

Combined Application of Gamma-Aminobutyric Acid and Carnauba Wax as Edible Coating on Pomegranates in Cold Storage

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ABSTRACT

Pomegranate is a popular fruit, rich in antioxidants and minerals but sensitive to postharvest storage. The efficiency of 5 and 10 mM γ -Aminobutyric Acid (GABA) combined with 0.5% carnauba wax as edible coating was investigated on extending the cold storage life of pomegranate fruit (Cv. *Malas Saveh*) after 45 and 90 days. Coatings maintained fruit freshness, inhibited the chilling injury symptoms, reduced peel malondialdehyde formation (minimum of 0.74 nM g⁻¹), reduced loss of aroma/taste, and increased aril antioxidant activity (maximum of 94.9%). Aril anthocyanin content was more stable in 5 mM GABA coated than uncoated fruits. However, the coatings caused more weight loss on 45th day (11.0% in 10 mM GABA and 8.3% in the control). This parameter was similar in coated and uncoated fruits after 90 days. Aril phenolic content in coated fruits was higher on 90th day but not on 45th day (maximum of 0.08 mg.100g⁻¹ in the control on day 45). Aril lightness increased in the control sample on 45th day while coated fruits were more similar to harvest time. After 90 days, the control and treated samples were similar. The color scales (a*, b*, and Chroma) values of peels and arils declined by storage without any significant effects of coatings. Peel and aril Hue did not change by storage time or coatings. Results suggested some benefits of coatings for retaining the postharvest quality of pomegranate fruits.

Keywords: Antioxidant activity, Fruit quality, *Punica granatum* L, Shelf life.

INTRODUCTION

Preservation of fresh fruit rich in health benefits and aroma compounds has become a challenge to horticulture and the food industry, because deterioration of fresh fruits occurs gradually during postharvest storage (Kumar *et al.*, 2020). For years, the edible coatings have been used to preserve fruits in a natural way and are defined as a thin layer made up of edible components covering the fruit surface for modifying the internal atmosphere and reducing the respiration rate and metabolic reactions (Kumar and Neeraj, 2019).

Some materials that are used as edible coatings are originally endogenous natural substances in plants that accumulate in

response to biotic and abiotic stresses. Gamma-Aminobutyric Acid (GABA) is an example. Some information is available on the effect of GABA on quality maintenance of horticultural crops postharvest (Shang *et al.*, 2011; Wang *et al.*, 2014; Rastegar *et al.*, 2020; Ebrahimzadeh *et al.*, 2019). Natural waxes are also widely used as edible coatings, to block the transpiration and to improve the outer surface appearance of different foods. Carnauba wax, which is obtained from the leaves of *Copernicia cerifera* palm tree, has been shown to retain the postharvest quality of some crops such as eggplant (Singh, *et al.*, 2016) and guava (Germano *et al.*, 2019).

Pomegranate (*Punica granatum* L.) is a desirable fruit rich in natural antioxidants

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such as anthocyanins, flavonoids, phenolic compounds, vitamins and minerals (Meighani, *et al.*, 2014), but highly perishable in cold storage. Positive effects of some types of edible coatings on extending the storage life of pomegranates have been reported including putrescine and carnauba wax (Barman *et al.*, 2011), chitosan (Varasteh *et al.*, 2012), chitosan, carnauba wax and resin wax (Meighani, *et al.*, 2014), and chitosan, ascorbic acid and citric acid (Zarbakhsh *et al.*, 2019).

Despite the beneficial properties of GABA and carnauba wax, they have not been combined as a coating. Therefore, the aim of this study was to evaluate the effect of GABA-carnauba wax edible coating on the postharvest quality and physiology of pomegranate fruits stored at cold conditions.

MATERIALS AND METHODS

Plant Material, Coating and Storage

The ripened and uniform-sized pomegranate fruits (*Cv. Malas Saveh*) were obtained from Shahreza orchards, Isfahan, Iran. Fruit harvest index was the ratio of soluble solids (17%) to total acid (5%). Six fruits were used for each replication. The treatments were the combination of GABA (Sigma Aldrich, Germany) with carnauba wax (Merck, Germany) in three levels (0.5% carnauba+5 mM GABA, 0.5% carnauba+10 mM GABA and distilled water as control). The fruits were immersed for 15 minutes in each treatment. After the surface of the fruits was dried, they were placed in plastic baskets, transferred to cold storage at $4\pm1^{\circ}\text{C}$ and $85\pm5\%$ relative humidity and stored for 90 days for further investigations.

Physiological Loss in Weight (PLW)

Weight loss was obtained from the difference between the weight of the first day and the weight at the storage day and

was expressed as a percentage of the initial fresh weight with the following equation:

$(\text{FW}-\text{DW})/\text{FW}\times 100$ [FW: Fresh Weight, DW: Dry Weight]

Chilling Injury and Malondialdehyde

Brown spots on the peel surface and internal separating segments, and pale aril color were considered as symptoms of chilling injury (Mirdehghan and Rahemi, 2005). The degree of chilling injury was calculated based on a range from zero to five: Zero: No symptoms of chilling injury, One: 1 to 20%, Two: 20 to 40%, Three: 40 to 60%, Four: 60 to 80%, and Five: More than 80% (Wang *et al.*, 2006). For malondialdehyde, 0.25 g of peel was ground in 5 mL of 0.1% trichloroacetic acid and centrifuged. One mL of extract was added to 4 mL of 20% trichloroacetic acid containing 0.5% thiobarbituric acid. The concentration was determined with absorbance measurement at 450, 532 and 600 nm with the following equation (Shekari *et al.*, 2021):

$$\text{MAD (nmol g}^{-1}\text{ FW)} = 6.45 (\text{OD}_{532} - \text{OD}_{600}) - 0.56 \text{OD}_{450}$$

MAD means Malondialdehyde, and OD means optical density.

