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Blood oranges maintain bioactive compounds and nutritional quality by postharvest treatments with γ-aminobutyric acid, methyl jasmonate or methyl salicylate during cold storage

Fariborz Habibi[a,b], Asghar Ramezanian[a], Fabián Guillén[b], María Serrano[c], Daniel Valero[b,*]

[a] Department of Horticultural Science, School of Agriculture, Shiraz University, Shiraz, Iran
[b] Department of Food Technology, University Miguel Hernández, Ctra. Beniel km. 3.2, 03312 Orihuela, Alicante, Spain
[c] Department of Applied Biology, University Miguel Hernández, Ctra. Beniel km. 3.2, 03312 Orihuela, Alicante, Spain

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ABSTRACT

The effects of postharvest treatments with γ-aminobutyric acid (GABA), methyl jasmonate (MeJA) or methyl salicylate (MeSA) on antioxidant systems and sensory quality of blood oranges during cold storage were evaluated (150 days at 3 °C plus 2 days at 20 °C, shelf life). Fruit firmness, titratable acidity (TA), total antioxidant activity (TAA) and ascorbic acid (AA) decreased during cold storage, all these changes being delayed in treated fruit, with the greatest differences observed with the 50 μmol L^{-1} MeJA and 100 μmol L^{-1} MeSA treatments. Total phenolic content (TPC), total anthocyanin content (TAC) and the major individual anthocyanins, cyanidin 3-glucoside and cyanidin 3-(6″-malonylglucoside), were found at higher concentration in treated fruit than in control during the whole cold storage period. Overall, 100 μmol L^{-1} MeSA was the most effective for maintaining fruit quality and maintained higher anthocyanin concentration due to higher phenylalanine ammonia-lyase (PAL) and lower polyphenol oxidase (PPO) activities.

1. Introduction

Blood orange (*Citrus sinensis* L. Osbeck) fruit are a rich source of phytonutrient and bioactive compounds such as anthocyanins, flavonoids, hydroxycinnamic acids, and ascorbic acid (Habibi & Ramezanian, 2017). Anthocyanin content is considered an important quality index of blood orange fruit and also important for human health due to its antioxidant activity (Rapisarda, Bellomo, & Intelisano, 2001). Anthocyanins of blood orange fruit maybe useful for human health in preventing cancer cells growth, diabetes, heart disease (arteriosclerosis), viral activity as well as other age-related diseases (Habibi & Ramezanian, 2017).

There has been an increasing interest in the use of natural compounds such as γ-aminobutyric acid (GABA), methyl jasmonate (MeJA) and methyl salicylate (MeSA) to maintain fruit quality during cold storage (Sheng et al., 2017; Sayyari et al., 2011). GABA is recognized as a GRAS (Generally Recognized as Safe) compound. However, limited research has been focused on GABA postharvest treatments to reduce chilling injury (CI) and induce resistance to some postharvest pathogens (Sheng et al., 2017). GABA treatment has been shown to have a positive effect on orange fruit quality ('Olinda Valencia' and 'Newhall Navel') by reducing citrate consumption during cold storage (Sheng et al., 2017).

Volatile compounds such as MeJA and MeSA which are derived from jasmonic acid and salicylic acid, respectively, have shown efficacy on maintaining fruit quality during storage. Thus, postharvest treatment with MeJA and MeSA have been reported to maintain quality and bioactive compounds during cold storage in pomegranate (Sayyari et al., 2011), sweet cherry (Giménez et al., 2016) and peach (Yu et al., 2016).

Although blood oranges are considered as non-climactic fruit, their bioactive compounds change during long-term cold storage. For example, anthocyanin synthesis continued after harvest and its concentration in blood oranges increased during cold storage (Habibi & Ramezanian, 2017). However, cold storage can lead to some losses in other quality attributes such as taste, sensory-influencing qualities of juice, and bioactive compounds such as ascorbic acid (Rapisarda et al., 2001). Furthermore, storage of blood oranges at ambient temperature increases respiration, transpiration and fungal decay and accelerates senescence, while affects changes in bioactive compounds and results in other substances which impart flavour or aroma to the fruit leading to a short shelf life (Rapisarda, Bianco, Pannuzzo, & Timpanaro, 2008). Therefore, novel techniques are necessary to maintain quality and

* Corresponding author.
E-mail address: daniel.valero@umh.es (D. Valero).

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increase the storability of blood orange fruit at cold storage.

Recently it has been shown that GABA, MeJA or MeSA postharvest treatments reduced CI symptoms in the rind of 'Moro' blood orange cultivar (Habibi et al., 2019). However, to date there is no available literature regarding the use of these treatments to maintain blood orange fruit quality during prolonged cold storage. Therefore, this study was aimed to examine the effects of postharvest GABA, MeJA or MeSA treatments on bioactive compounds and nutritional and sensory quality of blood orange cv. 'Moro' fruit during long-term cold storage.

2. Materials and methods

2.1. Fruit treatments and storage conditions

Blood oranges (*Citrus sinensis* L. Osbeck cv. 'Moro') fruit were harvested at commercial maturity stage according to TSS/TA ratio from a commercial orchard located in Jahrom (28° 30′ N, 53° 31′ E), Fars province, Iran. Trees were 7 years-old and grafted on *Citrus aurantifolia*. Fruit were transported to the postharvest laboratory of Shiraz University and visually checked for no peel injuries, selected on the basis of size uniformity. They were also disinfected with 2% NaOCl solution and then rinsed with distilled water and placed at ambient temperature for drying of the fruit. Fruit were treated with GABA (20 and 40 mmol L^{-1}, Sigma Aldrich, Germany), MeJA (50 and 100 μmol L^{-1}, Merck, Germany) and MeSA (50 and 100 μmol L^{-1}, Merck, Germany) before evaluating bioactive compounds, organoleptic and nutritional quality parameters during long-term cold storage. These concentrations of GABA, MeJA and MeSA were selected due to their efficacy for reducing external symptoms of CI (Habibi et al., 2019). GABA was applied by vacuum infiltration at 30 kPa for 8 min while the MeJA and MeSA vapour treatments were performed by placing 30 fruit in a 20 l plastic container in which the appropriate volumes of either MeJA or MeSA solutions were placed on filter paper at the bottom of the container and then sealed for 18 h at 20 °C. Control fruit had no treatment. Treatments were performed in triplicate and after that fruit were placed in polyethylene bags containing 16 holes to allow gas exchange and stored for up to 150 days at 3 °C and 90% RH. After 1, 30, 60, 90, 120 and 150 days of cold storage 5 orange fruits were taken at random from each treatment and replicate and transferred to 20 °C for 2 days (to simulate shelf life conditions), and then, the following parameters were measured.

2.2. Physicochemical parameters

For determination of weight loss (WL), fruit of each replicate of treatment were weighed before storage as initial weight (IW) and at each sampling time as final weight (FW). Percentage of WL reported with the following equation (Habibi & Ramezanian, 2017):

$$WL(\%) = \frac{IW - FW}{IW} \times 100$$

Firmness of each fruit was determined with a texture analyzer (TA-XT2, UK) equipped with a 3.5 mm diameter probe. The probe compressed 10% of equatorial diameter of fruit and stopped after breaking the fruit peel and data were reported as Newton (N) (Njombolwana et al., 2013). After that, the 5 fruits of each replicate were longitudinally cut in two valves one of them being used for sensory analysis and the other one used to obtain segments which were combined and used half of them to obtain juice in which total soluble solids and titratable acidity were measured and the other one to be lyophilized for sugars, organic acids and individual anthocyanins determinations.

Total soluble solids (TSS) was measured in the combined juice sample of each replicate with a hand-held refractometer (TI-RBX0032A, Singapore) and reported as percentage (%). Titratable acidity (TA) was determined in the same juice by titration with NaOH 0.1 N to pH 8.2 as endpoint with pH meter. Maturity index (TSS/TA) was expressed as the ratio of TSS to TA (Habibi & Ramezanian, 2017).

