreased compared to the fruit at harvest, while a^* value increased (Table 1). As shown in Table 1, post-harvest application of EtOH and SBC had a significant effect on L^* and a^* of aril. The highest L^* value after 10 weeks of storage was recorded in treated fruit with EtOH (20.2), and the lowest L^* obtained in control fruit (11.6). The highest a^* value (28.1) obtained from EtOH treatment, and the lowest (24.3) observed in control. However, EtOH and SBC treatments had no significant effect on b^* , chroma, and hue parameters. The results showed that treatments had a significant impact on the L^* and a^* parameters of pomegranate peel. In fact, these treatments caused to a lighter and redder both the peel and aril color.

As shown in Table 2, all the color parameters of aril and peel fruit were significantly different compare to fruit at harvest. Most of the parameters (except a^*) were reduced. In addition, the type of packaging had a significant effect on the L^* and a^* values. The highest L^* (18) and a^* (25.7) were obtained in vacuum-packed fruit. However, the type of packaging had no significant effect on b^* , hue

Table 1 - Effect of EtOH and NaHCO₃ dipping on color properties of pomegranate fruit (cv. Shishe-Kab) aril and peel after 10 weeks of cold storage at 5°C

Pre-storage treatments	Aril					Peel				
	L^*	a^*	b^*	h°	C^*	L^*	a^*	b^*	h°	C^*
At harvest	23.2±1.7 a	22.5±1.4 d	6.7±0.1 a	19.2±1.1 a	28.8±2.2 a	44.6±3.1 a	37.4±2.8 c	13.4±1.2 a	19.8±1.4 a	46.7±3.9 a
Control	11.6±0.8 d	24.3±2.0 c	7.6±0.8 a	16.7±0.6 b	26.2±1.7 b	35.4±3.4 c	43.6±2.7 b	15.1±1.9 a	18.7±1.2 a	45.7±3.7 a
Ethanol (10%)	20.2±1.9 b	28.1±1.6 a	7.4±0.8 a	17.7±1.3 b	25.0±1.8 b	39.4±2.9 b	45.4±4.5 a	14.3±1.0 a	18.1±1.9 a	46.4±4.1 a
SBC (1%)	18.7±1.2 c	26.1±0.9 b	6.8±0.4 a	17.9±1.2 b	25.3±1.9 b	39.2±3.1 b	43.2±2.8 a	16.9±1.5 a	19.3±1.1 a	46.9±3.4 a
Level of Sig.	**	**	NS	*	**	*	**	NS	NS	NS
LSD	5.46	6.52	3.62	4.65	6.52	5.04	5.02	5.47	4.21	4.57

Means \pm SE followed by different letters in the same column for the same evaluated parameter are significantly different ($P \leq 0.01$) according to the LSD test.

SBC= Sodium bicarbonate.

Table 2 - Effect of different MA packaging on color properties of pomegranate fruit (cv. Shishe-Kab) aril and peel after 10 weeks of cold storage at 5°C

Packaging	Aril					Peel				
	L^*	a^*	b^*	h°	C^*	L^*	a^*	b^*	h°	C^*
At harvest	23.6±1.4 a	23.5±1.8 c	7.5±0.8 a	21.7±1.1 a	27.8±2.0 a	48.0±2.5 a	32.4±2.9 b	16.9±1.8 a	23.1±1.6 a	35.9±3.4 b
Passive MAP	16.3±1.3 c	24.4±1.9 b	6.8±0.5 b	20.5±1.9 b	23.0±1.8 b	40.3±2.8 b	44.2±3.8 a	14.8±0.9 b	20.9±1.9 b	47.8±3.5 a
Vacuum	18.0±2.1 b	25.7±2.1 a	6.1±0.9 b	20.4±1.7 b	25.4±1.4 b	37.7±3.7 b	44.0±3.4 a	15.2±1.5 b	18.7±1.2 b	46.9±3.9 a
Level of Sig.	**	**	*	**	**	**	*	**	**	*
LSD	4.46	5.32	1.45	6.06	5.32	4.12	4.10	4.38	5.95	3.73

Means \pm SE followed by different letters in the same column for the same evaluated parameter are significantly different ($P \leq 0.01$) according to the LSD test.

angle, and chroma of pomegranate aril (Table 2). After 10 weeks of cold storage, passive MAP and vacuum packaging had no significant effect on the peel color attributes of the pomegranate fruit.