Total Antioxidant Activity, Anthocyanins Content and Total Phenolic Content

Total antioxidant activity of arils was determined by the 2,2-DiPhenyl-1- Picryl-Hydrazil (DPPH) radical-scavenging method. The absorbance was measured at 517 nm, and was expressed as the inhibition percentage of the DPPH radical (Rastegar *et al.*, 2020). For anthocyanins content, 1 mL of aril extract was made to 25 mL by buffer (0.2N KCl)/(0.2N HCl) with pH= 1. Another 10 mL of the same extract was made to 25 mL by buffer (1N $\text{CH}_3\text{CO}_2\text{Na}$)/(1N HCl) with pH= 4.5. The adsorption rate of these two samples was read at 510 nm and the

amount of anthocyanin was calculated by the following equation and was expressed as mg L^{-1} of fruit juice (Rapisarda *et al.*, 2000):

$$C_{\text{mg/L}} = (\text{AbS}_{\text{pH1}} - \text{AbS}_{\text{pH4.5}}) 482.82 \times 0.0402 \times \text{Dilution factor}$$

For determination of total phenolic content, 5 g of arils was ground with 10 mL of phosphate buffer and centrifuged. Then, 100 mL of supernatant was mixed with 400 mL of phosphate buffer, 2.5 mL of Folin–Ciocalteu (1:10 diluted), and 2 mL of 7.5% sodium carbonate. Absorbance measurement was done at 760 nm using gallic acid for preparing the standard curve. The results were expressed as mg of gallic acid in 100 g of fresh weight (Orthofer and Lamuela-Raventos, 1999).

Color Indicators

Color values of peels and arils were directly measured with a color meter (Minolta Chroma Meter Model CR-400, Minolta, Japan). The color was measured as the Lightness (L^*), red-green (a^*) and Blue-yellow (b^*). The Chroma value and Hue angle were calculated by the following equations (Moradinezhad *et al.*, 2020):

$$\text{Chroma} = \sqrt{(a^*)^2 + (b^*)^2} \quad \text{Hue angle} = \tan^{-1}\left(\frac{b^*}{a^*}\right)$$

Sensory Evaluation

The sensory analysis included a panel test with 10 semi-trained panelists in each of the four replications (a total number of 40 panelists). The panelists evaluated the aroma/taste and freshness of fruits. Excellent was shown with 5, very good with 4, good with 3, moderate with 2, poor with 1, and extremely poor with 0 (Moradinezhad *et al.*, 2020)

Statistical Analysis

The study was a factorial experiment based on a completely randomized design

with four replications. Sources of variation were storage time (0, 45 and 90 days), coatings (control, 0.5% carnauba+5 mM GABA, and 0.5% carnauba+10 mM GABA) and their interaction. Mean values were reported as the mean±standard error of means. Data were analyzed by SAS 9.1 statistical software package and the Least Significant Difference (LSD) test at $P=0.05$ was used to compare the means. The charts were plotted in MS-Excel software package.

RESULTS AND DISCUSSION

Fruit Weight Loss

As the storage time lengthened, the weight of the fruits decreased significantly ($P=0.05$). Coatings showed higher weight loss on 45th day (8.3% in control; 10.3 and 11.0% in GABA 5 and 10 mM, respectively), but after 90 days there was no significant difference between control and coatings (15.4% in control; 15.1 and 14.0% in GABA 5 and 10 mM, respectively) (Figure 1-a). The degree of changes in metabolic reactions and the level of reduction in weight loss in fruits that are covered by edible coatings depends on the type and thickness of the coating (Dhall, 2013). Although the edible coatings are usually expected to reduce the weight loss, in our study, the coatings accelerated it until 45th day, which may be due to carnauba concentration. Chiumarelli and Hubinger (2014) reported that coatings with 0.2% carnauba wax were better barriers to moisture and gas exchange and the formulations with higher carnauba wax resulted in rigid coatings.

Chilling Injury

During storage, the chilling injury symptoms were increased (maximum score of 2.6 for the control on day 90). Carnauba 0.5% plus GABA 5 mM inhibited the chilling injury at 45th day, and both coatings



had positive effects on controlling the injury on 90th day (Figure 1-b). Storage at low temperatures results in a reduction in both internal and external fruit quality. The recommended safe storage temperatures for pomegranates are 5°C for up to 2 months, and 7.2°C for longer periods, but in this temperature, microbial growth may occur (Kashash *et al.*, 2016). GABA is naturally induced when plants are exposed to abiotic stresses. It increases the antioxidants activity, proline accumulation, and the content of saturated fatty acids and the integrity of membranes (Rastegar *et al.*, 2020). GABA is effective on postharvest tolerance to chilling injury in banana (Wang *et al.*, 2014), peach (Shang *et al.*, 2011) and walnut kernel (Ebrahimzadeh *et al.*, 2019). Carnauba wax has also been shown to elevate the tolerance to chilling temperatures through reducing gas exchange between the food and the surrounding environment. Carnauba wax in combination with putrescine decreased chilling injury of pomegranates (Barman *et al.*, 2011). Positive effect of carnauba on chilling injury has also been reported on guava (Germano *et al.*, 2019), grapefruit (Dou, 2004), and citrus (Kellerman *et al.*, 2014). At any duration of storage, the response of fruits to the edible coatings depends very much on the composition and concentration of the coating (Dhall, 2013). Therefore, it can be deduced from the results of this study that carnauba (0.5%) plus GABA (0.5 mM) is a better formulation for short storage periods, while carnauba (0.5%) plus GABA (1 mM) is more effective for longer storage periods.

