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Use of 1-methylcyclopropene to complement refrigeration and ameliorate chilling injury symptoms in summer squash

Uso del 1-metilciclopropeno para complementar la refrigeración y reducir los síntomas de daño por frío en zapallito de tronco

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To elucidate the role of ethylene in summer squash [*Cucurbita maxima* var. Zapallito (Carr.) Millan] postharvest responses, harvested fruit was treated with the inhibitor of ethylene action 1-methylcyclopropene (1-MCP, 1 $\mu\text{L L}^{-1}$) and stored at 10 or 0 °C for 14 or 19 days, respectively. Deterioration, chilling injury (CI), weight loss, surface color, firmness, respiration rate, acidity, sugars, and antioxidants were determined. At 10 °C 1-MCP treated fruit showed lower deterioration and weight loss. The inhibition of ethylene action delayed yellowing, softening, and respiration, and prevented the increase of acidity. Chilling injury, manifested as shrunken areas and surface depressions, yellowing and softening were reduced in 1-MCP-treated fruit stored at 0 °C. Sugars and antioxidants were not affected. Results suggest that ethylene is involved in senescence and CI development in summer squash. Inhibition of its action by 1-MCP could be useful to prevent deterioration and maintain quality.

Keywords: ethylene; senescence; postharvest; quality; storage

Para elucidar el rol del etileno en la poscosecha de zapallito [*Cucurbita maxima* var. Zapallito (Carr.) Millan] se trataron frutos con el inhibidor de la acción del etileno 1-metilciclopropeno (1-MCP, 1 $\mu\text{L L}^{-1}$) y se almacenaron a 10 ó 0 °C por 14 ó 19 días, respectivamente. Se evaluó el deterioro, daño por frío (DF), pérdida de peso, color superficial, firmeza, respiración, acidez, azúcares y antioxidantes. A 10 °C los frutos tratados con 1-MCP mostraron menor deterioro que los controles. El tratamiento retrasó también la pérdida de peso, el amarillamiento, el ablandamiento, la respiración y el incremento de la acidez. A 0 °C el DF, la pérdida de color verde y el ablandamiento fueron significativamente retrasados por la inhibición de la acción del etileno. Los azúcares y antioxidantes no mostraron variaciones como consecuencia del tratamiento. Los resultados muestran que el etileno se encuentra involucrado en la senescencia y desarrollo de daño por frío en zapallito y que el 1-MCP podría ser de utilidad para mantener su calidad.

Palabras claves: etileno; senescencia; poscosecha; calidad; almacenamiento

Introduction

The *Cucurbitaceae* family includes some important fruits consumed in different regions of the globe such as gourd, melon, cucumber, watermelon, pumpkin, and squash (Robinson & Decker-Walters, 1999). The information available for various species within this group is still fragmentary and some general aspects regarding their quality and postharvest responses are poorly understood. Summer squashes [*Cucurbita maxima* var. Zapallito (Carr.) Millan] are native of South America. Even though in some regions they can make a significant contribution to overall vegetable consumption, their postharvest physiology has received little attention. They are non-climacteric fruits and are picked in an immature stage, before complete seed development and peel hardening (Kader, 2007). The main quality attributes of summer squashes are size, shape uniformity, firmness, green surface color, gloss, absence of physical damage and rots (Kader, 2007). Summer squashes are highly susceptible to dehydration, wounding and decay by both bacteria and fungi

and extensive postharvest deterioration usually results from softening and rind yellowing. In order to minimize the postharvest losses, rapid cooling and refrigeration are recommended, but even under optimal conditions their shelf life is relatively short (Kader, 2007). The potential benefits of cold storage cannot be fully exploited because the fruit is chilling-sensitive (Balandrán-Quintana et al., 2002; Gualanduzzi et al., 2009). Several groups have studied physiological aspects of zucchini squash (Martínez-Tellez, Ramos-Clamont, Gardea, & Vargas-Arispuro, 2002; Zheng, Fung, Wang, & Wang, 2008) and looked for strategies to improve quality maintenance (Lucera, Costa, Mastromatteo, Conte, & Del Nobile, 2010; Wang, 1995, 1996). Modified atmosphere (MAP) has been tested with moderate success. Low O₂ atmospheres (3–5 kPa) delayed yellowing and decay for a few days while high CO₂ levels reduced chilling injury (Izumi, Watada, & Douglas, 1996; Serrano, Pretel, Martínez-Madrid, Romojaro, & Riquelme, 1998). However, since fruit shelf life is not markedly extended, MAP is not commonly used.

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Ethylene regulates ripening and senescence in many horticultural products (Kader, 1986; Saltveit, 1999) and is associated with stress responses (Dong, Zhou, Sonego, Lers, & Lurie, 2001; Lafuente, Sala, & Zacarias, 2004; Pesis et al., 2002; Selvarajah, Bauchot, & John, 2001). 1-methylcyclopropene (1-MCP), an inhibitor of ethylene action, was launched to market in the last decade. It has become a useful tool for studies aiming at understanding ethylene responses and to inhibit deleterious responses mediated by the hormone (Blankenship & Dole, 2003; Huber, 2008). Given the central role of ethylene in climacteric fruit ripening, initial efforts focused on the evaluation of 1-MCP in these commodities (Watkins, 2006). Besides its potential for delaying fruit ripening, 1-MCP exposure may reduce some physiological disorders. Superficial scald development was reduced in apple (Sabban-Amin, Feygenberg, Belausov, & Pesis, 2011). Chilling injury severity was significantly decreased in 1-MCP treated persimmon, avocado, and guava (HersHKovitz, Saguy, & Pesis, 2005; Salvador, Arnal, Monterde, & Cuquerella, 2004; Singh & Pala, 2008). Recent work has shown that the inhibition of ethylene action can be also beneficial in some non-climacteric fruits. Treatments with 1-MCP reduced pulp browning, softening and dehydration, in eggplant (Massolo, Concellón, Chaves, & Vicente, 2011). The aim of this work was to investigate the role of ethylene on summer squash postharvest responses and to evaluate the potential of 1-MCP to control deterioration, complement refrigeration and delay chilling injury.

