

Effects of 1-MCP on postharvest quality and internal browning of white-flesh loquat fruit during cold storage

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Summary

Introduction – Treatments with 1-methylcyclopropene (1-MCP), an inhibitor of ethylene perception, were investigated on white-flesh loquat fruit to extend its shelf life and to prevent chilling injury. **Materials and methods** – The loquat (*Eriobotrya japonica* Lindl.) white-flesh fruits (cv. Claudia) were submitted to applications of 1-MCP at 1 °C for 20 h and at concentrations from 1 to 5 $\mu\text{L L}^{-1}$. The treated fruits were stored at 1 °C for 21 days before removal to 20 °C for ripening (shelf life). Fruit quality was analyzed at harvest, at the end of each storage period (7, 14, 21 days at 1 °C) and at 0, 2 and 5 days during the shelf life at 20 °C. **Results and discussion** – The 1-MCP treatment slowed fruit softening depending on concentration, and extended the period before the fruit became over-soft. The untreated fruits exhibited severe chilling symptoms manifested as flesh leatheriness and internal browning after 21 days of storage at 1 °C. The treatment with 1 $\mu\text{L L}^{-1}$ 1-MCP significantly inhibited increases in fruit firmness and internal browning index and maintained fruit weight during cold storage and shelf life, thereby delaying the development of chilling injury and maintaining fruit quality. **Conclusion** – Application of 1-MCP can extend postharvest life of rapidly softening, perishable fruit like white-flesh loquat.

Keywords

Italy, loquat, *Eriobotrya japonica*, 1-methylcyclopropene, chilling injury, fruit firmness, phenolics, shelf life

Résumé

Efficacité du 1-méthylcyclopropène sur la qualité post-récolte et le brunissement interne des fruits du loquat à chair blanche après stockage au froid.

Introduction – Des traitements au 1-méthylcyclopropène (1-MCP), un inhibiteur des récepteurs de l'éthylène, ont été étudiés sur les fruits du loquat à chair blanche afin de prolonger leur durée de vie et de prévenir les lésions liées au froid. **Matériel et méthodes** – Les fruits du néflier du Japon (*Eriobotrya japonica* Lindl.) à chair blanche (cv. Claudia) ont été soumis à des applications de 1-MCP à 1 °C pendant 20 h et à des concentrations de 1 à 5 $\mu\text{L L}^{-1}$. Les fruits traités ont été conservés à 1 °C pendant 21 jours avant de les placer à 20 °C pour maturation (durée de conservation). La qualité des fruits a été analysée à

Significance of this study

What is already known on this subject?

- Applications of 1-MCP is useful also in non-climacteric fruits, reducing the development of chilling injury and maintaining quality in cold-stored loquat fruit.

What are the new findings?

- Application of 1-MCP can extend postharvest life of rapidly softening, perishable fruit like white-flesh loquat.

What is the expected impact on horticulture?

- White-flesh loquat fruit marketing is limited by its high perishability; the combination of packaging and 1-MCP treatment can be helpful in its postharvest management.

la récolte, à la fin de chaque période de stockage (7, 14, 21 jours à 1 °C) et à 0, 2 et 5 jours au cours de la conservation à 20 °C. **Résultats et discussion** – Le traitement au 1-MCP a ralenti le ramollissement du fruit selon la concentration utilisée, et a prolongé la période avant laquelle le fruit devient trop mou. Les fruits non traités ont présenté de graves symptômes liés au froid tels que la chair qui se tanne (aspect cuirassé) et brunit en interne après 21 jours de stockage à 1 °C. Le traitement au 1-MCP à 1 $\mu\text{L L}^{-1}$ a inhibé de manière significative l'augmentation de fermeté des fruits ainsi que l'indice de brunissement interne, et a maintenu la masse des fruits pendant le stockage au froid et en conservation, retardant ainsi le développement de lésions liées au froid et maintenant la qualité des fruits. **Conclusion** – L'application de 1-MCP peut prolonger la vie post-récolte des fruits périssable ayant une tendance au ramollissement rapide tels que les nèfles à chair blanche.