Malondialdehyde

With increase in the storage time, the amount of malondialdehyde in peels increased. Both coatings, especially carnauba 0.5% plus GABA 10 mM, were able to reduce this increase (0.74 and 1.05 nM g⁻¹ on days 45 and 90, respectively) (Figure 1-c). Malondialdehyde is the product of fatty acid peroxidation caused by high

oxygen availability, and membrane damages, therefore, it can be used as an oxidative damage marker (Ebrahimzadeh *et al.*, 2019). Here, malondialdehyde formation can be the result of both chilling stress and drought stress upon weight loss. GABA acts as an inhibitor of malondialdehyde formation because it is capable of reducing H₂O₂ content and increasing antioxidant potential (Valenzuela *et al.*, 2017). In button mushrooms during 15 days of storage at 4°C, exogenous GABA increased the endogenous GABA content by increasing the expression of glutamate decarboxylase gene and decreasing the expression of GABA transaminase gene (Shekari *et al.*, 2021). It is also suggested that a possible cold stress protective mechanism might be induced by GABA, which alleviates cold storage symptoms of fruits (Wang *et al.*, 2014). Suppression of malondialdehyde formation by GABA in postharvest storage has been demonstrated in some other fruits like banana and orange (Wang *et al.*, 2014; Habibi *et al.*, 2019). Carnauba wax is a barrier against oxygen, therefore, capable of reducing oxidative damages. It was previously reported that GABA preserves pomegranate fruit quality during cold storage (Barman *et al.*, 2011). Carnauba inhibition of malondialdehyde formation is consistent with the results on guava (Germano *et al.*, 2019).

Antioxidant Activity

Antioxidant activity underwent little changes during storage. The coatings recorded higher antioxidant activity than the control after 90 days (minimum of 86.87% for the control on day 90; all other cases above 94%) (Figure 1-d). Because pomegranates are very rich in phytochemicals with antioxidant properties, the antioxidant capacity did not decline until 45 days, but a small decrease was observed after 90 days of storage in the control fruits. Antioxidant capacity of coated fruits was even stable after 90 days, because GABA

activates metabolic pathways involved in the maintenance of redox status and scavenging hydrogen peroxide (Valenzuela *et al.*, 2017). In addition, carnauba wax is a barrier against oxygen, therefore, reducing oxidative reactions. Similarly, higher antioxidant capacity by DPPH scavenging was found in GABA-treated mango fruits during 4 weeks of storage. The researchers stated that the role of GABA in increasing antioxidant activity was due to its ability to increase the production of antioxidant compounds such as phenols and flavonoids, as well as the activity of antioxidant enzymes (Rastegar *et al.*, 2020). Beta-aminobutyric acid enhanced antioxidant enzymes activity and caused higher levels of antioxidant activity through scavenging DPPH, superoxide and hydroxyl radicals in cherries postharvest (Wang *et al.*, 2016). In stored guava fruits, galactomannan-carnauba wax increased the activity of antioxidant enzymes, superoxide dismutase, and catalase, which led to 35% lower hydrogen peroxide content (Germano, *et al.*, 2019).

Anthocyanin Content

After 45 days, the anthocyanin content of fruits increased compared to harvest time, but carnauba 0.5% plus GABA 5 mM was able to keep this value stable (72.07 mg L⁻¹ for harvest time; 60.54 and 66.42 mg L⁻¹ for days 45 and 90, respectively). After 90 days, the anthocyanin content of all treatments was similar to harvest time (Figure 1-e). Pomegranate is a rich source of anthocyanins. Because anthocyanins have high antioxidant potential, their synthesis usually continues after harvest and at low-temperature storage (Zarbaksh *et al.*, 2019). Here, anthocyanin synthesis continued until 45th day in the control and fruits treated with carnauba 0.5% plus GABA 10 mM, but not in fruits treated with carnauba 0.5% plus GABA 5 mM. The latter fruits showed also less chilling injury on day 45, which may be the reason why they did not need anthocyanin synthesis. After 90

days, the anthocyanin content declined due to their probable involvement in radical scavenging. In contrary, Varasteh *et al.* (2012) reported that the total anthocyanin content of pomegranates decreased with storage time, but this change was reduced with chitosan treatments and at lower storage temperature (2°C as compared to 5°C). In another research, the level of pomegranate total anthocyanins increased significantly during 30 days of storage and a slight decrease was observed thereafter (Sayyari *et al.*, 2016). Anthocyanin content of pomegranates increased until 40 days of storage followed by a decrease and carnauba coating could delay declining anthocyanin content of pomegranates (Meighani *et al.*, 2014).

Phenolic Content

After 45 days, the amounts of phenolic content in coated fruits were lower than the control (0.087, 0.050, and 0.032 mg 100 g⁻¹ for the control, GABA 5, and 10 mM, respectively), but after 90 days, this value was higher in coated fruits than the control (0.021, 0.044, and 0.038 mg 100 g⁻¹ for control, GABA 5, and 10 mM, respectively) (Figure 1-f). Phenolic compounds are associated with plant responses against stresses (Gohari Ardabili *et al.*, 2011). Total phenolic content is usually accumulated under low temperature storage due to acclimation process (Wang *et al.*, 2014). Lower phenolic content in treated fruits than the control fruits of this study on 45th day may be the result of higher weight loss of treated fruits at this time, which probably imposed some drought stress to fruit tissues. However, after 90 days, while the weight loss of treated and control fruits was similar, the phenolic content of treated fruits was higher because of the beneficial effects of GABA as an edible coating. A lower phenolic content of coated fruits than the control has also been reported in pomegranate arils coated by ascorbic acid (Azizi *et al.*, 2018). But, ultimately, control fruits will have lower phenolic content than coated fruits overtime.