2.3. Bioactive compounds

Total anthocyanin concentration (TAC) was determined by the pH differential method (Habibi & Ramezanian, 2017). Fruit juice were diluted (1:4) with potassium chloride buffer for pH 1.0 and sodium acetate buffer for pH 4.5. Absorbance was measured at 510 and 700 nm with a microplate spectrophotometer (Epoch, USA) and TAC was reported as mg L^{-1}.

Total phenolic content (TPC) was estimated using the Folin-Ciocalteu colorimetric method (Meyers, Watkins, Pritts, & Liu, 2003), with microplate spectrophotometer (Epoch, USA). Briefly, 32 μL of fruit juice was mixed with 900 μL of 2% sodium carbonate, and after 3 min, 180 μL of 50% Folin was added. Samples were then placed for 30 min at ambient temperature in a dark place and then the absorbance was measured at 750 nm. TPC was determined via a standard curve prepared with different concentrations of gallic acid and results were reported as mg gallic acid equivalents (GAE) L^{-1}.

Total antioxidant activity (TAA) of the samples were measured by adding 100 μL of fruit juice to 1 mL DPPH (0.1 mM) and 1 mL Tris-HCl (pH = 7.5) buffer and the mixture was maintained for 30 min at ambient temperature in a dark place. Absorbance was measured with microplate spectrophotometer (Dynamica, UK) at 517 nm and TAA reported with the following equation (Brand-Williams, Cuvelier, & Berset, 1995):

$$TAA(\%) = \left[1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}}\right] \times 100$$

Ascorbic acid (AA) content in fruit juice was determined spectrophotometrically using the to 2,6-dichlorophenol indophenol method and reported as mg L^{-1} (AOAC, 2000).

2.4. HPLC analyses

For individual anthocyanins, 500 mg of lyophilized blood orange flesh were grounded and mixed with 10 mL of methanol/formic acid/water (79:1:20, v/v/v), before being centrifuged at 10,500 × *g* for 10 min. After centrifugation, the supernatant was filtered through a 0.45 μm syringe filter and 20 μL injected to HPLC that was equipped with a Luna C18 column (25 cm × 0.46 cm i.d., 5 μm particle size; Phenomenex, Maccles field, UK) and a C18 security guard (4.0 × 3.0 mm) cartridge system (Phenomenex, Macclesfield, UK). Formic acid (99:1, v/v) (phase A) and acetonitrile (phase B) were used as mobile phases, with a flow rate of 1 mL min^{-1}. The linear gradient started with 8% of solvent B, reaching 15% at 25 min, 22% at 55 min, and 40% at 60 min, which was maintained up to 60 min. Data were collected at 280, 320, 360, and 520 nm. Major anthocyanins (cyanidin 3-glucoside and cyanidin 3-(6″-malonylglucoside)) of blood orange fruit were detected at 520 nm, quantified by comparison with known standards and expressed as mg 100 g^{-1} (Martínez-Esplá et al., 2014).

Individual sugars and organic acids were quantified by using 500 mg of lyophilized blood orange flesh grounded and mixed with 10 mL of 50 mmol L^{-1} phosphate buffer (pH = 7.8) and then centrifuged at 10,500 × *g* for 10 min. After centrifugation, one mL of the extract was filtered through a 0.45 μm syringe filter and 10 μL were injected to HPLC (Hewlett-Packard HPLC Series 1100). The elution system consisted of 0.1% phosphoric acid running isocratically with a flow rate of 0.5 mL min^{-1}. Sugars were eluted through a Supelco column (Supelcogel C-610H, 30 cm × 7.8 mm, Supelco Park, Bellefonte, USA) and detected by refractive index detector at 210 nm. For quantification of sugars (sucrose, glucose and fructose), standard curves of pure sugars were used and the results expressed as mg 100 g^{-1}. For detection of organic acids, the same HPLC, elution system, flow rate and column were used as with sugars. Organic acids

profiles (oxalic acid, citric acid, malic acid and succinic acid) were quantified by standard curve of pure organic acids and reported as mg 100 g^{-1} (Martínez-Esplá et al., 2014).

2.5. Enzymes assay

Enzymes activities were evaluated in the flesh of fruit spectrophotometrically. Catalase (CAT) and peroxidase (POD) activities were measured by the method described by Chance and Maehly (1955) at 240 and 470 nm, respectively. Ascorbate peroxidase (APX) activity was evaluated following the method of Nakano and Asada (1981) and measured at 290 nm. Superoxide dismutase (SOD) activity was determined using the method described by Beauchamp and Fridovich (1971) and measured at 560 nm. Phenylalanine ammonia-lyase (PAL) activity was determined by evaluation of the absorbance of *trans*-cinnamic acid at 290 nm (Liu et al., 2016). Polyphenol oxidase (PPO) activity was measured by the method described by Silva and Koblitz (2010) at 425 nm. Total protein content of extracts was measured according to the Bradford (1976) method and used for reporting of specific enzymes activities as U mg^{-1} protein.

2.6. Sensory quality evaluation

Descriptive sensory analysis tests were performed by a trained panel for market acceptability. Edible quality of blood orange fruit was evaluated separately by 10 trained panellists (5 men and 5 women) at each sampling time during cold storage. Fruit were peeled and carpel segments were hand-separated and placed in glass dishes which were assessed in random order. Panellists evaluated sensory acceptability of fruit as edible quality by 9-point scale from 1 as the lowest edible quality and 9 as the best edible quality according to quantitative descriptive sensory analysis (Khorram, Ramezanian, & Hosseini, 2017).

2.7. Statistical analysis

The treatments were distributed according to a complete randomized design (CRD) with three replicates. Data were analysed using two-factor (treatments and storage times) analysis of variance (ANOVA). Data analyses were done with SAS software package v. 9.4 for Windows. Mean comparisons were achieved using least significance difference (LSD) test ($P < 0.05$) with standard deviation (SD) of means.

3. Results

3.1. Physicochemical parameters

As expected, WL of fruit increased during cold storage. However, WL significantly was delayed in all treated fruit, the lowest values being found in 100 μmol L^{-1} MeSA and 50 μmol L^{-1} MeJA (Fig. 1A). At all removal times WL in control fruit was higher than all times. In this study, WL in treated fruit with GABA at 20 and 40 mmol L^{-1}, MeJA at 50 and 100 μmol L^{-1}, and MeSA at 50 and 100 μmol L^{-1} was 18%, 25%, 33%, 23%, 27% and 34% lower than untreated fruit, respectively at the end of cold storage.

Fruit firmness gradually decreased during cold storage, but, this decrease was significantly delayed with GABA, MeJA and MeSA treatments (Fig. 1B). At the end of storage, firmness of the fruit treated with GABA at 20 and 40 mmol L^{-1}, MeJA at 50 and 100 μmol L^{-1}, and MeSA at 50 and 100 μmol L^{-1} was 7%, 8%, 16%, 10%, 9% and 12%, respectively higher than in control (Fig. 1B). In general, the 50 μmol L^{-1} MeJA treatment was the most effective for maintaining fruit firmness during cold storage (Fig. 1B), but this was depending on storage time.

TSS slightly increased in blood orange fruit in all treatments from 1 to 90 days of cold storage and then decreased. There were significant differences between treated and control fruit at the end of storage, the highest TSS being observed in control fruit (data not shown).

TA decreased for all treatments during cold storage (Fig. 1D). At the end of storage, untreated fruit had the lowest TA (0.73 ± 0.2). The highest TA was found in fruit treated with 100 μmol L^{-1} MeSA and 50 μmol L^{-1} MeJA at the end of cold storage (Fig. 1D). The reduction of TA was 44%, 40%, 35%, 21%, 29%, 36 and 19% at the end of cold storage compared with initial sampling time in control, 20 and 40 mmol L^{-1} GABA, 50 and 100 μmol L^{-1} MeJA, 50 and 100 μmol L^{-1} MeSA treated fruit, respectively (Fig. 1D).

Maturity index (TSS/TA ratio) increased during cold storage (Fig. 1C). In this study, untreated blood orange fruit had the highest maturity index at the end of cold storage. Blood orange fruit treated with 100 μmol L^{-1} MeSA and 50 μmol L^{-1} MeJA had the lowest TSS/TA ratio after long-term cold storage (Fig. 1C).