Materials and methods

Effect of 1-MCP on quality of fruit stored at 10 and 0 °C

Squashes [*Cucurbita maxima* var. Zapallito (Carr.) Millan] were divided into two groups namely control and 1-MCP. The 1-MCP treatment was performed according to Massolo et al (2011). The mass of 1-MCP commercial formulation necessary to reach a concentration of $1 \mu\text{L L}^{-1}$ was put into a plastic chamber. After hermetically sealing the chamber, 10 mL of water were added with in order to release the 1-MCP. After 12 h the fruit was packed in groups of two in plastic (PET) trays, and covered with perforated PVC (50 μm thick). Subsequently, fruit was removed, packed in plastic (PET) trays and covered with perforated PVC (50 μm thick). Control and treated fruit was stored at:

- (1) 10 °C for 0; 7 or 14 days (RH 85–90%) plus 2 days at 20 °C for 2 days to simulate a commercial shelf-life.
- (2) 0 °C for 0; 10 or 19 days (RH 85–90%) plus 2 days at 20 °C for 2 days.

Twenty fruits were taken at each sampling date and immediately evaluated or otherwise frozen in liquid N₂ and stored at –80 °C until use. Deterioration index, weight loss, surface color (lightness and tone), firmness (resistance to deformation and distance to failure), respiration rate, acidity, sugars and antioxidants were evaluated. The whole experiment was repeated twice.

Quality evaluation

Deterioration index and chilling injury

A rating scale (ranging from 0 to 3, being: 0 = excellent; 1 = good 2 = acceptable; 3 = poor) was used to evaluate fruit deterioration during storage. For fruit stored at 10 °C the parameters considered were dehydration signs, softening, rind color and decay. In the case of squashes stored at 0 °C, we evaluated surface depressions, pitting, softening and yellowing. The deterioration index (DI) and the chilling injury index (CI) were calculated according to the following equation.

DI or CI =

$$\frac{\sum(\text{deterioration level or injury level} \times \text{number of fruits in this level})}{\text{total number of fruits in the treatment}}$$

Weight loss

Fruit was weighed individually before packing and during storage. Weight loss (WL) was calculated from initial (IW) and final weights (FW) as described below:

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

Results were expressed as percentage of weight loss. Twenty fruits were used for each treatment and storage time considered.

Surface color

Fruit color was evaluated with a colorimeter (Minolta, Model CR-400, Osaka, Japan) by measuring the parameters L*, a* and b* in the equatorial zone of the fruit. The hue angle was calculated as: $\text{hue} = 180 - \text{tg}^{-1} \text{ b}^*/\text{a}^*$. Fifteen fruits were evaluated for each treatment and storage time considered, and two measurements were done on each fruit ($n = 30$).

Firmness

Texture was measured using a texture analyzer (TA.XT2, Stable Micro Systems Texture Technologies, Scarsdale, NY, USA) fitted with a 3-mm flat probe. Each fruit was compressed 10 mm at the equatorial zone at a rate of 1 mm s^{-1} . The slope of the force-deformation curves and the distance required to tissue failure were recorded. Results were expressed as N mm^{-1} and mm, respectively. Forty-five measurements were done for each treatment and storage time considered.

Respiration rate

Three fruits were placed into a hermetic flask and held for 15 min at 20 °C. Carbon dioxide in the headspace was determined with an IR sensor Alnor Compu-flow sensor (Model 8650, Alnor CA, USA). Fruit respiration was calculated and results were expressed as $\text{mmol kg}^{-1} \text{ h}^{-1}$. Carbon dioxide levels were always below $5000 \mu\text{L L}^{-1}$ to prevent inhibition of respiration. Three independent determinations were performed for each treatment and storage time considered.

Acidity

Longitudinal wedges of five different fruit were frozen in liquid nitrogen, ground in a mill, and 10 g of the resulting powder were added to 100 ml of water. Acidity was determined titrimetrically with NaOH (0.025 mol L^{-1}) until pH 8.2 (AOAC, 1980). Four samples were evaluated for each treatment and storage time considered. Results were expressed as $[\text{H}^+]$ mmol kg^{-1} .

Sugars

Frozen tissue was processed in a mill and 1 g of the resulting powder was extracted with 5 mL of ethanol. The mixture was vortexed and centrifuged at $13,000 \times g$ for 10 min at 4°C . Total sugars were measured with the anthrone reagent (Yemm & Willis, 1954). Briefly, aliquots of the extracts ($10\text{--}25 \mu\text{L}$) were taken and brought to $500 \mu\text{L}$ with water. One milliliter of 0.5 g L^{-1} anthrone, prepared in 98% (w/w) H_2SO_4 , was added slowly to the test tubes in a water-ice bath. The samples were boiled at 100°C for 10 min, cooled in water and the absorbance at 620 was measured in a spectrophotometer (Beckman Model UV Mini-1240, CA, USA). Glucose was used as a standard and results were expressed as g kg^{-1} . Four measurements were done for each treatment and storage time considered.