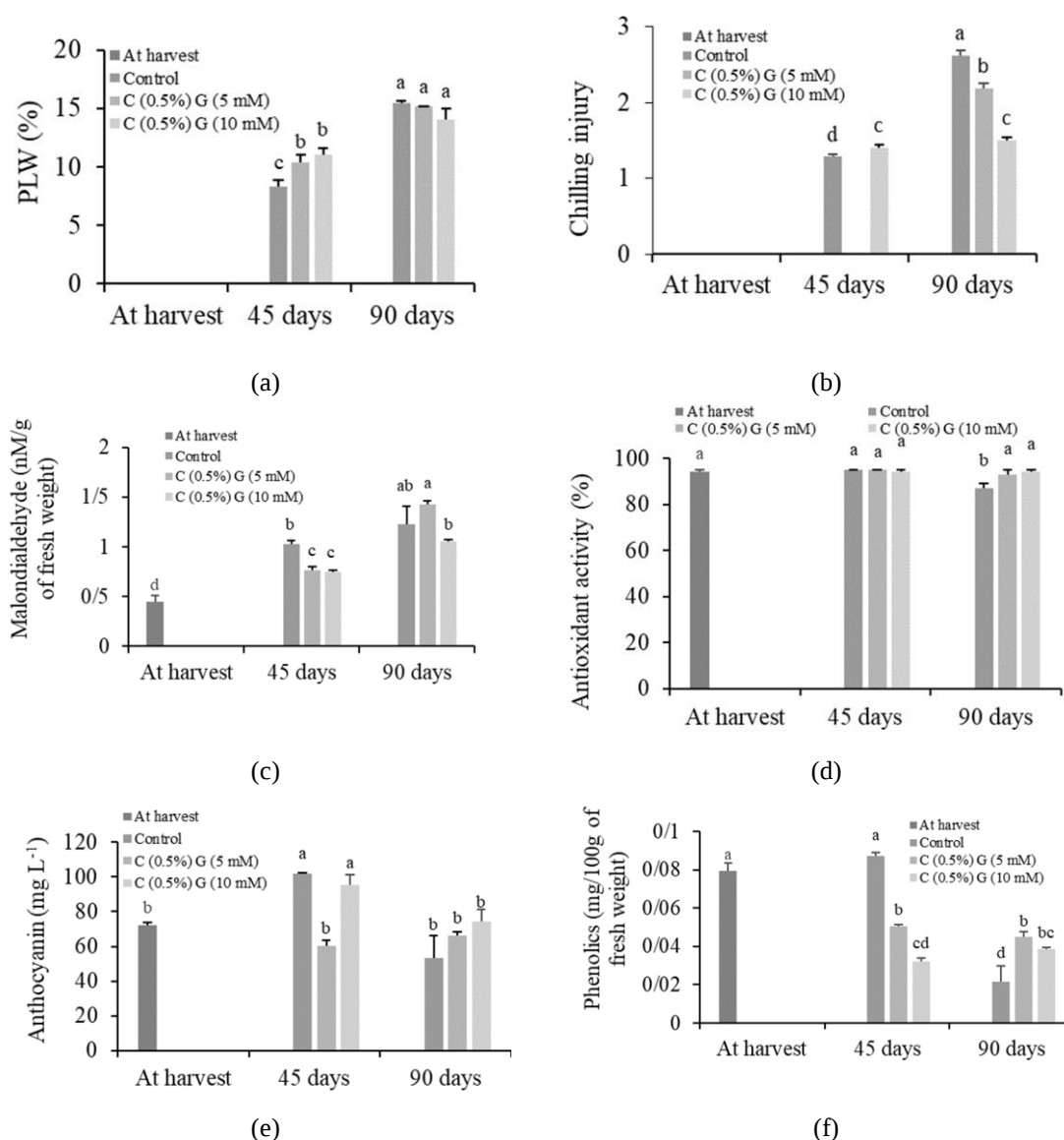


Figure 1. Interaction effect of GABA plus carnauba coating and storage time on: (a) pomegranate fruit weight loss; (b) pomegranate chilling injury; (c) pomegranate malondialdehyde content; (d) pomegranate antioxidant activity; (e) pomegranate anthocyanin content; (f) pomegranate phenolic compounds.

The final loss of phenolic content in the control tissues is due to senescence and breakdown of cell structure. Edible coating can delay the senescence by controlling the metabolic rate, retaining phenolic content for a longer storage period (Kumar *et al.*, 2020). Total phenolic content of banana peel stored at 7°C increased until 10 days followed by a decrease on 15th day, and again increased until 20th day. Bananas with GABA pretreatment showed higher phenolic content during storage

time (Wang *et al.*, 2014). In guava fruits, galactomannan-carnauba wax coating lowered polyphenol oxidase activity during 15 days of cold storage (Germano, *et al.*, 2019).

Color Indicators

Color is an important trait of fruits and determines the quality and consumer acceptability. In the present study, the Hue

value of peels and arils was not affected by time, coatings and their interaction (Table 1). For arils, a^* , b^* and Chroma were only affected by time, and declined on 90th day (a^* : 10.39; b^* : 5.82; Chroma: 11.93 on day 90) (Figures 2-a, -b, and -d). Aril lightness was influenced by the interaction of coatings and time, and showed an increase in the control sample on 45th day compared with harvest time, but the arils of coated fruits were more similar to harvest time. After 90 days, control and treated samples were similar to each other (Figure 2-c). For peels, only the effect of storage time was significant on a^* , b^* , lightness and Chroma. These values decreased by time (a^* : 25.89; b^* : 10.73; Lightness: 30.88; Chroma: 28.07 on day 90) (Figures 2-e, -f, -g, and -h).