Total soluble solids (TSS), titratable acidity (TA), TSS/TA ratio

The TSS and TSS/TA ratio in all treatments increased compared to fruit at harvest time. However, TA value in all treatments decreased compared to fruit at harvest (Table 3). Data analysis showed that the highest content of TSS and TSS/TA found in control (18.54 °Brix, and 18.05, respectively), and the lowest content obtained from treated fruit with EtOH (16.31°Brix, and 9.24, respectively). Also, the highest and lowest TA values obtained from EtOH (1.94%) and control (1.02%) respectively. In addition, the evaluation of MA packaging showed that the highest content of TSS and TSS/TA were related to passive MAP (respectively, 18.40 °Brix, and 16.78), and the lowest content obtained from vacu-

um packaging (respectively, 17.05°Brix, and 9.34). Also, the highest amount of TA observed in vacuum-packed fruit (1.94 %) (Table 4).

Anthocyanin content

According to Table 3, anthocyanin at harvest time was 21.51 mg L⁻¹. After 10 weeks of storage, its value in the control group was 23.19 mg L⁻¹ (about 8% more than harvest time). While, anthocyanin content was higher (34.27 mg L⁻¹) in EtOH-treated fruits (about 40% higher than harvest time). Also, anthocyanin in the SBC treatment was significantly higher than in the control. However, the highest amount of anthocyanin was obtained from ethanol treatment after 10 weeks of storage, and the lowest amount was observed in control. As shown in Table 4, the anthocyanin content at harvest time was 22.41 mg L⁻¹. After 10 weeks of storage, the amount of anthocyanin significantly increased than harvest time. However, there was no significant difference between different packaging treatments.

Table 3 - Effect of EtOH and NaHCO₃ dipping on biochemical attributes of pomegranate fruit (cv. Shishe-Kab) after 10 weeks of cold storage at 5°C

	TSS (°Brix)	TA (%)	TSS/TA	Anthocyanin (mg l ⁻¹)	Antioxidant activity (%)
At harvest	15.74±0.98 d	2.42±0.03 a	7.21±1.82 d	21.51±2.03 d	61.7±5.24 c
Control	18.54±1.51 a	1.02±0.01 d	18.05±1.28 a	23.19±1.17 c	68.2±6.50 b
Ethanol (10%)	16.31±0.84 c	1.94±0.01 b	9.24±1.87 c	34.27±1.41 a	77.3±5.74 a
SBC (1%)	17.72±1.22 b	1.26±0.04 c	13.28±1.09 b	26.88±1.26 b	70.9±6.01 b
Level of Sig.	**	**	**	**	**
LSD	0.96	0.12	2.13	4.07	9.42

Means ± SE followed by different letters in the same column for the same evaluated parameter are significantly different (P≤0.01) according to the LSD test.

SBC= Sodium bicarbonate.

Table 4 - Effect of different MA packaging on biochemical attributes of pomegranate fruit (cv. Shishe-Kab) after 10 weeks of cold storage at 5°C

Packaging	TSS (°Brix)	TA (%)	TSS/TA (°Brix/%)	Anthocyanin (mg l ⁻¹)	Antioxidant activity (%)
At harvest	16.02±1.78 c	2.28±0.01 a	7.13±1.48 c	22.41±2.07 b	63.5±4.54 b
Passive MAP	18.40±1.28 a	1.12±0.04 c	16.78±0.98 a	28.63±1.35 a	79.1±4.91 a
Vacuum	17.05±1.47 b	1.94±0.02 b	9.34±1.04 b	29.84±1.09 a	77.2±5.20 a
Level of Sig.	**	**	**	*	**
LSD	0.78	0.09	1.74	3.33	7.68

Means ± SE followed by different letters in the same column for the same evaluated parameter are significantly different (P≤0.01) according to the LSD test.

The total antioxidant capacity

The antioxidant activity of pomegranate fruit was measured by neutralizing free radicals. In general, in all treatments antioxidant activity was higher compared to fruit at harvest (Tables 3 and 4). The highest and lowest antioxidant activity recorded from EtOH and control treatments, respectively. However, SBC had no significant effect on antioxidant activity. Also, there was no significant difference between vacuum packaging and passive MAP.