The concentration of sucrose, glucose and fructose were affected by the postharvest treatments during storage (Table 1). At the end of cold storage, the highest and the lowest sucrose were observed in control fruit and those treated with 40 mmol L^{-1} GABA, respectively. Glucose had a similar trend than sucrose (Table 1). At the end of storage, the lowest and the highest glucose content were measured in 40 mmol L^{-1} GABA and 50 μmol L^{-1} MeJA treated fruit, respectively (Table 1). Fructose increased at day 30 of cold storage and there was no significant difference among days 30, 60, 90 and 120. Fructose significantly decreased at the end of cold storage. At this time, fruit treated with 50 μmol L^{-1} MeJA had higher fructose than other treatments. In addition, the lowest fructose was measured in fruit treated with 40 mmol L^{-1} GABA, 100 μmol L^{-1} MeJA and 50 μmol L^{-1} MeSA without significant difference among them (Table 1).

The major organic acids of blood orange fruit were citric acid, malic acid, succinic acid and oxalic acid (Table 1). Oxalic acid was affected by treatment and storage time and increased at the end of cold storage (Table 1). At this time, 100 μmol L^{-1} MeSA and 50 μmol L^{-1} MeJA had higher oxalic acid than other treatments and the lowest oxalic acid content was found in fruit treated with 20 mmol L^{-1} GABA (Table 1). Citric acid increased up to 30 days and then slightly decreased to the end of cold storage (Table 1). Fruit treated with 100 μmol L^{-1} MeSA had a higher citric acid at the end of cold storage. The lowest citric acid content was measured in fruit treated with 40 mmol L^{-1} GABA, 100 μmol L^{-1} MeJA and 50 μmol L^{-1} MeSA without significant difference among them (Table 1). Malic acid slightly decreased during cold storage in comparison with initial sampling time (Table 1). Fruit treated with 100 μmol L^{-1} MeSA had the highest malic acid content among treated fruit. Fruit treated with 50 μmol L^{-1} MeJA, 100 μmol L^{-1} MeJA and 50 μmol L^{-1} MeSA had the lowest malic acid at the end of sampling time (Table 1). Succinic acid slightly increased at the end of cold storage; however no significant difference was found in comparison with initial sampling time. At the end of sampling time, 100 μmol L^{-1} MeSA had the highest succinic acid content among all treatments (Table 1).

3.2. Bioactive compounds and antioxidant activity

AA content gradually decreased in all treatments during cold storage (Fig. 2A). In this study, untreated fruit had the lowest AA content at the end of cold storage. Fruit treated with 50 μmol L^{-1} MeJA and 100 μmol L^{-1} MeSA had the highest AA content at the end of cold storage and no significant difference was observed between them (Fig. 2A).

The TPC of treated fruit gradually increased up to 90 days of cold storage and then decreased to the end of storage (Fig. 2B). Fruit treated with 100 μmol L^{-1} MeSA had the highest TPC after long-term cold storage. No significant difference was detected between 40 mmol L^{-1} GABA and 50 μmol L^{-1} MeJA for TPC at the end of cold storage. Untreated fruit had the lowest TPC during cold storage (Fig. 2B).

TAA decreased gradually in blood orange fruit in all treatments during cold storage (Fig. 2C). Significant difference ($P < 0.05$) was

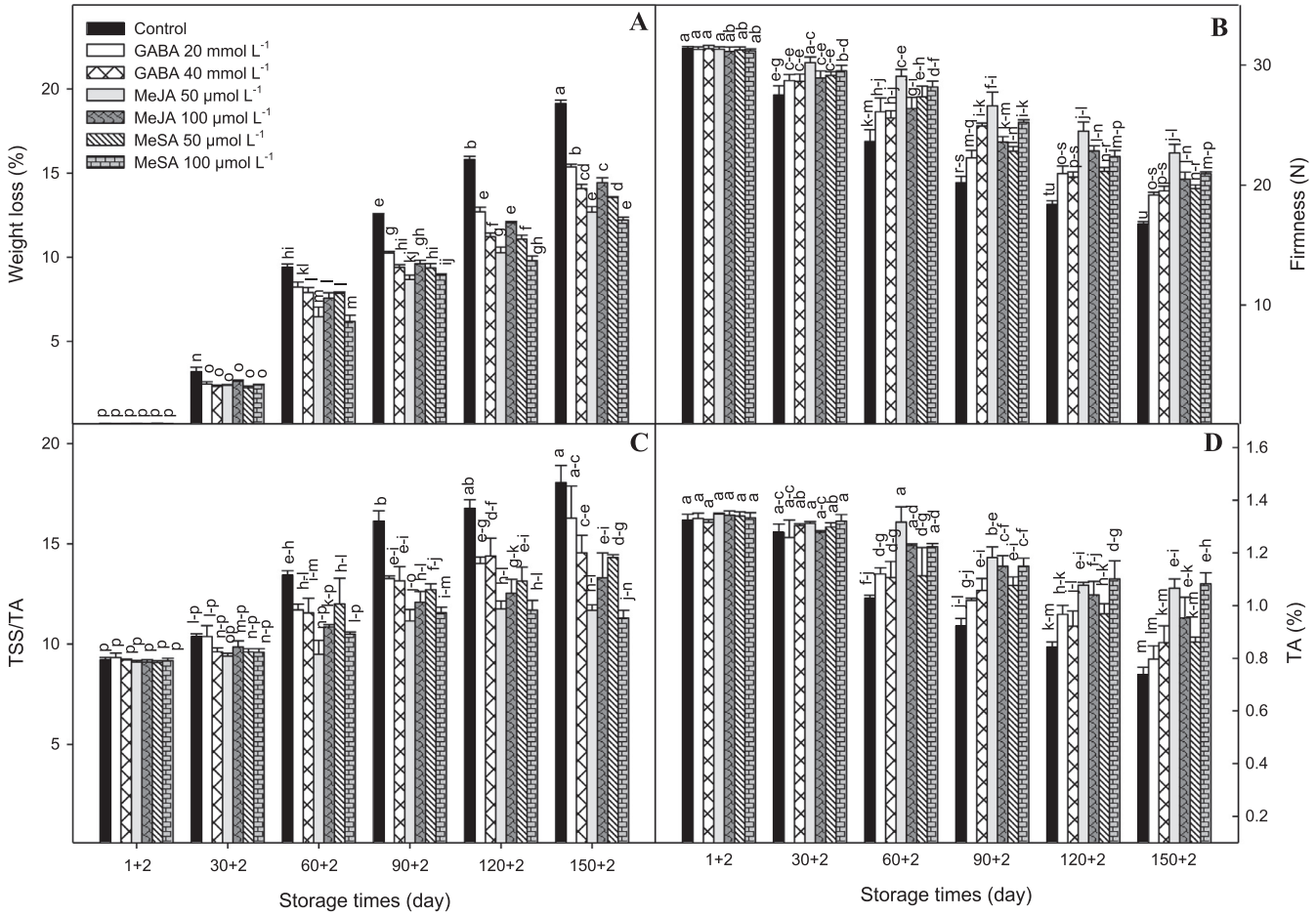

Fig. 1. Effect of treatments on physicochemical parameters; weigh loss (A), firmness (B), TSS/TA (C) and TA (D) of 'Moro' blood orange fruit during cold storage at 3 °C and 90% RH and subsequent 2 days storage at 20 °C. Vertical bars on columns represent ± standard deviation (SD) of means. Different letters above the bars on columns indicate significant difference at $P < 0.05$ level probability.

observed between treated and control samples during cold storage. TAA in fruit treated with GABA at 20 and 40 mmol L^{-1}, MeJA at 50 and 100 µmol L^{-1}, and MeSA at 50 and 100 µmol L^{-1} was 5%, 13%, 17%, 9%, 14% and 22% higher than untreated fruit, respectively. Overall, fruit treated with 100 µmol L^{-1} MeSA had the highest TAA after long-term cold storage (Fig. 2C).