Antioxidants

Antioxidants were determined according to Singleton, Orthofer, and Lamuela-Raventós (1999). Frozen pulp tissue was ground in a mill and approximately 1 g of the resulting powder was added to 5 mL of ethanol. The suspension was vortexed and centrifuged at $13,000 \times g$ for 10 min at 4°C . Fifty microlitres of 1:1 Folin-Ciocalteu reagent (in water) were pipetted into test tubes containing between 100 and $150 \mu\text{L}$ of ethanolic extracts (at 10°C and 0°C , respectively) and taken to 1.4 mL with water. Samples were vortexed and after 3 min at 20°C , $100 \mu\text{L}$ of 20% (w/v) Na_2CO_3 in 0.1 mol L^{-1} NaOH were added. Then, each test tube was vortexed and incubated at 20°C for 1 h. The absorbance at 760 nm was measured. Two extracts were prepared for each treatment and storage time analyzed and each one was measured in triplicate. A standard curve with chlorogenic acid was performed and results were expressed as g kg^{-1} .

Statistical analysis

Experiments were performed in a factorial design being the factors treatment, storage time at 0, 10 or 20°C . Data were analyzed by ANOVA and means were compared by a Fisher test at a significance level of 0.05.

Results and discussion

Effect of 1-MCP on quality of fruit stored at 10°C

Deterioration index, weight loss and respiration

To assess the role ethylene on summer squash, quality of control and 1-MCP treated fruit during storage at 10°C and after shelf-life period was evaluated. The deterioration index (DI) was calculated on the basis of fruit surface yellowing, dehydration, softening and decay. 1-MCP treated fruit

showed lower DI than the control both during storage at 10°C and after a shelf-life period at 20°C for 2 days (Figure 1A). Due to their soft rind and incompletely developed cuticle, summer squashes are prone to dehydration. Fruit dehydration increased during storage in both control and treated fruit. Exposure to 1-MCP resulted in a lower weight loss after long-term storage ($14+2$) (Figure 1B).

During the first week of storage a reduction in the respiration rate was observed as expected for non-climacteric fruits. Previous reports showed that the inhibition of ethylene action can result in a reduction of fruit CO_2 production (Massolo et al., 2011). 1-MCP treated squashes showed lower respiration rate than the control after 2 days at 20°C (Table 1). Afterwards, no significant differences were detected.

The role of ethylene in postharvest deterioration of cucurbits is highly variable. Winter squash has low sensitivity

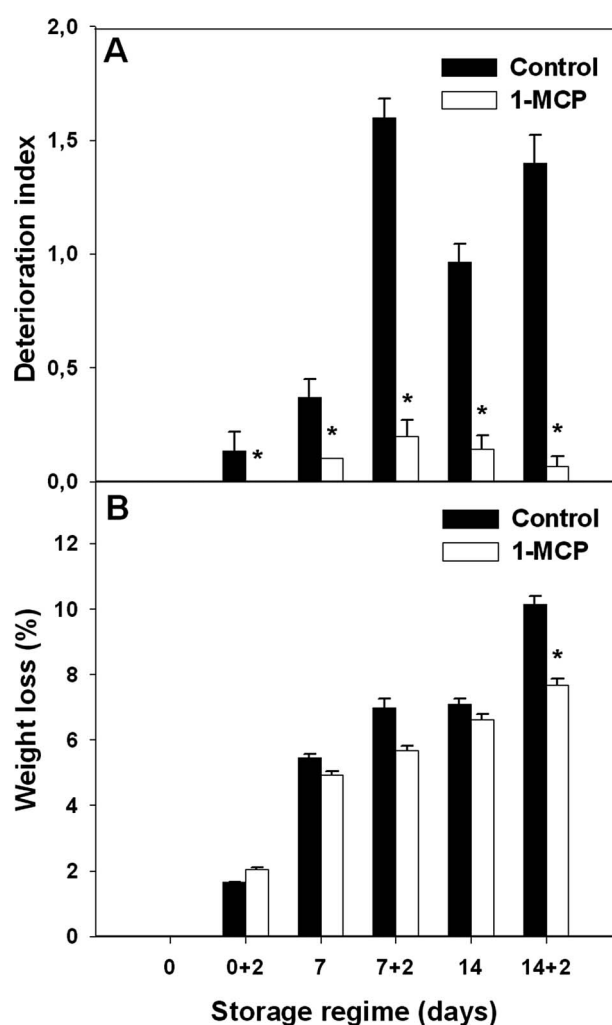


Figure 1. (A) Deterioration index and (B) weight loss of control and 1-MCP treated ($1 \mu\text{L L}^{-1}$, 12 h) summer squashes stored for 0, 7 and 14 days at 10°C and subsequently transferred to 20°C for 2 days ("++"). The asterisks indicate significant differences on a Fisher test at $P < 0.05$. LSD (A) = 0.13; LSD (B) = 2.03.

Figura 1. (A) Índice de deterioro y (B) pérdida de peso de zapallitos control y tratados con 1-MCP ($1 \mu\text{L L}^{-1}$, 12 h) almacenados por 0, 7 y 14 días a 10°C y luego transferidos a 20°C por 2 días ("++"). Los asteriscos indican diferencias significativas en un test de Fisher con $P < 0.05$. LSD (A) = 0,13; LSD (B) = 2,03.