Sensorial quality

The appearance of the product is effective in consumer preference. Figure 1 A, and B shows the effect of treatments on the scores of acceptance, taste, texture, appearance, and color of arils during storage at 5°C. EtOH treatment had the best sensory quality as

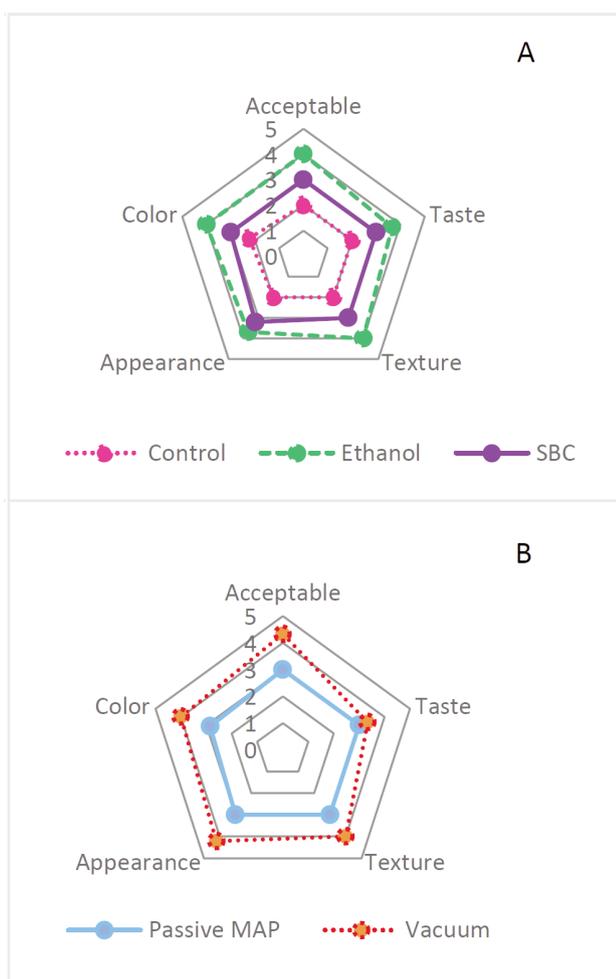


Fig. 1 - Effect of different chemical treatments (A) and MA packaging (B) on sensorial assessments of pomegranate arils (cv. Shishe-Kab) after 10 weeks of cold storage at 5°C. SBCO= Sodium bicarbonate.

scored higher by panelists. However, SBC treatment also achieved acceptance scores. Besides, sensory properties were improved in vacuum-packed fruit. Interestingly, Passive MAP fruit scored acceptable quality by panelists.

Decay

As shown in Figure 2, EtOH treatment significantly controlled fruit decay. The percentage of fruit rot under EtOH treatment was 12.57%, while in control samples was 36.21% (3-fold higher than EtOH). Also, vacuum packaging reduced the fruit decay more effectively than passive MAP.

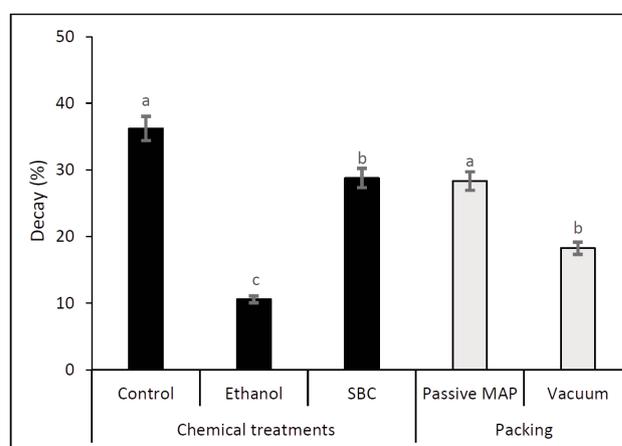


Fig. 2 - Effect of different chemical treatments and MA packaging on decay of pomegranate arils (cv. Shishe-Kab) after 10 weeks of cold storage at 5°C. Error bars represent the error (percentage). Identical letters mean there is no significant difference between them, at $P \leq 0.01$ (LSD). SBC= Sodium bicarbonate.