In another study on pomegranate, the Hue angle of peel was the highest at harvest time and declined on 60th day of storage both in the control and carnauba waxed fruits. But Chroma value was the lowest at harvest and increased after 60 days without any significant difference between the control and waxed fruits. The authors stated that high Hue and low Chroma indicate the immaturity of fruits (Barman *et al.*, 2011). In our study, completely mature fruits were harvested, therefore, the Hue angle, which shows the basic color, was not influenced by time, neither in the control nor in treated fruits. The a^* and b^* values of arils and peels declined by time, indicating a reduction in redness and a reduction in green pigments. Therefore, peel and aril Chroma, which indicates the color intensity, undergone a reduction by time as well. The

changes in peel and aril Chroma may be related to the anthocyanin synthesis or degradation (Figure 1-e), such that, in arils, Chroma increased slightly until 45th day and decreased thereafter.

The decrease in peel lightness is probably related to wilting and dehydration overtime. It can also be due to tissue browning through polyphenol oxidase activity and membrane damage caused by cold stress. The reduction in fruit lightness with time is reported in sweet cherries while β -aminobutyric acid inhibited this reduction (Wang *et al.*, 2016). Similar results were reported in fresh cut apples, in which the decrease in lightness was lower in GABA treated group (Gao *et al.*, 2018). Here, however, the coatings were not effective in keeping peel lightness stable. In another study on pomegranates, color parameters were not significantly affected by pre-treatment applications (Moradinezhad *et al.*, 2020). In our study, aril lightness showed an increase followed by a decrease in the control fruits and the coatings were able to inhibit these changes. In another research on pomegranates, aril lightness gradually decreased with time. Ascorbic acid coatings could control this reduction, probably by prevention of anthocyanin degradation (Zarbakhsh *et al.*, 2019). Meighani *et al.* (2014) reported a decrease in pomegranate aril lightness until 40th day of storage followed by an increase until 120th day. They related these changes to anthocyanin synthesis and degradation, respectively. In our study, the changes in aril lightness in the control fruits may be related to substances other than anthocyanins.

Table 1. Effect of storage time and GABA plus carnauba wax as edible coating on Hue of pomegranate peels and arils.

		Aril			Peel		
		Control	Carnauba (0.5%) GABA (5 mM)	Carnauba (0.5%) GABA (10 mM)	Control	Carnauba (0.5%) GABA (5 mM)	Carnauba (0.5%) GABA (10 mM)
Hue	At harvest	0.5198 a	0.5198 a	0.5198 a	0.3801 a	0.3801 a	0.3801 a
	45 days	0.5177 a	0.4802 a	0.4448 a	0.3827 a	0.3265 a	0.3708 a
	90 days	0.5437 a	0.5242 a	0.4671 a	0.4088 a	0.3960 a	0.3752 a