Table 1. Respiration, acidity, sugars and antioxidants in control and 1-MCP treated ($1 \mu\text{L L}^{-1}$, 12 h) summer squashes stored for 0, 7 and 14 days at 10°C and subsequently transferred to 20°C for 2 days ("++").

Tabla 1. Respiración, acidez, azúcares y antioxidantes de zapallitos control y tratados con 1-MCP ($1 \mu\text{L L}^{-1}$, 12 h) almacenados por 0, 7 y 14 días a 10°C y luego transferidos a 20°C por 2 días ("++").

		Storage regime (days)						LSD
		0	0+2	7	7+2	14	14+2	
Respiration ($\text{mmol kg}^{-1} \text{h}^{-1}$)	Control	1.29 ± 0.06	1.52 ± 0.09	1.12 ± 0.12	1.14 ± 0.15	1.25 ± 0.08	1.17 ± 0.19	0.37
	1-MCP	1.46 ± 0.03	$1.08 \pm 0.12^*$	0.90 ± 0.10	1.20 ± 0.05	1.03 ± 0.11	1.02 ± 0.13	
Acidity (mmol kg^{-1})	Control	12.80 ± 0.32	14.00 ± 1.35	17.59 ± 0.96	17.15 ± 0.74	16.82 ± 0.62	19.60 ± 2.38	3.37
	1-MCP	15.40 ± 2.25	14.40 ± 0.71	15.86 ± 0.17	14.11 ± 0.21	$13.27 \pm 0.19^*$	$14.56 \pm 2.06^*$	
Sugars (g kg^{-1})	Control	42.7 ± 2.3	42.5 ± 1.5	42.0 ± 1.0	42.7 ± 0.8	47.7 ± 1.2	42.0 ± 1.0	4.3
	1-MCP	46.2 ± 0.3	45.0 ± 2.0	44.5 ± 2.5	43.2 ± 1.8	46.7 ± 1.0	39.7 ± 1.0	
Antioxidants (mg kg^{-1})	Control	510 ± 18	545 ± 13	697 ± 48	539 ± 24	530 ± 22	534 ± 19	101
	1-MCP	604 ± 26	576 ± 24	599 ± 76	572 ± 32	612 ± 31	552 ± 28	

Note: The asterisks indicate significant differences from the control on a Fisher test at $P < 0.05$. The least significant difference (LSD) is shown.

Nota: Los asteriscos indican diferencias significativas respecto del control en un test de Fisher, $P < 0.05$. Se muestra la diferencia mínima significativa (LSD).

to ethylene, while watermelon would rapidly soften upon exposure to the hormone (Saftner, Luo, McEvoy, Abbott, & Vinyard, 2007). In cucumber the sensitivity to exogenous ethylene depends on the developmental stage (Hurr, Huber, & Vallejos, 2010). The central role of ethylene in postharvest management has led to the development of a number of strategies to control its effects (Kader, 2007). Several works have successfully tested 1-MCP in climacteric fruits (Watkins, 2006), but far fewer reports have characterized the responses of non-climacteric commodities. 1-MCP reduced browning of pineapple and eggplant (Massolo et al., 2011; Selvarajah et al., 2001). Results from this work, suggest that the inhibition of ethylene action reduce deterioration and weight loss of summer squash. Although the largest differences between control and treated fruit were observed after the shelf-life period, even in fruit held continuously in cold storage the treatments decreased quality loss.

Color

Lightness (L^*) increased during storage, and after 7 or 14 days at 10°C , 1-MCP treated fruit maintained greener than the control (Figure 2A). The same trend was detected after the shelf-life period. The hue value increased in 1-MCP treated squashes from 121 to around 124 after 1 week at 10°C , decreasing afterwards (Figure 2B). After 7 or 14 days of storage at 10°C control squashes showed more yellowing than 1-MCP treated fruit. Previous works have shown that 1-MCP can retard the loss of color in green vegetables by delaying senescence (Watkins, 2006). Rind and peel degreening have been shown to be ethylene dependent in melon and banana (Golding, Shearer, Wyllie, & McGlasson, 1998; Pech, Bouzayen, & Latché, 2008). Results suggest that inhibiting ethylene action is useful to prevent color deterioration of summer squash

Texture, sugars, acidity and antioxidants

The loss of firmness of control fruit, as determined by resistance to deformation, was higher than that of 1-MCP treated squashes (Figure 3A). The distance to tissue failure in tissue compression tests increased as fruit softened. After 7 and 14 days at 10°C followed by a shelf-life period it was