TAC increased in blood orange fruit up to 90 days of cold storage and then slightly decreased (Fig. 2D). Control fruit had the lowest TAC in comparison with treated fruit at the end of cold storage (Fig. 2D). 40 mmol L^{-1} GABA, 50 µmol L^{-1} MeJA, and 100 µmol L^{-1} MeSA were the most effective treatments for increasing and maintaining TAC in blood orange fruit after long-term cold storage (Fig. 2D). These differences on TAC between control and treated fruit can be observed in Fig. 3 in which the internal colour appearance of 'Moro' blood orange fruit during cold storage is shown.

Individual HPLC analysis showed that cyanidin 3-glucoside and cyanidin 3-(6″-malonylglucoside) were the major individual anthocyanins in blood orange fruit (Fig. 2E and F). Cyanidin 3-glucoside and cyanidin 3-(6″-malonylglucoside) were higher in fruit treated fruit with 50 µmol L^{-1} MeJA and 100 µmol L^{-1} MeSA, than in contol after 90 days of cold storage (Fig. 2E and F).

3.3. Enzymes activity

POD activity was not detected in any treatments or sampling times.

CAT activity increased in all treated fruit up to 30 days of cold storage, remained relatively constant at 60 and 90 days of sampling time before it generally decreased to the end of cold storage (Fig. 4A). Fruit treated with 50 µmol L^{-1} MeJA and 100 µmol L^{-1} MeSA had a higher CAT activity at the end of cold storage. However, there was no significant difference between them (Fig. 4A).

APX activity was generally higher in treated fruit in comparison with control fruit follow cold storage at 30 days. The most APX activity was found after 30 days of cold storage and remained constant at day 60 and then slightly deceased to the end of storage. However, there was no significant difference among 90, 120 and 150 days of cold storage (Fig. 4B).

SOD activity increased in all treatments up to 60 days of cold storage, and then generally decreased (Fig. 3C). After 60 days of storage, SOD activity in fruit treated with 20, 40 mmol L^{-1} GABA and 100 µmol L^{-1} MeSA was significantly ($P < 0.05$) higher than other treated or untreated fruit (Fig. 4C).

PAL activity was significantly enhanced through postharvest treatments during cold storage (Fig. 4D). PAL activity increased in flesh of blood orange fruit in all treatments up to 90 days of cold storage and then decreased to the end of storage. At this time, the PAL activity in fruit treated with 20 and 40 mmol L^{-1} GABA, 50 and 100 µmol L^{-1} MeJA, 50 and 100 µmol L^{-1} MeSA was 10%, 14%, 12%, 17%, 8% and 19% higher than in control fruit, respectively (Fig. 4D). The most effective treatments for PAL activity enhancement during cold storage were 100 µmol L^{-1} MeSA and 50 µmol L^{-1} MeJA (Fig. 4D).

PPO activity increased in all treatments up to 90 days of cold storage and no significant differences were observed at days 90, 120 and 150 of cold storage (Fig. 4E). However, PPO activity was significantly lower in treated fruit in comparison with control samples during cold storage. The most effective treatment on reducing PPO activity during cold

Table 1
Effect of treatments on individual sugars (mg 100 g^{-1}) and individual of organic acids (mg 100 g^{-1}) of 'Moro' blood orange fruit during cold storage at 3 °C and 90% RH and subsequent 2 days storage at 20 °C.