Mots-clés

Italie, néflier du Japon, *Eriobotrya japonica*, fermeté du fruit, lésions liées au froid, composés phénoliques, aptitude à la conservation

Introduction

Loquat (*Eriobotrya japonica* Lindl.) is a subtropical evergreen tree belonging to the *Rosaceae*, subfamily *Maloideae*, and it had its origin in south-eastern China. It blooms in fall and early winter and its white flowers give rise to yellow-fleshed or white-fleshed spherical-oval pomes. Loquat is cultivated in the subtropical regions of southern

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China, Japan, northern India and Israel (Lin *et al.*, 1999) and, more recently, the production has expanded in subtropical and tropical areas of many countries including Turkey, Brazil, Spain and Italy (Lin, 2007). Even though in the Mediterranean countries it was considered among the underutilized fruit tree species for its production and consumption (Llácer, 1996), loquat is well adapted, cultivated and commercially diffused in many areas (Gisbert *et al.*, 2007). In Italy more than 98% of the internal production, about 6,000 t, comes from Sicily (ISTAT, 2014). The fruit ripening period is concentrated in a few months of the year: March, April, and May (Calabrese *et al.*, 2003). Loquat cultivation is based on commercial cultivars and local ecotypes. The international high-quality cultivars were imported from Spain, California, China whereas local cultivars were originated from breeding programs carried out in Italy (Baratta *et al.*, 1995).

Loquat has been classified as a non-climacteric fruit (Blumenfeld, 1980): recent studies confirm that loquat fruit lacks the ability of autocatalysis of ethylene production, which is an essential feature characterizing climacteric fruits (Reig *et al.*, 2016). The development of loquat fruit occurs in two phases: a growth phase (growth of the seed), and a maturation phase with ripening-related changes (decreasing of organic acid contents, color changes and softening of the pulp, increasing of bioactive compound contents) (Agustí *et al.*, 2006; Koba *et al.*, 2007). In the last phase fruit senescence, titratable acidity declines, accompanied by a loss of flavor and taste, and juiciness decreases. Ethylene and CO₂ production gradually decline during fruit maturation (González *et al.*, 2003).

After harvest, loquat fruit are very perishable and sensitive to mechanical injury and microbial decay. Low temperature has been reported to inhibit fruit respiration and ethylene production, and thus extend storage life, although significant chilling injury occurs at 1 °C (Cai *et al.*, 2006b). Chilling injury, browning and purple spot are major problems, and the fruit are susceptible to various postharvest diseases, especially following mechanical injury (Pareek *et al.*, 2013).

Softening of fruit tissue is often the most apparent change that occurs after harvest in both climacteric and non-climacteric fruit. In loquat, however, firmness tends to increase during ripening and senescence (Cai *et al.*, 2006b). Several studies have shown an unusual increase in postharvest firmness at low temperatures, described as a chilling injury symptom, due to tissue lignification and development of a leathery (flesh leatheriness) and juiceless pulp (Zheng *et al.*, 2010). Lurie and Crisosto (2005) observed more frequently chilling injury symptoms, like internal browning and reddening, in white-flesh peach cultivars. There is a lack of information about chilling injury incidence and postharvest performance of white-flesh sub-acid loquat fruit that is highly appreciated by consumers.

The 1-methylcyclopropene (1-MCP), an inhibitor of ethylene perception, was found to delay ripening and improve postharvest quality of climacteric fruits; an application of 5 µL L⁻¹ 1-MCP could extend shelf life of rapidly softening and perishable fruits such as early season, melting and white-flesh stone fruits like peach or nectarine (Liguori *et al.*, 2004; Lurie and Paliyath, 2008). Applications of 1-MCP were useful also in non-climacteric fruits as strawberry fruit, maintaining firmness and color and extending postharvest fruit life (Jiang *et al.*, 2001). Several authors reported that an application of 1-MCP reduced the development of chilling injury and maintained quality in cold-stored loquat fruit (Lurie and Paliyath, 2008; Cai *et al.*, 2006b). Liguori *et al.*

(2014) demonstrated that 1-MCP treatment was effective in terms of softening inhibition in a short cold storage period in white- and yellow-flesh loquat fruits; however, different aspects remain unclear, such as the different behavior of white-flesh cultivars during extended cold storage and the effect of 1-MCP treatment on those fruit, that more than often are very perishable.