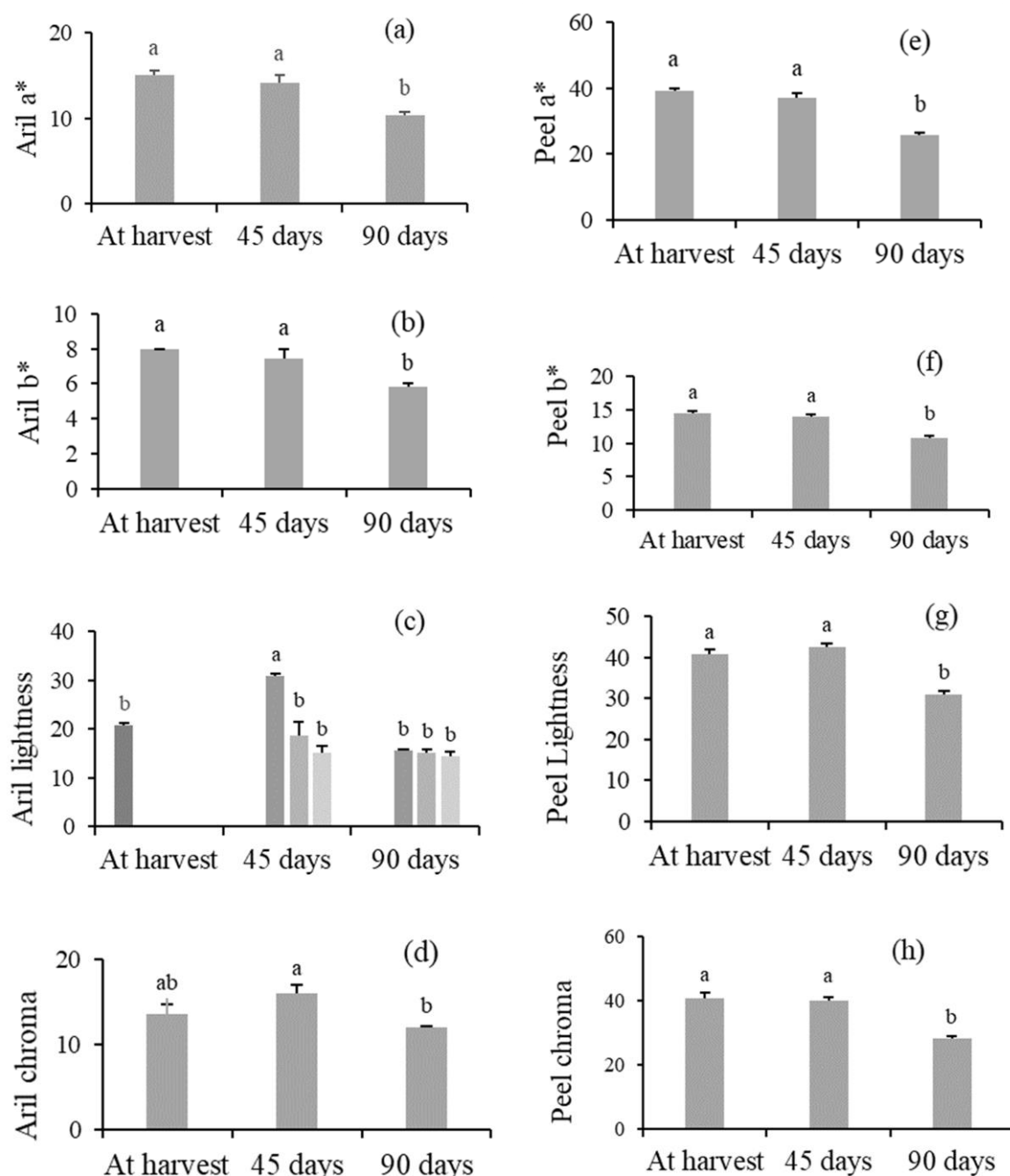


Figure 2. Simple effect of storage time on aril a* (a), aril b* (b), aril Chroma (d), peel a* (e), peel b* (f), peel lightness (g), peel Chroma and interaction effect of GABA plus carnauba coating and storage time on aril lightness (c) of pomegranate.

Higher stability in aril lightness in coated fruits than the control is probably due to lower metabolic reactions inside the coated fruits.

Sensory Evaluation

According to panel test, the fruits had the best score (5= Excellent) of aroma and taste

at harvest. The aroma and taste of fruits decreased with time, but carnauba 0.5% plus GABA 10 mM could decrease this reduction on 90th day (2.87 and 3.3 degree for control and GABA 10 mM, respectively) (Figure 3-a). No off-flavor was distinguished by the panelists. The highest score of fruit freshness belonged to harvest time as well; however, the storage time reduced this trait. The coatings had positive effects on keeping fruit freshness on 45th day, but not on 90th day (Figure 3-b). Cold stress causes cell membrane damages leading to loss of water, volatiles, organic acids and sugars and, therefore, loss of sensory qualities. Textural changes occur along with ripening as well. Edible coatings have the ability to reduce these losses through lowering the enzymatic and biochemical reactions. In this study, carnauba 0.5% plus GABA 10 mM could decrease the reduction of aroma and taste on 90th day (Figure 3 a). In another research, it was found that GABA treatment could be employed for maintaining the sensory and nutritional quality of button mushrooms during cold storage (Shekari *et al.*, 2021). Guava fruits waxed by carnauba remained firmer and the ripening process was delayed (McGuire, 1997). Grape fruits waxed by carnauba also remained shinier and more acceptable than the control (Dou, 2004).

CONCLUSIONS

In conclusion, pomegranate fruits undergo some qualitative and biochemical changes during cold storage. The use of GABA plus carnauba wax as edible coating imposed more weight loss until 45 days, which is not desirable for the producer, although this parameter was similar in the control and treated fruits on 90th day. At the same time, the coatings had some beneficial effects for storage in terms of retarding the chilling injury and the changes triggered by time, such as color changes and loss of firmness. It appears that pomegranates can be kept for almost three months along with preservation of their quantitative and qualitative traits, if

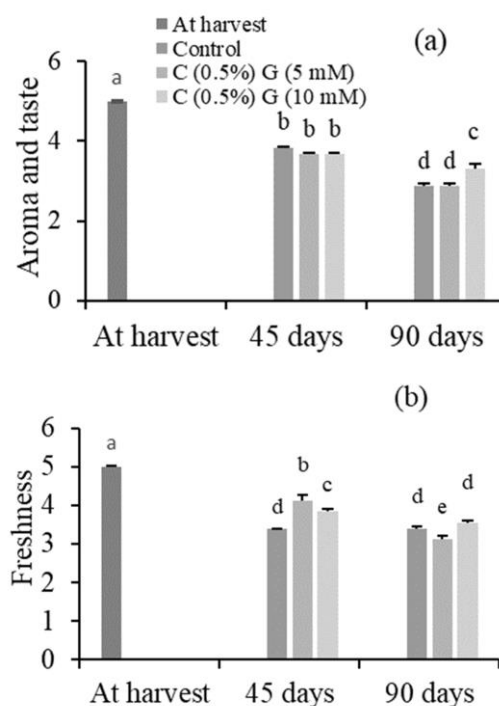


Figure 3. Effect of GABA plus carnauba coating and storage time on pomegranate (a) Aroma and taste, and (b) Freshness of fruit texture.

they are kept at $4\pm1^{\circ}\text{C}$ and are coated with edible GABA plus carnauba wax. Regarding chilling injury signs, carnauba (0.5%) plus GABA (5 mM) was a better treatment for 45 days of storage, but carnauba (0.5%) plus GABA (10 mM) is preferentially suggested for 90 days. Application of GABA and carnauba on arils (ready to eat pomegranate) is an issue that deserves research in the future. Application of lower concentrations of GABA and carnauba may also be examined in the next experiments in the hope that their negative effects on weight loss will be eliminated.