4. Discussion and Conclusions

The L^* represents lightness changes from 0, which has no lightness (absolute black) to 100, which is maximum lightness (absolute white) (Morales *et al.*, 2020). a^* varies between green and red, negative values of a^* indicate green colors, and positive values represent red colors. b^* varies between blue and yellow, negative values of b^* indicate blue colors and positive values represent yellow colors. Hue is the color tone, or color name of a color. Chroma is the amount of saturation of a color. Colors of high chroma are said to be clear, bright or brilliant. Dull (pastel) colors have a low chroma (Morales *et al.*, 2020).

Jin *et al.* (2013) reported that the application of

EtOH on melon sweet fruit preserves the color and freshness of the fruit. They showed that after 16 days of storage, the highest L^* value related to EtOH 0.5 ml treatment, which is inconsistent with the results of the present report. The results showed that during the storage period, the L^* of fruit peel decreased or darkened, which indicates a decrease in the peel quality compared to harvest time. The researchers showed that the synthesis of ethylene, followed by senescence, causes the enzymes associated with oxidative reactions and ultimately leads to darkening (low L^* value) of the color (Hasan *et al.*, 2018; Morales *et al.*, 2020). In line with the findings of Abdi *et al.* (1998), our results showed that the highest L^* value obtained in EtOH treatment, maybe due to the inhibition of ethylene biosynthesis which delaying fruit senescence and changes of senescence-related pigments. The results of this study on fruit color characteristics are also in line with the findings of Ponzo *et al.* (2018) on guava fruit. Moradinezhad and Dorostkar (2020) found that fresh jujube fruit under vacuum packaging had a higher L^* value. They stated that vacuum packaging might inhibit enzymatic reactions and delay the darkening of fruit color. Similar results were reported by Moradinezhad *et al.* (2019) on pomegranate fruit. They showed that passive MAP and vacuum packaging did not have a significant effect on the pomegranate peel color attributes. Therefore, it concluded that the treatments used in the present study did not have adverse effects on the peel of pomegranate fruit and did not reduce the marketable value of the fruit.

The increase in a^* , is related to the increased biosynthesis and accumulation of anthocyanin pigments, which are responsible for the intense red color of ripe pomegranate fruit (Lyu *et al.*, 2020). Moradinezhad *et al.* (2019) reported that the a^* value of pomegranate peel in vacuum-packed fruit was higher than the control. They stated that vacuum packaging modified the atmosphere around the fruit, extending its shelf-life and improving the color of the fruit, which is similar to the results of the present study. Vacuum-packed fruits had the lowest respiration rate, probably because of reduced O_2 concentration and ethylene removal from the intercellular spaces (Rana *et al.*, 2018).

In most fruits and vegetables, sugar makes up the main component of TSS, which is thus a reasonable indicator of the values sugar levels (Huang *et al.*, 2021). As the results showed, the TSS content of pomegranate fruit increased slightly after 10 weeks

of storage compared to harvest time, because pomegranate fruit is classified as a non-climacteric fruit. Generally, most non-climacteric fruits have a minor change on TSS during the storage period, mainly due to low starch accumulation content during growth and development. However, one of the most significant changes that occur during fruit ripening is the hydrolysis of starch to sugar, which changes the taste and texture of the product. Likely, any treatment that delays maturation reduces soluble solids content. Previous studies have shown that EtOH inhibits the production and action of ethylene (Podd and Van Staden, 1998) as a ripening hormone. EtOH prevented the conversion of ACC to ethylene (Podd and Van Staden, 1998). This compound reduces ethylene synthesis by reducing ACC synthase activity (a key enzyme in ethylene synthesis). In the present study, we found that 10% EtOH preserves TSS of the pomegranate fruit. Liu *et al.* (2019) showed that post-harvest application of 50% EtOH in cassava for 12 and 24 hours reduced TSS compared to control samples. This decrease in TSS is probably due to a reduction in the respiration rate. A similar result has been reported on blueberry (Ji *et al.*, 2021).