The present study was conducted to determine if 1-MCP applied during storage at low temperature (1 °C) (Cao *et al.*, 2011) could be used on a white-flesh, high perishable, loquat fruit to maintain postharvest fruit quality and prevent internal browning and leathery flesh (chilling injury) during cold storage. Our aim was to understand the effect of 1-MCP treatment on fruit senescence, on chilling injury symptoms, on some biocompounds (total phenolics) and the antioxidant capacity during cold storage.

Materials and methods

Fruit treatment

Loquat white-flesh sub-acid fruits (cv. Claudia) were harvested from a commercial orchard, located in Palermo (38°04'N, 13°22'E, 150 m a.s.l.). Fruits were hand-picked at the ripe stage (light orange peel), suitable for the fresh fruit market. Immediately after harvest fruit quality parameters were analyzed.

The fruits were placed at 1 °C and 95% relative humidity (RH) in 30-L closed containers and treated with 1 and 5 µL L⁻¹ 1-MCP for 20 h. The 1-methylcyclopropene formulation (Smartfresh, Italy), a.i. of 0.14%, was weighed into a test tube and capped. Just before treatment, 5 mL of 40 °C water was added to the test tube. The tube was immediately closed, vortexed and placed in the jar with the fruit, and opened. The 30-L plastic jar was immediately sealed.

Three replicates of ten (10) fruits for each of the control and treatments (0, 1 and 5 µL L⁻¹ 1-MCP) were stored at 1 °C and 95% RH for 7, 14, 21 days, and weight loss was measured at the end of the storage period on 10 fruits per treatment. A total number of 300 fruits ($3_{\text{blocks}} \times 10_{\text{repl.}} \times 3_{\text{treatments}} \times 3_{\text{storage periods}} + 30_{\text{fruits}}$) were used after storage to measure firmness, total soluble solids, titratable acidity, browning index and flesh leatheriness. At the end of the 21 days storage period another set of fruit ($n=300$) were left at 20 °C for 2 and 5 days to simulate shelf life conditions.

Quality parameters: firmness, total soluble solids, titratable acidity and flesh disorders

The fruits were analyzed at harvest, at the end of each storage period (7, 14, 21 days at 1 °C) and at 0, 2 and 5 days during the shelf life at 20 °C. Firmness was measured on opposite paped cheeks of each fruit with a digital penetrometer (model 53205, Tr Turoni, Forlì, Italy) incorporating an 8-mm diameter probe, after removal of a small piece of peel. A wedge-shaped slice of flesh was taken longitudinally from each fruit and ten fruit wedges were peeled and juiced. Total soluble solids (TSS, in °Brix) were determined by digital refractometer (Palette PR-32, Atago Co., Ltd.) and titratable acidity (TA) by titration of 10 mL juice with 0.1 N NaOH to pH 8.1 and expressed as % malic acid (model S compact titrator, Crison Instruments, Barcelona, Spain). Ten fruits for each treatment were periodically weighed to observe their weight loss. After measurement of firmness and sampling for TSS and TA, fruits were halved and examined visually for flesh disorders and browning. Flesh browning was assessed by measuring the extent of browned area on each fruit on the

following scale: 0 = no browning; 1 = less than 1/4 browning; 2 = 1/4–1/2 browning; 3 = 1/2–3/4 browning; 4 = more than 3/4 browning. The browning index (BI) was calculated, using the following formula:

$$BI = \frac{(1 N_1 + 2 N_2 + 3 N_3 + 4 N_4) \times 100}{4 N}$$

where N is the total number of fruit measured and N₁, N₂, N₃ and N₄ are the number of fruits showing different degrees of browning (Wang *et al.*, 2005). Data were submitted to analysis of variance (ANOVA) and means were separated with Tukey's test at $P \leq 0.05$. The statistical analysis was carried out using Systat 10 (Systat, USA).