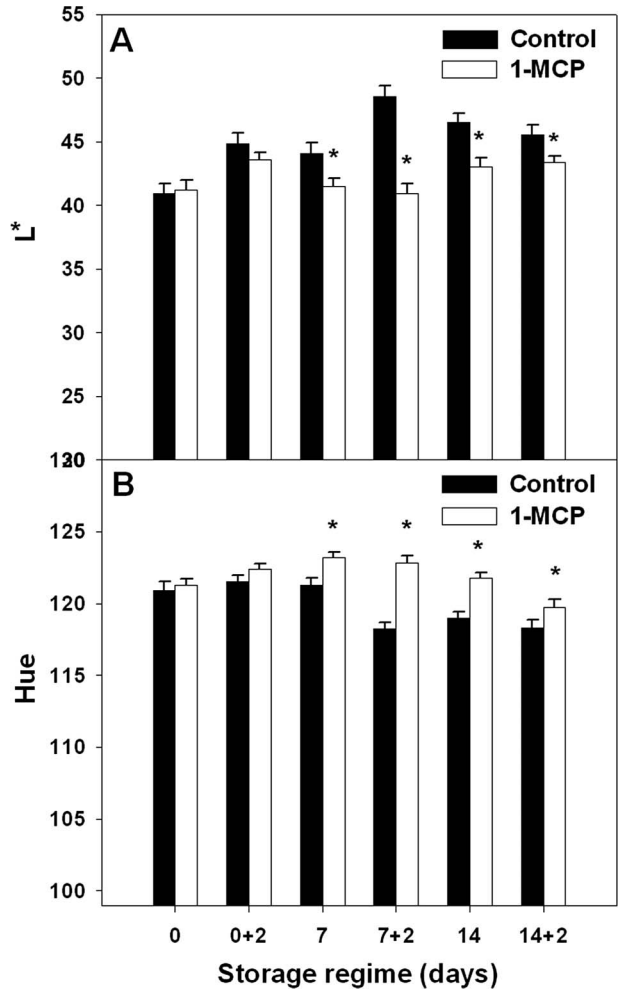


Figure 2. (A) Lightness (L^*) and (B) color tone (hue) of control and 1-MCP treated ($1 \mu\text{L L}^{-1}$, 12 h) summer squashes stored for 0, 7 and 14 days at 10°C and subsequently transferred to 20°C for 2 days ("++"). The asterisks indicate significant differences on a Fisher test at $P < 0.05$. LSD (A) = 2.06; LSD (B) = 1.31.

Figura 2. (A) Brillo (L^*) y (B) tono de color (hue) de zapallitos control y tratados con 1-MCP ($1 \mu\text{L L}^{-1}$, 12 h) almacenados por 0, 7 y 14 días a 10°C y luego transferidos a 20°C por 2 días ("++"). Los asteriscos indican diferencias significativas en un test de Fisher, $P < 0.05$. LSD (A) = 2,06; LSD (B) = 1,31.

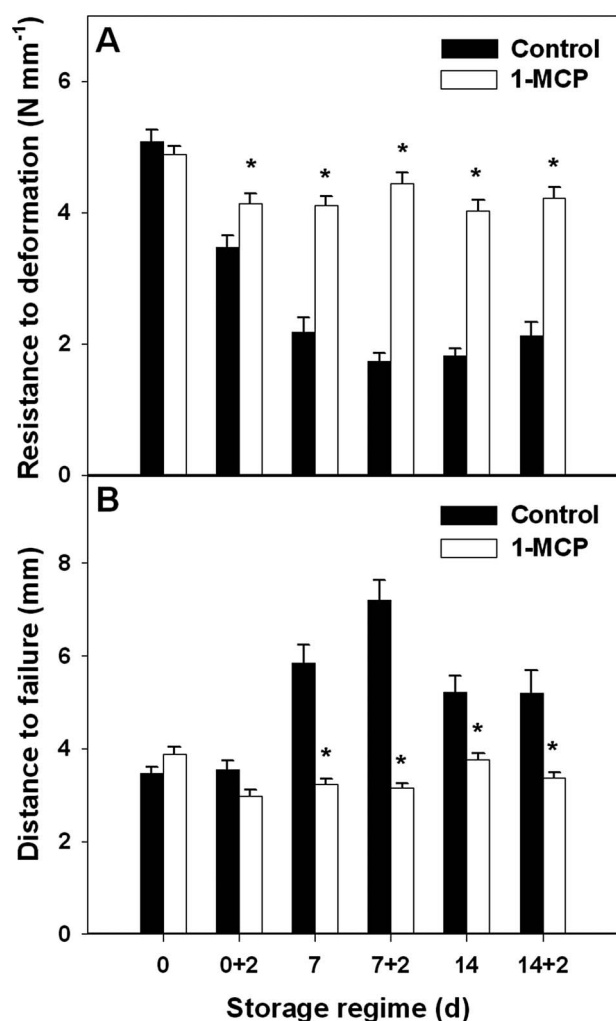


Figure 3. (A) Resistance to deformation (N mm^{-1}) and (B) distance to failure (mm) of control and 1-MCP treated ($1 \mu\text{L L}^{-1}$, 12 h) summer squashes stored for 0, 7 and 14 days at 10°C and subsequently transferred to 20°C for 2 days ("++"). The asterisks indicate significant differences on a Fisher test at ($P < 0.05$). LSD (A) = 0.49; LSD (B) = 0.73.

Figura 3. (A) Resistencia a la deformación (N mm^{-1}) y (B) distancia de ruptura (mm) de zapallitos control y tratados con 1-MCP ($1 \mu\text{L L}^{-1}$, 12 h) almacenados por 0, 7 y 14 días a 10°C y luego transferidos a 20°C por 2 días ("++"). Los asteriscos indican diferencias significativas en un test de Fisher, $P < 0,05$. LSD (A) = 0,49; LSD (B) = 0,73.

lower in 1-MCP treated indicating that softening was delayed (Figure 3B). Firmness loss of climacteric cucurbits such as melon has been reported to comprise both ethylene-dependent and independent components (Pech et al., 2008). Results from the present work suggest that despite of their non-climacteric nature; ethylene regulates, at least in part, the textural modifications of summer squash. Evaluation of the effect of ethylene applications on softening would be useful to support this. The higher firmness retention in treated fruit could be the result of a reduced action of cell wall degrading enzymes, as has been shown in other fruits exposed to 1-MCP (Blankenship & Dole, 2003). However, changes in electrolyte balance that might indirectly affect texture have been reported in some ethylene treated cucurbits (Huber, 2008). Further studies would be of interest to address this issue.