Treatment	Storage time	Individual sugars			Individual organic acids			
		Sucrose	Glucose	Fructose	Oxalic acid	Citric acid	Malic acid	Succinic acid
Control	1 + 2	977 ± 62 f–k	586 ± 26 k–p	646 ± 13 p–q	0.74 ± 0.4 n–r	331 ± 3 e–h	74 ± 9 b–g	36 ± 5 g–j
GABA 20 mmol L^{-1}		1057 ± 18 d–f	612 ± 13 g–n	644 ± 18 p–q	1.08 ± 0.2 h–o	281 ± 3 m–o	75 ± 3 b–f	35 ± 3 g–j
GABA 40 mmol L^{-1}		1017 ± 9 e–i	612 ± 6 g–n	673 ± 6 n–p	0.69 ± 0.1 p–s	277 ± 1 n–p	64 ± 5 g–l	30 ± 1 g–j
MeJA 50 µmol L^{-1}		990 ± 3 f–j	594 ± 4 i–p	647 ± 5 p–q	2.61 ± 0.6 a	229 ± 9 *t*–u	51 ± 6 n–p	60 ± 9 a–f
MeJA 100 µmol L^{-1}		999 ± 5 e–j	558 ± 3 o–p	618 ± 10 q–r	0.51 ± 0.1 r–s	238 ± 4 s–t	52 ± 1 m–p	23 ± 6 h–j
MeSA 50 µmol L^{-1}		923 ± 22 j–m	578 ± 14 l–p	648 ± 12 o–q	1.11 ± 0.1 g–m	255 ± 3 q–s	64 ± 3 h–l	33 ± 5 g–j
MeSA 100 µmol L^{-1}		1037 ± 21 d–g	612 ± 9 g–n	659 ± 21 n–q	1.09 ± 0.2 h–n	289 ± 9 l–n	84 ± 6 b	61 ± 6 a–e
Control	30 + 2	1195 ± 12 c	775 ± 4 a	812 ± 8 f–h	0.79 ± 0.3 m–r	340 ± 3 d–g	76 ± 3 b–e	44 ± 4 e–h
GABA 20 mmol L^{-1}		1231 ± 2 b–c	765 ± 54 a–b	843 ± 15 d–g	1.00 ± 0.1 i–p	365 ± 2 b	73 ± 1 c–h	40 ± 2 1 e–i
GABA 40 mmol L^{-1}		1216 ± 6 b–c	712 ± 16 b–c	902 ± 13 a–c	0.89 ± 0.1 l–q	323 ± 5 g–j	74 ± 3 b–g	37 ± 3 g–j
MeJA 50 µmol L^{-1}		1103 ± 27 d	623 ± 16 f–m	723 ± 39 k–m	1.37 ± 0.2 d–h	282 ± 9 m–o	81 ± 8 b–c	46 ± 7 d–g
MeJA 100 µmol L^{-1}		1316 ± 16 a	709 ± 6 b–c	822 ± 4 e–g	0.86 ± 0.1 m–r	320 ± 2 h–j	75 ± 3 b–f	32 ± 3 g–j
MeSA 50 µmol L^{-1}		1225 ± 6 b–c	666 ± 4 c–g	814 ± 83 f–h	0.78 ± 0.2 m–r	356 ± 8 b–d	72 ± 4 c–i	32 ± 1 g–j
MeSA 100 µmol L^{-1}		1261 ± 7 a–c	693 ± 6 c–e	830 ± 2 d–g	0.73 ± 0.1 o–r	285 ± 3 l–o	71 ± 4 c–i	21 ± 2 i–j
Control	60 + 2	989 ± 1 f–j	694 ± 2 c–d	856 ± 2 d–f	1.23 ± 0.1 f–l	277 ± 1 n–p	73. ± 5 c–h	41 ± 6. e–f
GABA 20 mmol L^{-1}		1028 ± 8 d–h	649 ± 37 d–i	859 ± 32 c–e	1.90 ± 0.1 b–c	263 ± 7 p–q	67 ± 6 d–j	31 ± 9 g–j
GABA 40 mmol L^{-1}		1048 ± 1 d–f	651 ± 10 d–i	830 ± 12 d–g	0.98 ± 0.1 j–p	365 ± 6 b	66 ± 2 e–k	35 ± 5 g–j
MeJA 50 µmol L^{-1}		907 ± 3 k–n	688 ± 70 c–e	868 ± 38 b–d	1.70 ± 0.2 c–d	271 ± 2 o–q	67 ± 8 d–k	42 ± 5 e–i
MeJA 100 µmol L^{-1}		826 ± 18 n–p	538 ± 152 p–r	693 ± 42 m–o	1.64 ± 0.3 c–e	242 ± 4 r–t	54 ± 15 l–p	68 ± 4 a–c
MeSA 50 µmol L^{-1}		995 ± 5 f–j	664 ± 65 c–g	815 ± 31 e–h	1.14 ± 0.1 f–m	331 ± 5 e–h	75 ± 6 b–f	36 ± 2 g–j
MeSA 100 µmol L^{-1}		954 ± 3 h–k	637 ± 89 d–k	819 ± 50 e–g	1.49 ± 0.1 d–f	326 ± 7 f–i	69. ± 9 d–i	75 ± 7 0 a
Control	90 + 2	1043 ± 42 d–f	551 ± 31 o–q	704 ± 38 l–n	0.72 ± 0.1 p–r	297 ± 2 k–m	65 ± 4 f–k	45 ± 4 d–g
GABA 20 mmol L^{-1}		1296 ± 30 a–b	655 ± 16 c–h	870 ± 22 b–d	0.36 ± 0.2 s	403 ± 5 a	71 ± 2 c–i	35 ± 5 g–j
GABA 40 mmol L^{-1}		1031 ± 42 d–h	630 ± 23 f–l	859 ± 25 c–e	1.05 ± 0.1 h–p	345 ± 5 c–e	70 ± 8 d–i	41 ± 4 e–i
MeJA 50 µmol L^{-1}		1047 ± 12 d–f	610 ± 7 g–n	800 ± 24 g–i	0.80 ± 0.1 m–r	325 ± 2 f–j	63. ± 4 h–l	39 ± 5 f–i
MeJA 100 µmol L^{-1}		1017 ± 5 e–i	599 ± 2 h–o	839 ± 6 d–g	1.31 ± 0.1 e–g	288 ± 7 l–o	81 ± 9 b–c	43 ± 7 e–h
MeSA 50 µmol L^{-1}		1036 ± 14 d–g	647 ± 32 d–j	843 ± 24 d–g	0.55 ± 0.1 q–s	199 ± 3 v	31 ± 2 q	17 ± 2 j
MeSA 100 µmol L^{-1}		849 ± 3 m–o	583 ± 7 k–p	758 ± 7 i–k	1.36 ± 0.1 d–i	357 ± 2 b–d	62 ± 4 i–m	39 ± 4 e–i
Control	120 + 2	1020 ± 4 e–i	552 ± 3 o–q	742 ± 5 j–l	1.08 ± 0.1 h–o	362 ± 9 b–c	69 ± 3 d–i	46 ± 3 c–g
GABA 20 mmol L^{-1}		1104 ± 3 d	674 ± 2 c–f	924 ± 5 a	0.81 ± 0.1 m–r	258 ± 7 q–r	72 ± 1 c–i	37 ± 6 g–j
GABA 40 mmol L^{-1}		982 ± 5 f–k	570 ± 2 m–p	830 ± 23 d-j	0.94 ± 0.1 k–p	276 ± 9 n–p	71 ± 2 c–i	32 ± 5 g–j
MeJA 50 µmol L^{-1}		962 ± 4 g–k	663 ± 2 c–g	911 ± 10 ab	0.87 ± 0.1 m–r	331 ± 5 e–h	67 ± 3 d–j	39 ± 3 e–i
MeJA 100 µmol L^{-1}		845 ± 78 m–o	403 ± 28 t	591 ± 39 r–s	1.26 ± 0.1 f–k	230 ± 12 *t*–u	58 ± 9 j–n	45 ± 8 d–j
MeSA 50 µmol L^{-1}		1077 ± 24 d–e	630 ± 7 f–l	871 ± 24 b–d	0.82 ± 0.1 m–r	341 ± 2 d–f	78 ± 1 b–d	50 ± 6 b–g
MeSA 100 µmol L^{-1}		1040 ± 30 d–g	568 ± 9 m–p	774 ± 16 h–j	1.01 ± 0.14 h–p	340 ± 6 d–g	67 ± 2 e–k	40 ± 5 e–i
Control	150 + 2	1031 ± 20 d–f	551 ± 15 o–q	723 ± 19 k–m	1.46 ± 0 d–g	340 ± 5 d–g	96 ± 2 a	66 ± 5 a–d
GABA 20 mmol L^{-1}		992 ± 28 f–j	590 ± 38 j–p	694 ± 69 m–n	0.73 ± 0.1 n–r	312 ± 3 i–k	45 ± 8 p	31 ± 5 g–j
GABA 40 mmol L^{-1}		750 ± 20 p	464 ± 11 s	573 ± 20 r–s	1.69 ± 0.2 c–d	219 ± 4 u	56 ± 6 k–o	40 ± 6 e–i
MeJA 50 µmol L^{-1}		1004 ± 8 e–i	636 ± 4 e–k	855 ± 7 d–f	2.16 ± 0.8 b	303 ± 9 k–l	53 ± 9 l–p	69 ± 3 a–b
MeJA 100 µmol L^{-1}		798 ± 8 op	498 ± 1 q–s	572 ± 29 s	1.08 ± 0.1 h–o	217 ± 6 u	47 ± 4 n–p	35 ± 3 g–j
MeSA 50 µmol L^{-1}		870 ± 4 l–o	483 ± 4 r–s	571 ± 9 s	1.66 ± 0.1 c–e	309 ± 1 j–k	49 ± 1 n–p	44 ± 7 e–h
MeSA 100 µmol L^{-1}		945 ± 14 k–j	576 ± 1 l–p	766 ± 9 i–k	2.22 ± 0.2 b	357 ± 9 b–d	84 ± 2 b	48 ± 10 b–g

Different letters in the same column indicate significant difference at $P < 0.05$ level probability. The results are represented ± standard deviation (SD) of means.

storage was 100 µmol L^{-1} MeSA (Fig. 4E).

3.4. Sensory quality

In this study, edible quality of blood orange fruit was evaluated for each treatment and sampling time (Fig. 5A–D). The edible quality of fruit was similar in all treated fruit at days 1 and 30 of cold storage, therefore results are not shown. Acceptability of edible quality decreased after 60 days of cold storage in untreated fruit, but it was maintained similarly in all treatments at this sampling date (Fig. 5A). The edible quality decreased in treated fruit after 90 days of cold storage; however, control fruit had the lowest sensory acceptability after 120 and 150 days of cold storage (Fig. 5B–D). Regarding sensory evaluation, MeJA and MeSA treatments were more effective than vacuum infiltration of GABA for acceptability of edible quality. Overall, fruit treated with 100 µmol L^{-1} MeSA efficiently maintained edible quality of 'Moro' blood orange fruit after long-term cold storage (Fig. 5D).

4. Discussion

With the aim to improve quality and enhance the content of bioactive compounds of blood orange during prolonged cold storage, postharvest treatments with GABA, MeSA and MeJA were performed. All treatments reduced WL compared with control fruit during 150 days of storage (Fig. 1A). Transpiration of fruit occurs due to water diffusion through peel surface and it is one of the most important factors which can affect the WL of fruit during storage. Furthermore, WL is caused by metabolic activities, such as respiration, and cellular breakdown due to CI damages at low temperature (Habibi & Ramezanian, 2017). Thus, the reduction of WL found in treated fruit could be attributed to their effect on maintaining membrane integrity at low temperature according to previous report on pomegranate (Sayyari et al., 2011).

Firmness is one of the most important factors for determining the acceptability of fruit (Khorram et al., 2017). In this study, all treated fruit in the late removals had higher firmness than control. Pectic compounds are considered as cell wall structural polysaccharides are responsible for the fruit firmness and softening occurs when cell-wall degrading enzymes are activated leading to pectin depolymerization