Total phenolics and antioxidant capacity

To prepare the fruit extracts, 5 g flesh tissue from each sampling data (harvest, 7, 14, 21 days of storage) was homogenized with 5 mL of 950 mL L⁻¹ cold ethanol and centrifuged at 10,000 × *g* for 15 min, and a further 5 mL of 800 mL L⁻¹ cold ethanol extracted the residua again. The supernatants were combined to make a final volume of 25 mL. The ethanol extract was used for analysis of total phenolics and antioxidant capacity. Total phenolic content in loquat fruit extracts was determined according to the Folin-Ciocalteu procedure (Slinkard and Singleton, 1977), and results were expressed as g of gallic acid equivalent (GAE) kg⁻¹ fresh weight (FW).

The DPPH radical scavenging activity of the sample extract was estimated following the method of Larrauri *et al.* (1998). An aliquot (0.1 mL) of the ethanol extract was added to 2.9 mL DPPH (120 μmol L⁻¹) in methanol. The absorbance at 517 nm was measured after the reaction mixtures were incubated for 30 min at 30 °C in the dark. A solvent containing 120 mol L⁻¹ DPPH without mixing with the sample solution was used as control. The result was calculated according the following formula:

$$\text{radical scavenging activity (\%)} = 100 - \frac{\text{absorbance of sample}}{\text{absorbance of control}} \times 100$$

A statistical analysis was performed using analysis of variance (ANOVA) and the least significant difference (LSD) test to determine statistically different values at a significance level of $P < 0.01$ and $P < 0.001$ with the SPSS software package (version 13).

Sensory evaluation

At the end of cold storage period (21 days at 1 °C) and shelf life (20 °C), 10 fruits for each treatment were subjected to sensory evaluation. The sensory profile (UNI 10957, 2003) was defined by a panel of 10 judges that were trained to recognize the qualitative characteristics to be assessed and, in a preliminary meeting, generated 17 attributes, on the basis of frequency of citation (>60%) sorted by groups: 2 for aspect (flesh color, ground color), 3 for odor (loquat, herbaceous, floral), 4 for taste (sourness, sweetness, acidity, astringency), 4 for rheological (hardness, easy peel, easy stone, juiciness), 3 for flavor (loquat, herbaceous, floral) and finally an overall assessment. A discontinuous scale was utilized for evaluation. The left side of the scale corresponded to the absence of the sensation (value 1.0) and the right side corresponded to the highest intensity (value 9.0). The evaluations were carried out from 10:00 to 12:00 a.m. in individual booths illuminated by white light (UNI EN ISO

8589, 2010). The order of presentation was randomized for each judgement and water was provided for rinsing between fruit samples. A computerized data collection program was used (FIZZ, Software Solutions for Sensory Analysis and Consumer Tests, Biosystemes, Couternon, France). The sensory data for each attribute were submitted to one way Analysis of the Variance (ANOVA) using samples as factors.

Results and discussion

Effects of 1-MCP on fruit quality aspects

The treatments with 1-MCP, an inhibitor of ethylene perception, resulted in lower chilling injury symptoms and smaller flesh leathery development, when compared to the control fruit. Fruit firmness, weight loss and internal browning were used to evaluate the effect of 1-MCP on the development of chilling injury symptoms during cold storage of white flesh loquat fruit. In particular, in loquat fruit, firmness increased while extractable juice rate decreased after low-temperature storage, and finally resulted in a dry and firm texture (leatheriness) (Agustí *et al.*, 2006).