The changes in acids, sugars, and antioxidants were measured to evaluate whether or not the treatments caused any modification in attributes related to taste or nutritional value. In the first week of storage, there was an increase in control fruit acidity, while no modifications were detected in treated fruit (Table 1). The differences became significant after 14 or 14+2 days of storage. This could be associated with fermentative reactions that may occur upon severe fruit damage. In contrast, acidity remained unchanged in 1-MCP treated squashes. Sugars content was near 30 g kg^{-1} , and did not show major variations during storage in both control and 1-MCP treated fruit (Table 1). The content of hydrophilic antioxidants (AOX) was evaluated with the Folin-Ciocalteu reagent (Singleton et al., 1999). Phenolic compounds and ascorbic acid readily react with this reagent, so the assay might be used to estimation hydrophilic reducing substances (Huang, Ou, & Prior, 2005). The content of AOX was not affected by 1-MCP treatment (Table 1). In cantaloupe melon, despite of their typical climacteric behavior, it has been shown that sugar accumulation is ethylene-independent (Pech et al., 2008). Overall, results show that exposure to 1-MCP does not cause major modifications in AOX and sugars, but rather delays negative changes such as increased acidification and excessive firmness loss.

Effect of 1-MCP on chilling injury and quality of fruit stored at 0°C

Chilling injury, weight loss and respiration

Summer squashes were chilling damaged when stored below 5°C (Wang, 1995, 1996). The most important deterioration symptoms observed in the present work in fruit stored at 0°C were shrunk areas, surface depressions, softening and yellowing. The first CI manifestations appeared after 10 days of storage at 0°C , but irreversibility of chilling injury has been shown to occur long before the appearance of visible symptoms (Balandrán-Quintana, Mendoza-Wilson, Gardea-Béjar, Vargas-Arispuro, & Martínez-Téllez, 2003). After 10 days at 0°C followed by a 2 days shelf-life period, the CI symptoms were exacerbated in control fruit (Figure 4A). After 19 days at 0°C control squashes also showed higher deterioration. Untreated fruit also presented higher weight loss after 10, 10+2, or 19 days of storage at 0°C (Figure 4B). Fruit treated with 1-MCP showed after 10 days lower respiration rate than the control, but afterwards, no differences were found (Table 2). Results show that 1-MCP treatments ameliorate chilling injury in summer squashes. However, even treated fruit showed symptoms of deterioration and stored better at 10°C . Various strategies have been tested to control CI in zucchini squashes. Temperature conditioning, heat treatments high CO_2 concentrations prior to refrigeration reduced CI (Wang, 1995, 1996; Serrano et al., 1998). Some works suggested that polyamines could be involved in the acclimation to low temperature stress (Martínez-Téllez et al., 2002; Wang & Buta, 1994). Although increased ethylene production is frequently detected in plants subjected to chilling, the role of the hormone in this process has not been determined. In some species, ethylene exposure increased the manifestation of chilling injury (Candan, Graell, & Larrigaudière, 2008; Pesis et al., 2002; Selvarajah et al., 2001), but in other cases the disorder was delayed (Dong et al., 2001; Lafuente et al., 2004). Treatments with

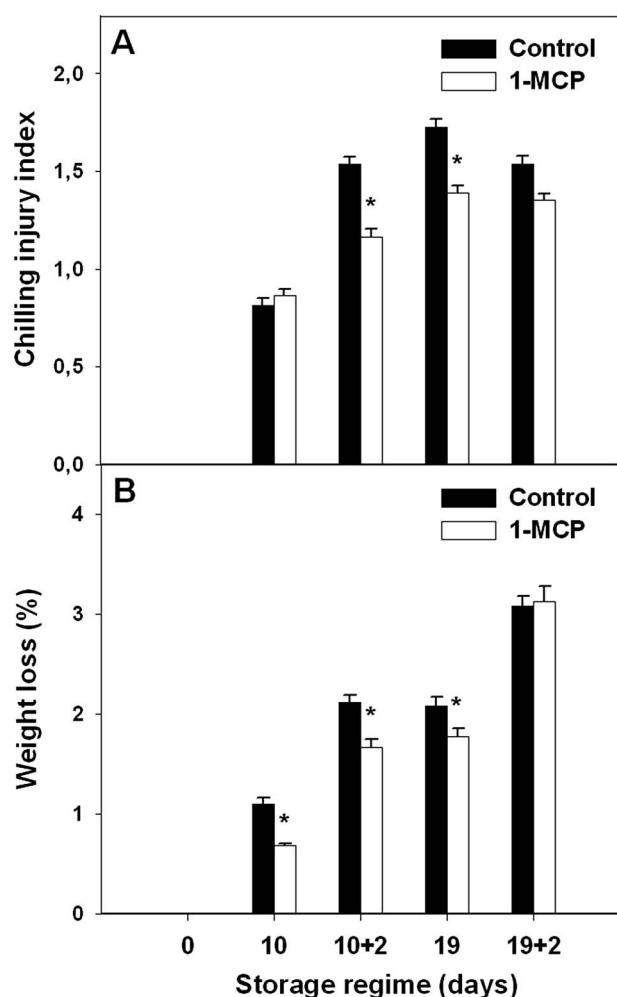


Figure 4. (A) Chilling injury and (B) weight loss of control and 1-MCP treated ($1 \mu\text{L L}^{-1}$, 12 h) summer squashes stored for 0, 10 and 19 days at 0°C and subsequently transferred to 20°C for 2 days ("++"). The asterisks indicate significant differences on a Fisher test at ($P < 0.05$). LSD (A)=0.337; LSD (B)=0.16.