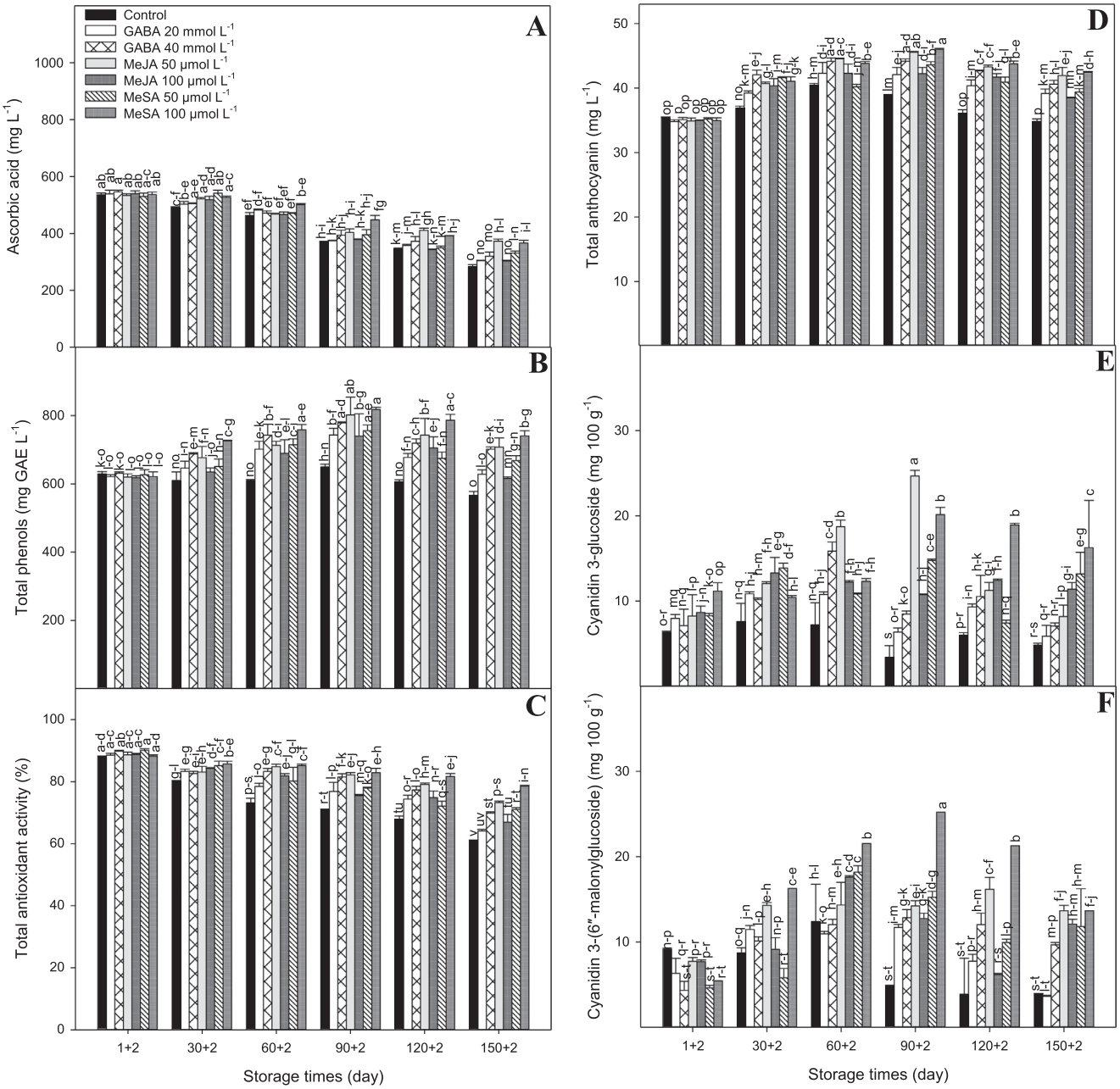

Fig. 2. Effect of treatments on ascorbic acid (A), total phenolic content (B), total antioxidant activity (C) total anthocyanin concentration (D), cyanidin-3-glycoside (E) and Cyanidin 3-(6″-malonylglucoside) (F) of 'Moro' blood orange fruit during cold storage at 3 °C and 90% RH and subsequent 2 days storage at 20 °C. Vertical bars on columns represent ± standard deviation (SD) of means. Different letters above the bars on columns indicate significant difference at $P < 0.05$ level probability.

associated with activities of polygalactrunase, pectin lyase, pectin methylesterase and cellulase (Valero & Serrano, 2010). Therefore, prevention of the activity of cell-wall degrading enzymes could be effective for maintaining fruit firmness as reported for sweet cherry fruit treated with MeJA (Giménez et al., 2016).

TSS increased up to 90 days of cold storage and then slightly decreased (data not shown) as did sucrose, the major sugar in blood orange fruit, and probably due to senescence or to their use in respiratory reactions for providing ATP during long-term storage as reported by Huang, Liu, Lu, and Xia (2008).

TA decreased in all treatments during storage, although in general, control fruit had lower TA values than control during the whole storage period. TA is related to the amount of organic acids, which highly affect fruit quality, while their reduction during cold storage is related to their use as main respiratory substrates for energy production during storage (Habibi & Ramezanian, 2017). Furthermore, sugars can be synthesised from organic acids and consequently, leading to reduce TA (Rapisarda et al., 2008). In fact, the main organic acids, citric, malic, succinic, oxalic and ascorbic acids found in blood orange fruit decreased during cold storage. However, it is worth noting that treated fruit had higher organic acid concentration than control which could be attributed to the reduction in the fruit respiration rate and to the delay of fruit senescence leading to maintenance of nutritional quality of fruit and to prolong storage period (Valero et al., 2011).

The maturity index (TSS/TA ratio) is also an important indicator for determining fruit quality and marketability (Khorram et al., 2017), and its increase during cold storage was due to the reduction of organic acids and the slight increase in sugars as storage advanced. However, all

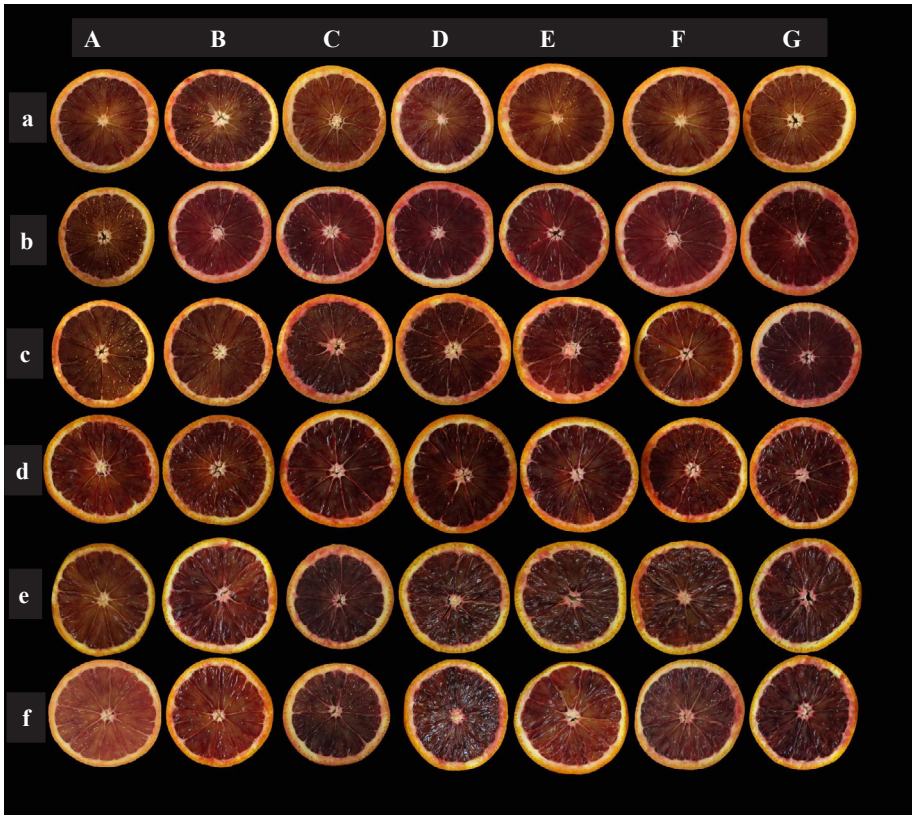

Fig. 3. Internal color appearance of 'Moro' blood orange fruit during cold storage at 3 °C and 90% RH; columns (A), control; (B), 20 mmol L^{-1} GABA; (C), 40 mmol L^{-1} GABA; (D), 50 μmol L^{-1} MeJA; (E), 100 μmol L^{-1} MeJA; (F), 50 μmol L^{-1} MeSA; (G), 100 μmol L^{-1} MeSA, rows (a), 1 days after storage plus 2 days at 20 °C; (b), 30 days after storage plus 2 days at 20 °C; (c), 60 days after storage plus 2 days at 20 °C; (d), 90 days after storage plus 2 days at 20 °C; (e), 120 days after storage plus 2 days at 20 °C; (f), 150 days after storage plus 2 days at 20 °C.

treatments delayed significantly the increase in maturity index, showing a possible effect on reducing the postharvest ripening process (Valero & Serrano, 2010).