Applications of 1-MCP (at 1 and 5 μL L⁻¹) delayed softening of white-flesh 'Claudia' loquat fruits during cold storage and prevented fruit flesh leatheriness. Untreated loquat fruits became firmer (leathery) during cold storage, with the process being prevented by 1-MCP (Figure 1A). Firmness of the untreated fruits was 17% higher than at harvest, instead of 1- and 5-μL L⁻¹ 1-MCP treated fruits, were 10 and 4% lower than at harvest, respectively, after 21 days at 1 °C (Figure 1A). Leathery flesh appeared in untreated and 5-μL L⁻¹ 1-MCP treated fruits at the subsequent shelf life (Figure 1B); after 5 days at 20 °C, fruit were, respectively, 6 and 4% firmer than at the beginning of the shelf life. Otherwise, an application of 1 μL L⁻¹ 1-MCP inhibited the increase in fruit firmness also during the shelf life (Figure 1A). TSS increased in untreated fruit after 21 days of cold storage, and was 10% higher than at harvest (Figure 1C); untreated fruit showed the same behavior during the subsequent shelf life, after 5 days at 20 °C, TSS was, 10% higher than at the beginning of the shelf life (Figure 1D). TSS in the 1-μL L⁻¹ 1-MCP treated fruits was 4% lower than at harvest (Figure 1D) and 3% lower after 5 days at 20 °C than at the beginning of the shelf life (Figure 1C). The 5-μL L⁻¹ 1-MCP treated fruits showed the same trend as the 1-μL L⁻¹ 1-MCP treated fruits until 14 days of cold storage, then TSS slightly increased (1% higher than harvest after 21 days at 1 °C) (Figure 1C); with 5-μL L⁻¹ 1-MCP treated fruits TSS was also 3% higher after 5 days at 20 °C than at the beginning of the shelf-life (Figure 1D). TSS increase was, probably, due to the weight loss that occurred in untreated and 5-μL L⁻¹ 1-MCP treated fruits, during cold storage and shelf life. The TA loss was not affected by 1-MCP in both concentrations (data not shown).

Untreated 'Claudia' white-flesh loquat fruits expressed severe chilling injury symptoms after two weeks of storage at 1 °C such as internal browning and leathery flesh. Similar irreversible symptoms observed in peach have been related to the low temperature (Ju *et al.*, 2000). On the other hand, 1-MCP treatments maintained fruit quality and significantly delayed internal browning incidence and flesh leatheriness development as reported in other studies at different storage temperatures (González *et al.*, 2003).

Weight loss progressively increased with storage time for all treatments, but was significantly reduced by 1-MCP treatment. Total weight loss of untreated, 1- and 5-μL L⁻¹ 1-MCP treated fruits, was 8.5, 2.0 and 4.5%, respectively,

TABLE 1. Changes in total phenolics and antioxidant capacity of untreated and 1-MCP treated white-flesh loquat (*Eriobotrya japonica* Lindl. 'Claudia') fruits during 21 days of storage at 1 °C and 95% RH and subsequent shelf life at 20 °C. Data are means ± standard errors ($n = 10$).

Storage period	Total phenolics (mg kg ⁻¹)				Antioxidant capacity (%)			
	Untreated	1 µl L ⁻¹ 1-MCP	5 µl L ⁻¹ 1-MCP	P	Untreated	1 µl L ⁻¹ 1-MCP	5 µl L ⁻¹ 1-MCP	P
Harvest	480.23 ± 16.92				42.44 ± 0.06			
7 days at 1 °C	420.97 ± 11.10 a ^v	499.91 ± 15.33 b	483.34 ± 14.43 b	0.01	41.24 ± 0.73 a	45.85 ± 1.20 b	42.46 ± 0.50 a	0.01
14 days at 1 °C	418.62 ± 14.85 a	530.51 ± 12.00 b	563.64 ± 12.90 b	0.00	40.72 ± 0.86 a	47.16 ± 1.02 b	48.94 ± 1.22 b	0.001
21 days at 1 °C	521.30 ± 13.00 a	563.53 ± 13.10ab	595.68 ± 14.30 b	0.01	33.06 ± 0.05 a	46.22 ± 0.90 b	47.84 ± 1.17 b	0.001
21 days at 1 °C plus 5 days at 20 °C (shelf life)	615.03 ± 12.72 b	588.28 ± 14.50ab	549.34 ± 12.20 a	0.01	42.64 ± 0.49 b	32.76 ± 2.15 a	33.54 ± 1.23 a	0.001

^v Letters are used to denote statistically significant differences between untreated and 1-MCP treated fruits.