Figura 4. (A) Daño por frío y (B) pérdida de peso de zapallitos control y tratados con 1-MCP ($1 \mu\text{L L}^{-1}$, 12 h) almacenados por 0, 10 y 19 días a 0°C y luego transferidos a 20°C por 2 días ("++"). Los asteriscos indican diferencias significativas en un test de Fisher, $P < 0.05$. LSD (A)=0.337; LSD (B)=0.16.

1-MCP have recently shown to reduce CI in some fruits (Cao, Zheng, Wang, Rui, & Tang, 2009; Singh & Pala, 2008). Similar to these reports, the inhibition of ethylene responses by 1-MCP could reduce chilling injury manifestations in summer squash.

Color

During the initial days of storage a reduction in fruit L^* was observed. This resulted from browning reactions. Afterwards, there was an increase in fruit lightness in the control, associated with rind yellowing and chlorophyll degradation (Figure 5A). The hue value showed a reduction in both control and treated fruit after 10 days of storage, but upon transfer to 20°C it decreased more rapidly in non-treated squashes (Figure 5B). After 19 or 19+2 days of storage, 1-MCP treated fruits remained greener.

Texture, acidity, sugars and antioxidants

Resistance to deformation did not show major changes during the first days of storage at 0°C . After the shelf-life period rapid firmness loss occurred in the control, but no changes were detected in 1-MCP treated squashes (Figure 6A). No differences in firmness were detected between control and treated fruit after 19 days, but after the shelf-life period softening was also delayed in 1-MCP treated squashes. At the end of the storage period the resistance to deformation of the control was less than half of that of 1-MCP treated fruit. The distance to failure did not show variations until the end of the storage period. After 19+2 days higher values were recorded in control fruit. Results suggest that chilling-induced softening of summer squash is ethylene dependent. In non-climacteric loquat some members of the ethylene signal transduction pathway have been to be induced by cold stress (Wang et al., 2010). It would be of interest to characterize these elements in cucurbits, especially considering the difference in susceptibility to chilling observed in different species of the family.

The changes in acidity, sugars, and antioxidants between treatments or during storage were minor (Table 2). After 19+2 days, higher acidity was found in the control, probably

Table 2. Respiration, acidity, sugars and antioxidants in control and 1-MCP treated ($1 \mu\text{L L}^{-1}$, 12 h) summer squashes stored for 0, 10 and 19 days at 0°C and subsequently transferred to 20°C for 2 days ("++").

Tabla 2. Respiración, acidez, azúcares y antioxidantes de zapallitos control y tratados con 1-MCP ($1 \mu\text{L L}^{-1}$, 12 h) almacenados por 0, 10 y 19 días a 0°C y luego transferidos a 20°C por 2 días ("++").

		Storage regime (days)					LSD
		0	10	10+2	19	19+2	
Respiration rate ($\text{mmol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$)	Control	1.63 ± 0.09	1.25 ± 0.19	1.16 ± 0.07	1.13 ± 0.05	1.09 ± 0.17	0.29
	1-MCP	1.75 ± 0.06	$0.92^* \pm 0.09$	$0.67^* \pm 0.09$	0.96 ± 0.03	1.05 ± 0.02	
Acidity (mmol kg^{-1})	Control	11.6 ± 2.1	6.7 ± 1.3	10.6 ± 1.7	7.8 ± 0.9	14.4 ± 1.0	3.8
	1-MCP	10.4 ± 0.8	9.1 ± 1.3	10.7 ± 0.1	6.5 ± 1.1	$10.0^* \pm 0.2$	
Sugars (g kg^{-1})	Control	29.1 ± 1.4	26.0 ± 2.6	28.1 ± 1.7	27.2 ± 1.3	28.1 ± 1.1	4.8
	1-MCP	28.5 ± 1.4	29.4 ± 1.3	27.7 ± 1.3	29.5 ± 1.5	25.5 ± 2.6	
Antioxidants (mg kg^{-1})	Control	380 ± 8	371 ± 19	434 ± 24	392 ± 21	435 ± 17	39
	1-MCP	392 ± 10	409 ± 8	434 ± 9	420 ± 8	403 ± 11	

Note: The asterisks indicate significant differences from the control on a Fisher test at $P < 0.05$. The least significant difference (LSD) is shown.

Nota: Los asteriscos indican diferencias significativas respecto del control en un test de Fisher, $P < 0.05$. Se muestra la diferencia mínima significativa (LSD).