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REFERENCES

1. Azizi, F., Erfani, M. J., Khademi, O. and Nourollahi, K. 2018. Effects of Oxalic Acid and Ascorbic Acid on Shelf Life of Pomegranate Arils. *Iran. J. Food Sci. Technol.*, **14**: 15–24.
2. Barman, K., Asrey, R. and Pal, R. K. 2011. Putrescine and Carnauba Wax Pretreatments Alleviate Chilling Injury, Enhance Shelf Life and Preserve Pomegranate Fruit Quality during Cold Storage. *Sci. Hortic.*, **130**: 795–800.
3. Chiumarelli, M. and Hubinger, M. D. 2014. Evaluation of Edible Films and Coatings Formulated with Cassava Starch, Glycerol, Carnauba Wax and Stearic Acid. *Food Hydrocoll.*, **38**: 20–27.
4. Dhall, R. K. 2013. Advances in Edible Coatings for Fresh Fruits and Vegetables: A Review. *Crit. Rev. Food Sci. Nutr.*, **53**(5): 435–450.
5. Dou, H. 2004. Effect of Coating Application on Chilling Injury of Grapefruit Cultivars. *HortScience*, **39**(3): 558–561.
6. Ebrahimzadeh, A., Pirzad, F., Tahanian, H. and Soleimani Aghdam, M. 2019. Influence of Gum Arabic Enriched with GABA Coating on Oxidative Damage of Walnut Kernels. *Food. Technol. Biotechnol.*, **57**(4): 554–560.
7. Gao, H., Wu, S., Zeng, Q., Li, P. and Guan, W. 2018. Effects of Exogenous γ -Aminobutyric Acid Treatment on Browning and Foodborne Pathogens in Fresh-Cut Apples. *Postharvest Biol. Technol.*, **146**: 1–8.
8. Germano, T. A., Aguiar, R. P., Bastos, M. S. R., Moreira, R. A., Ayala-Zavala, J. F. and de Miranda, M. R. A. 2019. Galactomannan-Carnauba Wax Coating Improves the Antioxidant Status and Reduces Chilling Injury of 'Paluma' Guava. *Postharvest Biol. Technol.*, **149**: 9–17.
9. Gohari Ardabili, A., Farhoosh, R. and Haddad Khodaparast, M. H. 2011. Chemical Composition and Physicochemical Properties of Pumpkin Seeds (*Cucurbita pepo* Subsp. *pepo* var. *Styriaca*) Grown in Iran. *J. Agr. Sci. Tech.*, **13**(7): 1053–1063.
10. Habibi, F., Ramezani, A., Rahemi, M., Eshghi, S., Guillen, F., Serrano, M. and Valero, D. 2019. Postharvest Treatments with γ -Aminobutyric Acid, Methyl Jasmonate, or Methyl Salicylate Enhance Chilling Tolerance of Blood Orange Fruit at Prolonged Cold Storage. *J. Sci. Food Agric.*, **99**(14): 6408–6417.
11. Kashash, Y., Mayuoni-Kirshenbaum, L., Goldenberg, L. and Choi, H. J. 2016. Effects of Harvest Date and Low-Temperature Conditioning on Chilling Tolerance of 'Wonderful' Pomegranate Fruit. *Sci. Hortic.*, **209**: 286–292.
12. Kellerman, M., Erasmus, A., Cronje, P. J. R. and Fourie, P. H. 2014. Thiabendazole Residue Loading in Dip, Drench and Wax Coating Applications to Control Green Mould and Chilling Injury on Citrus Fruit. *Postharvest Biol. Technol.*, **96**: 78–87.
13. Kumar, N., Pratibha, Neeraj, Ojha, A., Upadhyay, A., Singh, R. and Kumar, S. 2020. Effect of Active Chitosan-Pullulan Composite Edible Coating Enrich with Pomegranate Peel Extract on the Storage Quality of Green Bell Pepper. *LWT - Food Sci. Technol.*, **138**: 110435.
14. Kumar, N. and Neeraj. 2019. Polysaccharide-Based Component and Their Relevance in Edible Film/Coating: A Review. *Nutr. Food Sci.*, **49**(5): 793–823.
15. McGuire, R. G. 1997. Quarantine Treatment and Application of Carnauba Wax Coating. *HortScience*, **32**(2): 271–274.
16. Meighani, H., Ghasemnezhad, M. and Bakhshi, D. 2014. Effect of Different Coatings on Post-Harvest Quality and Bioactive Compounds of Pomegranate (*Punica granatum* L.) Fruits. *J. Food Sci. Technol.*, **52**(7): 4507–4514.
17. Mirdehghan, S. H. and Rahemi, M. 2005. Effects of Hot Water Treatment on Reducing Chilling Injury of Pomegranate (*Punica granatum*) Fruit during Storage. *Acta Hort.*, **682**: 887–892.
18. Moradinezhad, F., Ansarifard, E. and Mohammadian Moghaddam, M. 2020. Extending the Shelf Life and Maintaining Quality of Minimally-Processed Pomegranate Arils Using Ascorbic Acid Coating and Modified Atmosphere Packaging. *J. Food Meas. Charact.*, **14**: 3445–3454.
19. Orthofer, R. and Lamuela-Raventos, R. 1999. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin–Ciocalteu, Reagent. *Method Enzymol.*, **299**: 152–178.
20. Rapisarda, P., Fanella, F. and Maccarone, F. 2000. Reliability of Analytical Methods for Determining Anthocyanin in Blood Orange Juices. *J. Agri. Food Chem.*, **48**: 2249–2252.