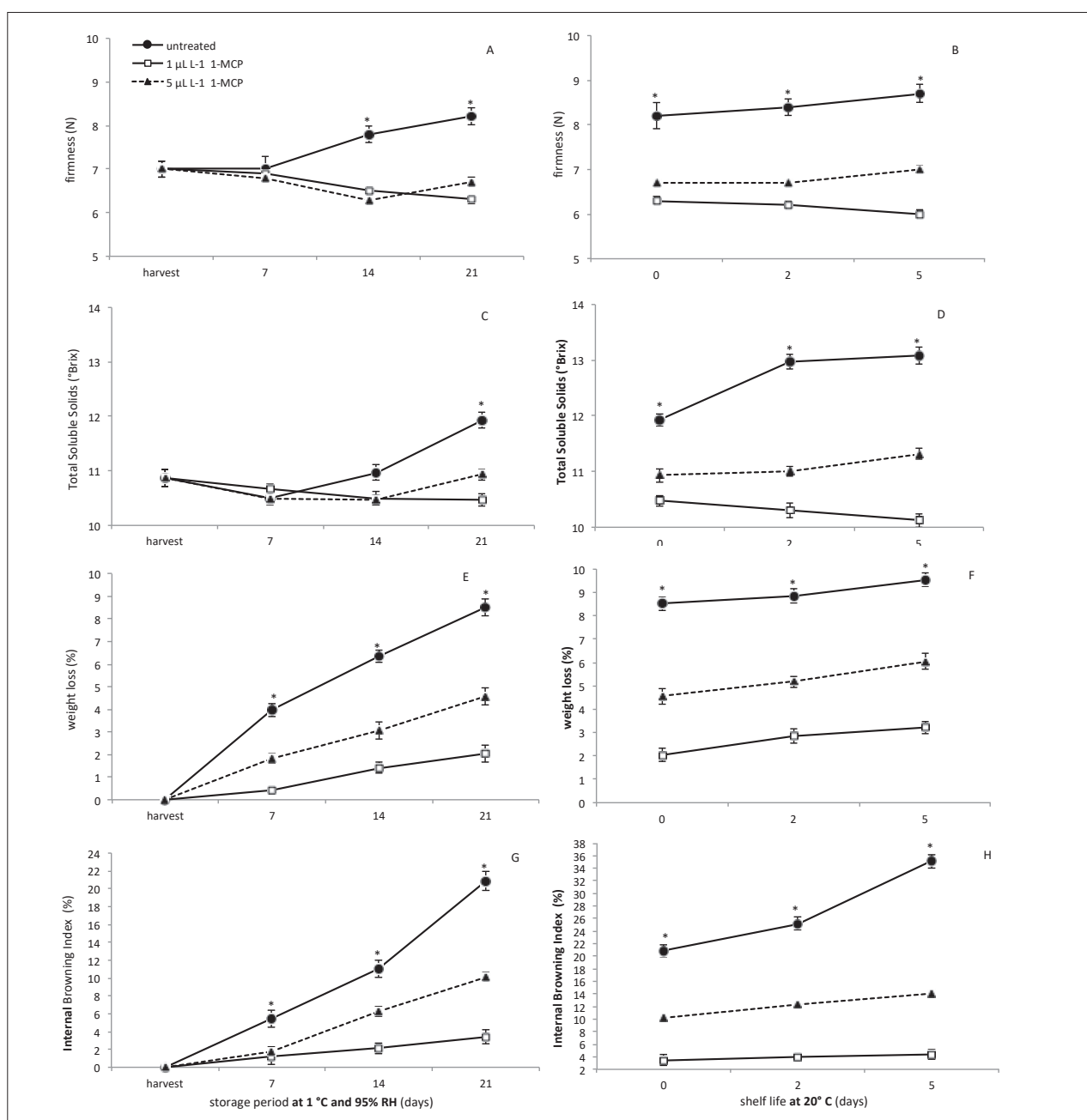


FIGURE 1. Changes in fruit firmness (A, B), total solid soluble (C, D), weight loss (E, F) and internal browning index (G, H) of untreated and 1-MCP treated white-flesh loquat (*Eriobotrya japonica* Lindl. 'Claudia') fruits during 21 days of storage at 1 °C (95% RH) and subsequent shelf life at 20 °C. Data are means ± standard errors ($n = 30$). The * indicate significant differences at $P < 0.05$ between untreated and 1-MCP treated fruits.

after 21 days of cold storage (Figure 1E), and 9.5, 3.2 and 6.0%, respectively, after 5 days of shelf life (Figure 1F). In both cases 1-MCP treatment was effective in preventing weight losses.