TAC increased in blood orange fruit for the first 90 days of cold storage. TAC of blood orange fruit after harvest has been shown to increase due to cold stress conditions and also to the treatments with chemical elicitors (Habibi & Ramezanian, 2017). Anthocyanin synthesis in blood oranges after harvest depends on the activity of PAL enzyme. The enhancement of PAL activity in blood orange is regulated through gene expression in response to low temperature (Lo Piero, Puglisi, Rapisarda, & Petrone, 2005). In this study, PAL activity significantly higher in all blood orange treated fruit than in control during the whole cold storage period. MeSA is involved for enhancing TAC by stimulating the activity of the PAL enzyme activity (Valero et al., 2011). Cyanidin 3-glucoside and cyanidin 3-(6″-malonylglucoside) have been reported as the two main anthocyanins in blood oranges (Lee, 2002). In this study, cyanidin 3-(6″-malonylglucoside) was generally greater than cyanidin 3-glucoside in blood orange fruit (Fig. 2E and F). Similar to TAC, cyanidin 3-glucoside and cyanidin 3-(6″-malonylglucoside) were higher in fruit treated with MeJA and MeSA.

Lower TAC was observed in untreated fruit after 90 days of cold storage. Some physical and chemical factors such as pH, temperature, oxygen availability, enzymes activity, AA, sugars and sugar degradation products can affect the anthocyanins stability and degradation (Lo Piero, 2015). Storage temperatures can also induce anthocyanin synthesis in response to chilling injury in fruit (Tsantili, Shin, Nock, & Watkins, 2010). Cell vacuoles are the main places for anthocyanin degradation (Lo Piero, 2015). During long-term cold storage, internal pigment of blood orange can undergo modifications that lead to the conversion of poly-condensed compounds. Furfural and hydroxyl methyl furfural are chemical indicators of sugar and AA degradation during polymerization process. The main reasons of the formation of these polymers are reactions between blood orange pigments and the previously mentioned intermediates compounds of degradation of sugar and AA in an acidic condition (Krifi & Metche, 2000). Therefore, anthocyanin degradation can happen after long-term cold storage.

Enzymatic studies of anthocyanin degradation have shown that PPO and POD can degrade anthocyanin during postharvest storage of oranges. These enzymes generally can act for hydrolysing and oxidizing the anthocyanin molecule during senescence of fruit (Catalano, Ingallinera, Todaro, Rapisarda, & Spagna, 2009). It has been reported that POD activity with direct oxidation and PPO activity are more probable for anthocyanin degradation (Lo Piero, 2015). However, in this study, POD activity was not detected in blood orange fruit, therefore it is not the candidate route for anthocyanin degradation. In addition, PPO activity seemed to have low action on anthocyanin degradation probably because of the blood oranges strongly acidic environment. Furthermore, higher content of citric acid and AA are considered as inhibitors of PPO activity (Catalano et al., 2009). In this study, treated fruit had a low pH and higher citric acid and AA in comparison with control samples. Therefore, low PPO activity may explain why the anthocyanin degradation was lower than in treated fruit. MeSA was the most effective treatment for maintaining TAC during cold storage, due to higher PAL and lower PPO activity (Fig. 4D and E).

Both enzymatic and non-enzymatic antioxidant systems are responsible for scavenging free radicals and reactive oxygen species (ROS) in the stored fruit under cold stress (Sayyari et al., 2011). TAA in blood oranges derives from antioxidant enzymes and bioactive compounds including TAC, TPC and AA that are responsible for the antioxidant system (Habibi & Ramezanian, 2017). Phenolic compounds, including anthocyanins, are considered as non-enzymatic antioxidant compounds sine they are electron donors contributing to neutralize free radicals (Proteggente, Saija, De Pasquale, & Rice-Evans, 2003; Valero et al., 2011). Indeed, the reduction of TPC previously reported in orange fruits during cold storage has been attributed to their enzymatic oxidation mainly due to PPO and POD activities (Rapisarda et al., 2008; Valero et al., 2011). However, the present results show than in blood orange the higher reduction in TPC found in control samples was due to higher PPO and lower PAL activities since no POD activity was

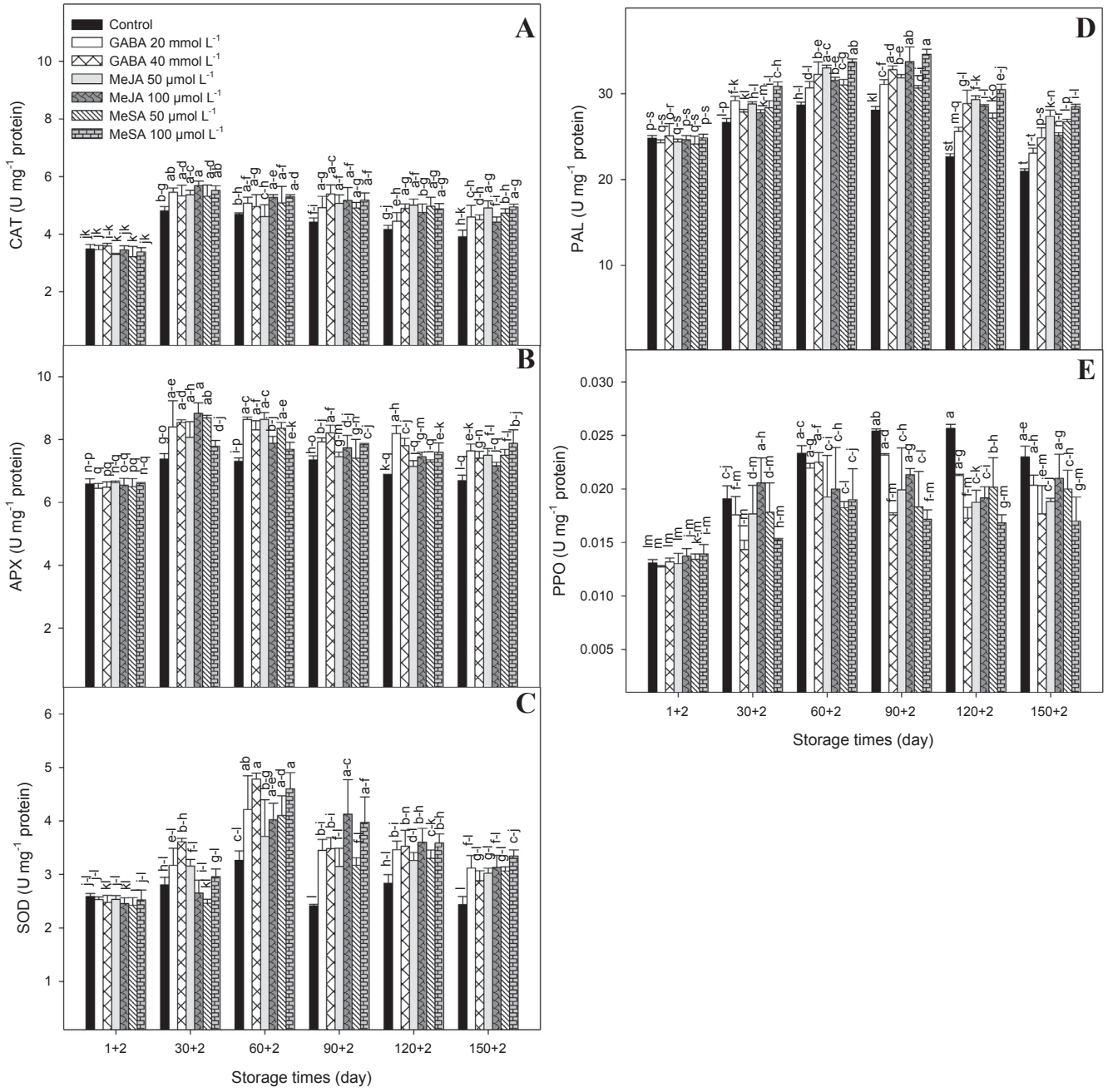

Fig. 4. Effect of treatments on activities of CAT (A), APX (B), SOD (C), PAL (D) and PPO (E) of 'Moro' blood orange fruit during cold storage at 3 °C and 90% RH and subsequent 2 days storage at 20 °C. Vertical bars on columns represent ± standard deviation (SD) of means. Different letters above the bars on columns indicate significant difference at $P < 0.05$ level probability.

detected.