21. Rastegar, S., Khankahdani, H. H. and Rahimzadeh, M. 2020. Effect of γ -Aminobutyric Acid on the Antioxidant System and Biochemical Changes of Mango Fruit during Storage. *Food Measur.*, **14**: 778-789.
22. Sayyari, M., Soleimani Aghdam, M., Salehi, F. and Ghanbari, F. 2016. Salicyloyl Chitosan Alleviates Chilling Injury and Maintains Antioxidant Capacity of Pomegranate Fruits during Cold Storage. *Sci Hortic.*, **211**: 110-117.
23. Shang, H., Cao, S., Yang, Z., Cai, Y. and Zheng, Y. 2011. Effect of Exogenous Gamma Aminobutyric Acid Treatment on Proline Accumulation and Chilling Injury in Peach Fruit after Long-term Cold Storage. *J. Agri. Food Chem.*, **59**: 1264-1268.
24. Shekari, A., Naghshiband Hassani, R. and Soleimani Aghdam, M. 2021. Exogenous Application of GABA Retards Cap Browning in *Agaricus bisporus* and its Possible Mechanism. *Postharvest Biol. Technol.*, **174**: 111434.
25. Singh, S., Khemariya, P., Rai, A., Rai, A. C., Koley, T. K. and Singh, B. 2016. Carnauba Wax-based Edible Coating Enhances Shelf-Life and Retain Quality of Eggplant (*Solanum melongena*) Fruits. *LWT*, **74**: 420-426.
26. Soleimani Aghdam, M., Razavi, F. and Karamneghad, F. 2015. Maintaining the Postharvest Nutritional Quality of Peach Fruits by γ -Aminobutyric Acid. *Iran. J. Plant Physiol.*, **5(4)**: 1457-1463.
27. Valenzuela, J. L., Manzano, S., Palma, F., Carvajal, F., Garrido, D. and Jamilena, M. 2017. Oxidative Stress Associated with Chilling Injury in Immature Fruit: Postharvest Technological and Biotechnological Solutions. *Int. J. Mol. Sci.*, **18**: 1467.
28. Varasteh, F., Arzani, K., Barzegar, M. and Zamani, Z. 2012. Changes in Anthocyanins in Arils of Chitosan-coated Pomegranate (*Punica granatum* L. cv. Rabbab-e-Neyriz) Fruit during Cold Storage. *Food Chem.*, **130**: 267-272.
29. Wang, L., Chena, S., Kong, W., Li, S. and Archbold, D. D. 2006. Salicylic Acid Pretreatment Alleviates Chilling Injury and Affects the Antioxidant System and Heat Shock Proteins of Peaches during Cold Storage. *Postharvest Biol. Technol.*, **41**: 244-251.
30. Wang, L., Zhang, H., Jin, P., Guo, X., Li, Y., Fan, C., Wang, J. and Zheng, Y. 2016. Enhancement of Storage Quality and Antioxidant Capacity of Harvested Sweet Cherry Fruit by Immersion with β -Aminobutyric Acid. *Postharvest Biol. Technol.*, **118**: 71-78.
31. Wang, Y., Luo, Z. and Huang, H. 2014. Effect of Exogenous γ -Aminobutyric Acid (GABA) Treatment on Chilling Injury and Antioxidant Capacity in Banana Peel. *Sci. Hortic.*, **168**: 132-137.
32. Zarbakhsh, S., Kazemzadeh-Beneh, H. and Rastegar, S. 2019. Quality Preservation of Minimally Processed Pomegranate cv. Jahrom Arils Based on Chitosan and Organic Acid Edible Coatings. *J. Food Safe.*, **40(2)**: 1-12.

کاربرد ترکیبی از گاما آمینوبوتریک اسید و واکس کارنوبا به عنوان پوشش خوراکی روی انار در ذخیره سرد

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چکیده

انار یک میوه محبوب و غنی از آنتی اکسیدان ها و مواد معدنی است، اما برای ذخیره پس از برداشت بسیار حساس است. تاثیر گاما آمینو بوتریک اسید (GABA) در غلظت های ۵ و ۱۰ میلی مولار همراه



با ۵/۰٪ واکس کارنوبا به عنوان پوشش خوراکی روی افزایش عمر ذخیره سرد میوه انار رقم 'ملس ساوه' بعد از ۴۵ و ۹۰ روز بررسی شد. پوشش‌ها توانستند سفتی پوست (حداکثر ۴/۰۲ کیلوگرم نیرو)، سفتی آریل (حداکثر ۰/۸۲ کیلوگرم نیرو) و تازگی میوه را حفظ کنند، نشانه‌های آسیب سرمایی را مهار کنند، تشکیل مالون دی‌آلدهید پوست (حداقل ۰/۷۴ نانومول در گرم) و از دست رفتن عطر و طعم را کاهش دهند، و فعالیت آنتی‌اکسیدانی آریل (حداکثر ۹۴/۹٪) را افزایش دهند. مقدار آنتوسیانین آریل در میوه‌های پوشیده شده با ۵ میلی‌مولار GABA نسبت به میوه‌های بدون پوشش پایدارتر بود. اما پوشش‌ها کاهش وزن را در روز ۴۵ افزایش دادند (۱۱/۰٪ در ۱۰ میلی‌مولار GABA و ۸/۳٪ در شاهد)، این پارامتر بعد از ۹۰ روز در میوه‌های پوشش‌دار و بدون پوشش مشابه بود. میزان ترکیبات فنولی آریل در میوه‌های پوشش‌دار در روز ۹۰ بالاتر بود اما در روز ۴۵ تفاوتی نداشت (حداکثر ۰/۰۸ میلی‌گرم در ۱۰۰ گرم). درخشندگی آریل در روز ۴۵ در شاهد افزایش یافت، اما میوه‌های پوشش‌دار بیشتر مشابه زمان برداشت بودند. بعد از ۹۰ روز، نمونه‌های شاهد و پوشش‌دار مشابه بودند. شاخص‌های a^* ، b^* و کرومای پوست و آریل با گذشت زمان کاهش یافتند و پوشش‌ها اثر معنی‌داری روی آن‌ها نداشتند. زاویه رنگ پوست و آریل تحت تاثیر زمان و پوشش‌ها قرار نگرفت. نتایج، مفید بودن پوشش‌ها را برای حفظ کیفیت پس از برداشت میوه انار پیشنهاد می‌کنند.