The 1-MCP treatment had a positive effect in terms of total solid soluble content retention; a slightly TSS increase occurred on untreated fruit, probably, due to the weight loss occurred in untreated, during cold storage and shelf life. Untreated fruits showed a significant weight loss during cold storage and the subsequent shelf life; the 1-MCP treatment was also effective in terms of weight retention.

Untreated loquat fruit showed an increase of internal browning during cold storage, with browning index (BI) values of 11 and 21% after 14 and 21 days, respectively, at 1 °C (Figure 1F); moreover, the internal browning index reached 35% at the end of the shelf life on untreated loquat fruits (Figure 1G). The 1- and 5- $\mu\text{L L}^{-1}$ 1-MCP treated fruits showed slight browning after 21 days at 1 °C and their BI (3 and 10%, respectively) were significantly lower than for untreated fruits (Figure 1G). The 1- $\mu\text{L L}^{-1}$ 1-MCP treatment was more effective than the 5- $\mu\text{L L}^{-1}$ 1-MCP one, especially during the shelf life, with a BI of 4% and 14%, respectively (Figure 1H).

During cold storage (1 °C) and shelf life (20 °C) conditions, the 1- $\mu\text{L L}^{-1}$ 1-MCP treatment completely inhibited the occurrence of leatheriness, demonstrating that a relatively low concentration of 1-MCP was already useful to extend highly perishable white-flesh loquat postharvest life. This is in contrast with other authors who described an increase in

fruit firmness during cold storage also in 1-MCP treated loquat fruits (Cai *et al.*, 2006a), probably due by the positive combination of the 1-MCP treatment and cold storage temperatures higher than 0 °C. The 1-MCP treatment significantly reduced internal browning manifestation; similar results were described by Zheng *et al.* (2010) on loquat fruit and by Pesis *et al.* (2002) on avocado fruit. These data suggest that 1-MCP may be commercially used to prevent chilling injury symptoms and flesh disorders during cold storage and to extend the shelf life of the highly perishable white-flesh loquat fruit.

Effects of 1-MCP on total phenolics and the antioxidant capacity

The 1-MCP-treated fruits showed higher total phenolic contents and higher antioxidant activities than untreated fruits during the cold storage; the 1- and 5- $\mu\text{L L}^{-1}$ 1-MCP treated fruits also showed an increase of their total phenolic contents of 22 and 14%, respectively, during cold storage compared with the levels at harvest (Table 1). Untreated fruits exhibited a slight increase of the total phenolic contents after 21 days of cold storage (29%) and during shelf life (19%), probably due to the high weight loss that occurred (Table 1).

Sensory evaluation

The 1-MCP treatment affects some sensory quality descriptors of the stored fruits compared with the untreated ones. There were differences among untreated and treated loquat fruit of 'Claudia'; in particular the 1- $\mu\text{L L}^{-1}$ 1-MCP