The organic acids present in foods influence the flavor, color, microbial stability keeping quality (Jawad *et al.*, 2020). Citric acid is the predominant acid in pomegranate fruit (Tozzi *et al.*, 2020). However, inorganic acids such as phosphoric and carbonic acids (arising from carbon dioxide in solution) often play an important and even predominant role in food acidulation (Jawad *et al.*, 2020). In plant tissue, EtOH and acetaldehyde can be converted to each other. Therefore, external application of EtOH increases the amount of acetaldehyde inside the fruit tissue, which causes production of more EtOH in the presence of oxygen (Podd and Van Staden, 1998). But, in the process of EtOH production, carbon dioxide is also produced as a byproduct (Podd and Van Staden, 1998). Increased carbon dioxide inhibits ethylene synthesis and reduces the respiration rate (Park *et al.*, 2021). As carbon dioxide increases, the synthesis of sugars (especially glucose) also increases, and also organic acids are not consumed in cellular respiration (Park *et al.*, 2021). Therefore, the use of EtOH causes the accumulation of organic acids, as a result, increasing TA. According to the proposed mechanism, we found that the post-harvest application of 10% EtOH preserves the TA in pomegranate fruit. Our finding is in consistent with the report of Shao *et al.* (2020) on wampee fruit.

The results also indicated that the lowest TSS/TA ratio obtained from EtOH treatment and vacuum packaging. It can be concluded that these treatments inhibit respiration rate and maintain the acidity of the pomegranate fruit. Selcuk and Erkan (2016) in Turkey found similar results on sweet pomegranate fruit. The reduction of TSS /TA ratio using MAP indicates a delay in the ripening of pomegranate fruit. This results are consistent with the findings of Venkatachalam and Meenune (2015) on longkong fruit.

Anthocyanins are commonly found in plant cell vacuoles in the form of glycosides (i.e., the combination of anthocyanins with simple sugars such as glucose, galactose, etc.) (Podd and Van Staden, 1998). The presence of sugar in the structure of anthocyanins makes them soluble in water. Interestingly, we found that 10% EtOH treatment increased the anthocyanin content by 50% compared to the control. Tzortzakis and Economakis (2007) showed that post-harvest application of EtOH (vapor) to tomatoes increases glucose, fructose and total soluble solids values. They found the increase in sugar was due to the weight loss of the tomatoes. Our findings support the above-mentioned study. Similarly, on bayberries, Wang *et al.* (2010) found that EtOH ($22.32 \mu\text{mol L}^{-1}$) increased anthocyanins. They stated that this increase in anthocyanin content was related to increased antioxidant activity. Liu *et al.* (2019) showed that treatment of 50% EtOH for 24 hours on cassava was able to increase the anthocyanin content by about 14% compared to control. They stated that this increase in anthocyanin content could be related to the effect of EtOH on reducing free radicals and increasing the activity of antioxidant enzymes. Similar results were reported by El Kereamy *et al.* (2002) on the grapes and Huang *et al.* (2015) on the blueberry. As presented in Table 4, the type of packaging did not affect the anthocyanin content of pomegranate fruit.

An unavoidable consequence of aerobic metabolism is reactive oxygen species (ROS). Environmental stresses lead to enhanced generation of ROS in plants due to disruption of cellular homeostasis (Wang *et al.*, 2021). All ROS are highly harmful to organisms at high concentrations. When the level of ROS exceeds the defense mechanisms, a cell is in a state of "oxidative stress" (Tang and Vashisth, 2020). Plant possess complex antioxidative defense systems comprising of non-enzymatic and enzymatic components to scavenge ROS (Wang *et al.*, 2021). The

antioxidants are substances capable of delaying or inhibiting oxidation processes (Hudson, 2012).