It has been reported that there was a positive correlation between TAC and TPC with TAA in blood orange cultivars (Habibi & Ramezanian, 2017). Treated fruit had higher TAA than control fruit and the reduction of TAA in control samples was probably related to fruit senescence after prolonged storage (Habibi & Ramezanian, 2017). In this study, treated fruit had more TPC and subsequently higher antioxidant capacity at the end of storage than control one. Accordingly, TPC enhanced antioxidant capacity in kiwifruit (Du, Li, Ma, & Liang, 2009). Moreover, fruit treated with MeSA had the highest TPC, which could be attributed to the highest stimulation found on PAL activity (Sayyari et al., 2011). Antioxidant enzymes, including CAT, APX and SOD can act as ROS scavengers (Giménez et al., 2016). In this study CAT, APX and SOD activities increased at initial storage time and decreased during cold storage, which could have decreased the TAA. Overall, non-enzymatic antioxidant system in blood orange fruit might be considered as the most important mechanism for scavenging ROS. Among the commercial citrus fruit, only blood oranges have anthocyanin and this pigment can affect the antioxidant capacity (Habibi & Ramezanian, 2017).

AA decreased during cold storage. AA is extremely susceptible to degradation due to oxidation during cold storage. Low temperatures can increase the accumulation of free radicals. AA is a main and prominent non-enzymatic antioxidant in citrus fruit that has the potential to eliminate free radical in the cells (Huang et al., 2008). Therefore, the main reason for reducing AA may be attributed to scavenging ROS by non-enzymatic system as an electron donor to neutralize free radicals during low temperature storage. AA degradation depends on

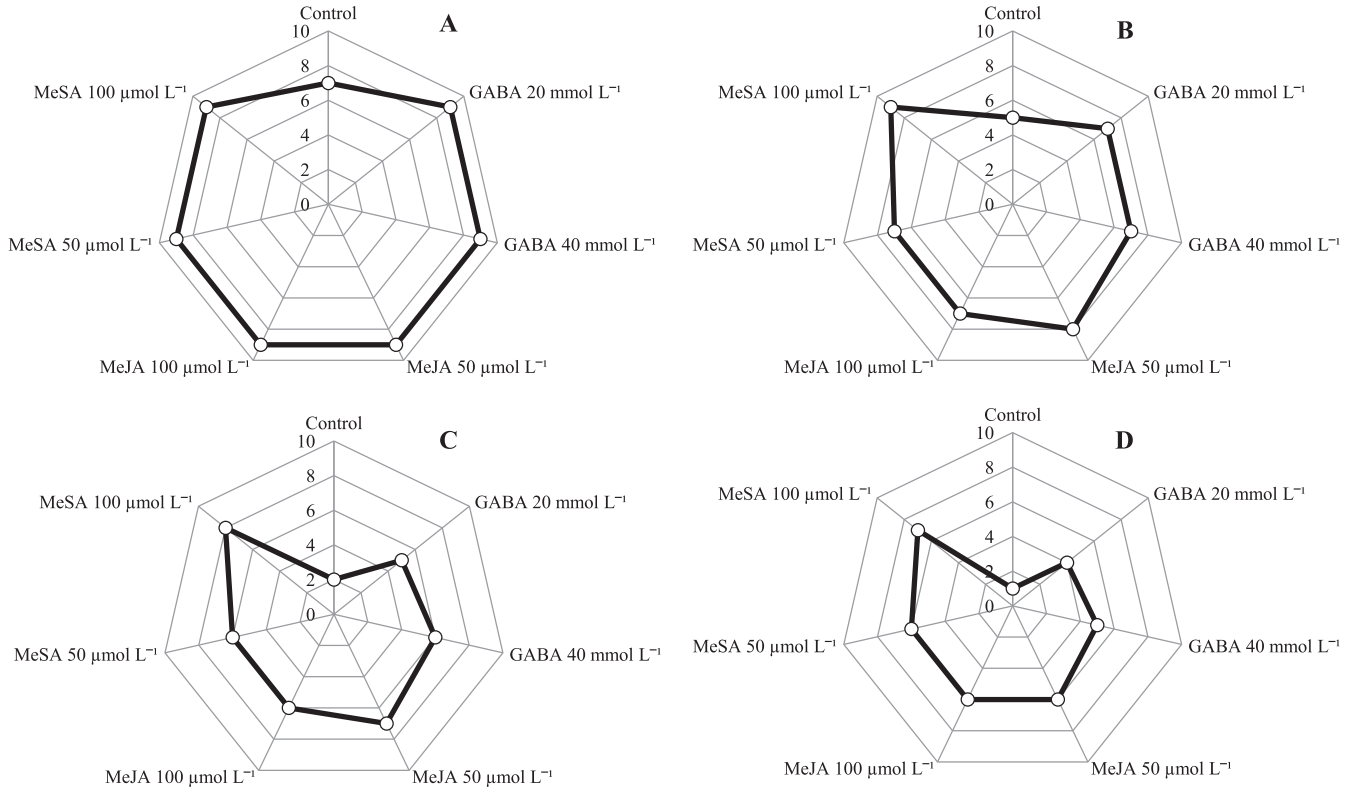

Fig. 5. Effect of treatments on edible quality of 'Moro' blood orange fruit. Descriptive sensory analysis tests were performed by a trained panel after 60 (A), 90 (B), 120 (C), and 150 (D) days of storage at 3 °C and subsequent 2 days storage at 20 °C.

endogenous oxidation due to the action of several enzymes including ascorbate oxidase and POD (Plaza et al., 2011). In addition, reduction of AA is associated with fruit senescence after prolonged storage (Habibi & Ramezanian, 2017). In this study, fruit treated with 50 µmol L^{-1} MeJA and 50 and 100 µmol L^{-1} MeSA had a higher AA content at the end of cold storage.

Although blood orange fruit are non-climacteric fruits, they exhibit some biochemical changes during the postharvest life, and the low metabolic activity of these fruit can lead to large effect on fruit quality during long-term cold storage (Habibi & Ramezanian, 2017). It has been reported that organic acids are reduced faster than sugars during storage, so it is expected that the fruit flavour changes after long-term storage. The fruit taste depends on the amount of organic acids and sugars and also on their ratio (Khorram et al., 2017). During prolonged storage, edible quality of blood orange fruit gradually decreased and control samples had the lowest sensory acceptability according to the test panel. The main reason for decreasing of edible quality probably is the production of compounds of the fermentative metabolism (Khorram et al., 2017). Sensory evaluation demonstrated that treated fruit had the best edible quality until 90 days of cold storage according to the panellist evaluation. However, 100 µmol L^{-1} MeSA was the best treatment for maintaining edible quality of 'Moro' blood orange fruit after long-term cold storage.

5. Conclusion

In this study, we reported for the first time that postharvest treatments with GABA, MeJA and MeSA are a new approach to maintain the bioactive compounds and nutritional quality of blood orange during prolonged cold storage, being also MeJA and MeSA treatments effective on maintaining sensory quality. Non-enzymatic compounds, mainly anthocyanins, were the major antioxidant system in blood orange fruit while antioxidant enzymes (CAT, SOD and APX) seem no to have a pivotal role in this process. Based on results, sensory quality was maintained up to 60 days of cold storage in control fruit, while this time could be extended in all treated fruit, based on bioactive compounds and edible quality, the highest extension, even up to 150 days, being obtained with 100 µmol L^{-1} MeSA treatment. In addition, anthocyanin degradation was attributed to PPO activity since it was reduced in treated fruit. Overall, vapour treatments of MeSA and MeJA had more positive effect than vacuum infiltration of GABA for maintaining bioactive compounds and nutritional and sensory quality of blood orange fruit during long-term cold storage throughout a delay of the fruit senescence process.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.125634.

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