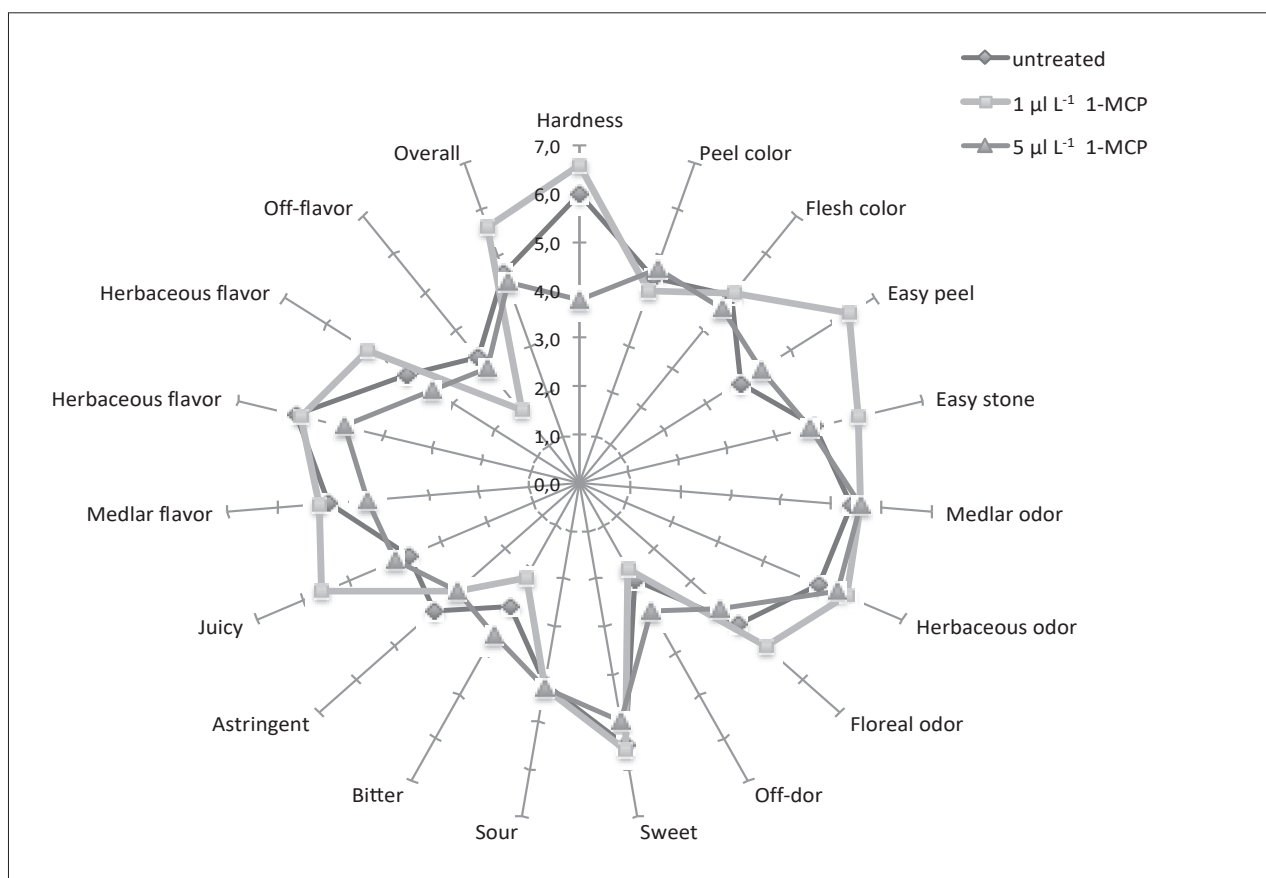


FIGURE 2. Sensory analysis of loquat (*Eriobotrya japonica* Lindl. 'Claudia') fruits treated with 1 and 5 $\mu\text{L L}^{-1}$ 1-MCP at harvest and stored at 1 °C (95% RH) for 21 days and then held at 20 °C for 5 days. Data are means of 10 panelists testing 10 fruits for each treatment.

treated fruits showed significant differences in terms of a high intensity of the descriptors juicy and easy peel. Judges, also, preferred the 1- $\mu\text{L L}^{-1}$ 1-MCP treated fruits in terms of overall quality (Figure 2).

Conclusion

The 1-MCP treated fruits showed higher levels of phenolics and a higher antioxidant activity than the untreated fruits during cold storage. The positive response of loquat to the ethylene inhibitor at low temperature allowed the fruit to be treated with cooling, thus making it possible to store when it cannot be marketed immediately. This 1-MCP positive effect was noticed also during the shelf life subsequent the 21 days cold storage, suggesting that white-flesh highly perishable loquat fruit could be marketed without chilling injury symptoms, also after a long cold storage period. The 1-MCP treatment influenced positively also the overall sensory quality of the cold stored fruit, resulting in a higher overall quality of treated fruit compared with untreated ones.

This treatment could expand the distribution areas of the highly perishable white-flesh loquat fruit and allow versatile planning for the growers in marketing the crop. This study demonstrated that an application of 1 $\mu\text{L L}^{-1}$ 1-MCP may be commercially used to prevent chilling injury symptoms and flesh disorders (flesh leatheriness and internal browning) during cold storage and to extend the shelf life of the highly perishable white-flesh loquat fruit. Since the global marketing of white-flesh loquat fruit is limited by its high perishability, the combination of packaging and 1-MCP treatment can be helpful in the postharvest management of this fruit.

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