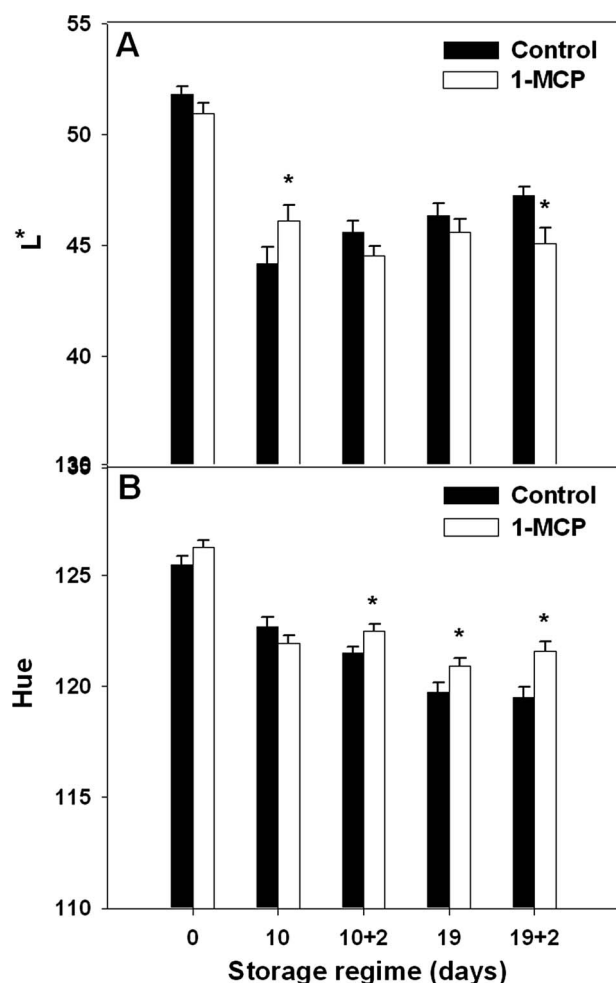


Figure 5. (A) Lightness (L^*) and (B) color tone (hue) of control and 1-MCP treated ($1 \mu\text{L L}^{-1}$, 12 h) summer squashes stored for 0, 10 and 19 days at 0°C and subsequently transferred to 20°C for 2 days ("++"). The asterisks indicate significant differences on a Fisher test at ($P < 0.05$). LSD (A) = 1.385; LSD (B) = 0.931.

Figura 5. (A) Brillo (L^*) y (B) tono de color (hue) de zapallitos control y tratados con 1-MCP ($1 \mu\text{L L}^{-1}$, 12 h) almacenados por 0, 10 y 19 días a 0°C y luego transferidos a 20°C por 2 días ("++"). Los asteriscos indican diferencias significativas en un test de Fisher, $P < 0.05$. LSD (A) = 1,385; LSD (B) = 0,931.

as a consequence of fermentative reactions associated with tissue disruption, as was also found in fruit stored at 10°C .

Conclusions

This work provides evidence about the importance of inhibiting ethylene action in summer squash. The use of 1-MCP delays deterioration, yellowing and softening without causing negative changes in other physico-chemical attributes, and could be useful to complement refrigeration. The treatments did not eliminate chilling injury in fruit stored at 0°C , but clearly ameliorate their tolerance to low-temperature disorders. 1-MCP exposure decrease surface pitting, weight loss, yellowing, and textural modifications. Overall, results suggest that 1-MCP could be a useful tool to complement refrigeration in summer squash.

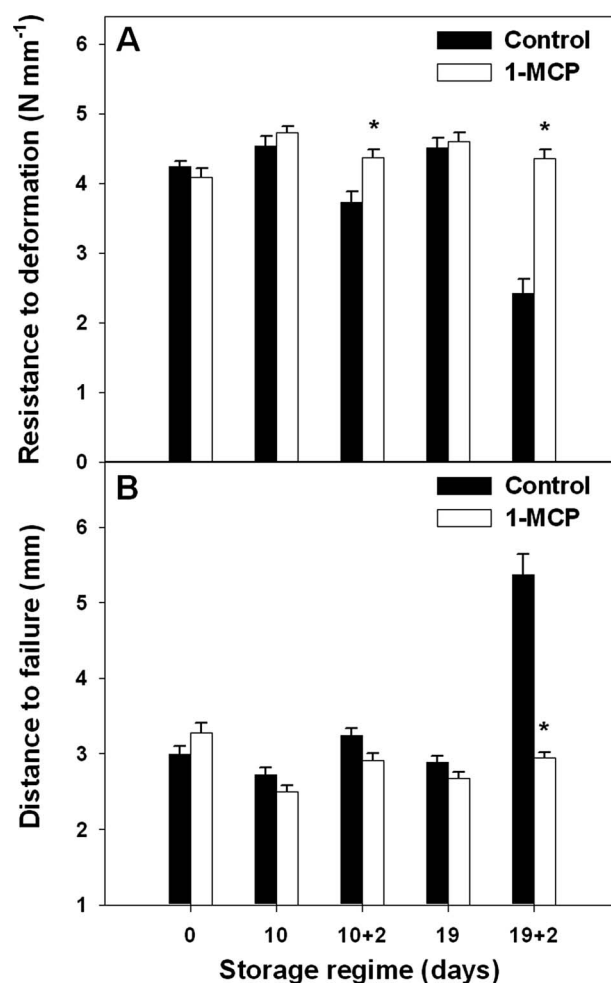


Figure 6. (A) Resistance to deformation (N mm^{-1}) and (B) distance to failure (mm) of control and 1-MCP treated ($1 \mu\text{L L}^{-1}$, 12 h) summer squashes stored for 0, 10 and 19 days at 0°C and subsequently transferred to 20°C for 2 days ("++"). The asterisks indicate significant differences on a Fisher test at ($P < 0.05$). LSD (A) = 0.371; LSD (B) = 0.333.

Figura 6. (A) Resistencia a la deformación (N mm^{-1}) y (B) distancia de ruptura (mm) de zapallitos control y tratados con 1-MCP ($1 \mu\text{L L}^{-1}$, 12 h) almacenados por 0, 10 y 19 días a 0°C y luego transferidos a 20°C por 2 días ("++"). Los asteriscos indican diferencias significativas en un test de Fisher, $P < 0.05$. LSD (A) = 0,371; LSD (B) = 0,333.

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