It has been shown that pomegranate juice, and even fermented pomegranate juice have high antioxidant activity (Li *et al.*, 2006). These activities may be related to diverse phenolic compounds present in pomegranate juice, including punicalagin isomers, ellagic acid derivatives and anthocyanins (delphinidin, cyanidin and pelargonidin 3-glucosides and 3,5-diglucosides). These compounds are known for their properties in scavenging free radicals and inhibiting lipid oxidation in vitro (Li *et al.*, 2006). Our results showed that postharvest use of EtOH maintained the antioxidant activity of pomegranate fruit. In line with these results, Wang *et al.* (2015) showed that EtOH ($300 \mu\text{l L}^{-1}$) increased H_2O_2 (a type of free radical). They stated that due to the balance between free radicals and antioxidants, the antioxidant content of loquat fruit increased. Natural antioxidants may exhibit one or more of the following roles: free radical scavenger, reducing agent, and quencher of singlet oxygen formation (Kim *et al.*, 2007). Moreover, anthocyanidins also have the potent antioxidant capacity and possible protective effects on human health (Kim *et al.*, 2007). Anthocyanins have greater antioxidant activity than either vitamin C or E (Kim *et al.*, 2007). These reports are in accordance with the results of anthocyanin content in the present study, which indicate the relationship between antioxidant activity and anthocyanin content in pomegranate aril.

Suzuki *et al.* (2004) showed that broccoli packaged with EtOH pads in a perforated film maintained their green color longer than control. Lurie *et al.* (2006) suggested EtOH as a promising alternative to SO_2 in grapes since it prevented the occurrence of quality decay associated with loss of freshness, glossy appearance, and browning. Similar to our results, Liu *et al.* (2012) reported that EtOH (6 ml/kg of fruit weight) improved the sweet melons sensory properties. They stated that the enhanced sweet melons' sensory properties might be more dependent on the EtOH treatment than the process of ripening and senescence. As mentioned before, pomegranate fruits are harvested during commercial maturity, so they have the highest sensory and taste quality at harvest time. According to Table 3, TSS and TSS/TA ration in ethanol treated fruit changed less than harvest time (compared to control and SBC). Therefore, ethanol treatment preserved the taste of treated fruit. In addition, the results of Table 3 in terms of TSS and TSS/TA ratio are consistent with the results of evalua-

tors. Also, as shown in Figure 1, vacuum packaging better retained the taste of pomegranate fruit, which is in line with the results of TSS, and TA in the present study. Similar results have been reported on the effect of vacuum packaging on preserving the sensory properties of jujube (Moradinezhad and Dorostkar, 2020) and litchi (Shah and Nath, 2006) fruit.

Researchers speculate that EtOH could kill the mitochondrial inner membrane of fungal spores, thereby preventing the spread of infection in the fruit (Gabler *et al.*, 2004). Similarly, in a recent study by Ji *et al.* (2019) the effect of EtOH on the physical properties of blueberries investigated. They found that EtOH treatment (1000 $\mu\text{L L}^{-1}$) significantly reduced fruit contamination (rotting was observed about 4% in EtOH treatment and 40% in control). This reduction in EtOH-induced decay may be due to the preservation of cell membranes in treated samples. Since low concentrations of EtOH can lower the temperature at which phospholipids undergo a phase change (Rowe, 1983), the increases in the spore mortality and decay control following the addition of EtOH may have resulted from a lowering of the phase-change temperature of mitochondrial membranes of the spores under these conditions (Margosan *et al.*, 1997). Also, possibly external application of EtOH has been shown to increase acetaldehyde produced by the alcohol dehydrogenase. Acetaldehyde directly attacks pathogens. EtOH has also been shown to increase resistance to other environmental stresses (such as chilling in cucumber) in addition to controlling diseases (especially fungal) (Frenkel and Erez, 1996). It had been proven that low oxygen in vacuum treatment significantly decreased ethylene production (Min *et al.*, 2019). On the other hand, a treatment that reduces ethylene synthesis has a good effect on infection control (Min *et al.*, 2019). Low ethylene production in a vacuum treatment may result in lower cell wall enzyme activity and cell integrity maintenance, considering the fact that cell wall enzymes are activated by ethylene. This will probably help maintain the strength of the cell wall and reduce decay (Ntsoane *et al.*, 2019). Similar results were obtained by Moradinezhad and Dorostkar (2020) on fresh jujube fruit.

The results of presented in this report show that the use of EtOH (10%) or vacuum packaging alone minimize decay, and maintain color, total soluble solids and titratable acidity of pomegranate fruit during storage. Also, these treatments increased biochemical properties such as anthocyanin content

and antioxidant capacity and improved sensory quality. Overall, it can be concluded that EtOH has the potential to control decay, enhance antioxidant systems, and extend the shelf-life of the pomegranate fruit.

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