International Food Research Journal 25(4): 1483-1488 (August 2018)

Journal homepage: *http://www.ifrj.upm.edu.my*

Rind color change and granulation in pummelo [*Citrus maxima* (Burm. ex Rumph.) Merr.] fruit as influenced by 1-methylcyclopropene

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Article history

Received: 21 March 2017
Received in revised form: 21 May 2017
Accepted: 25 May 2017

Keywords

Yellowing
1-MCP
Visual quality
Vesicle drying

Abstract

The rind color, granulation, and some physico-chemical characteristics of 'Magallanes' pummelo [*Citrus maxima* (Burm. ex Rumph.) Merr.] fruit treated with 1-methylcyclopropene (1-MCP) for 8 hours at 0, 50 or 500 nL•L^{-1} then stored at ambient conditions (26.39±0.66ºC, 83.31±4.03% RH) for 12 weeks were evaluated. Peel color became more yellow as L^*, a^*, b^* and hue values gradually increased during storage. Chroma slightly increased. Juice content, and electrolyte leakage did not vary among treatments. However, weight loss, visual quality, shriveling, decay, pH, total soluble solids (TSS), titratable acidity (TA) and TSS:TA were affected by 1-MCP. Compared to the control, weight loss in treated fruit was lower at 6 weeks after treatment (WAT) with better visual quality at 6 and 9 WAT because of lesser shriveling. At 12 WAT, fruit treated with 500 nL•L^{-1} exhibited the highest TA and lowest TSS:TA ratio. Granulation or vesicle drying was most frequently observed initially in the middle and stylar-end sections of the fruit segment. Granulated samples exhibited higher pH and lower TSS than the non-granulated segments. Application of 1-MCP at 50 and 500 nL•L^{-1} did not influence the yellowing and granulation of 'Magallanes' pummelo fruit but it maintained good fruit visual quality for three weeks longer than untreated fruit.

Introduction

Pummelo [*Citrus maxima* (Burm. ex Rumph.) Merr.] is a flavorful and nutritious tropical fruit (Abirami *et al*., 2014). For these reasons, there is a persistent demand for pummelo fruit both in local and export markets (Fernandez and de Guzman, 2013). In 2012, the Philippines was the 20th major producer of pummelo aggregated with grapefruit worldwide with its production of 30,472 MT (FAO, 2012).

Like most citrus crop, pummelo is affected by granulation, a physiological disorder. Granulation is also known as crystallization and section drying indicated by gel formation within the juice vesicles and shriveling of juice sac (Ritenour *et al*., 2004). Granulation affects the eating quality of fruit thus lowering the overall fruit quality. The affected fruit develops a flat taste as it loses sugar and acid to some extent (Hofman *et al*., 2011). Chen *et al.* (2005) suggested that granulated juice sacs undergo senescence. Granulation is a result of various biotic and abiotic stresses (Ladaniya, 2008). Some authors (Burns and Albrigo, 1997; Ritenour *et al*., 2004) have reported factors that influence granulation in citrus such as storage duration, fruit size and maturation.

The ripening of citrus fruits is affected by ethylene which causes a rise in respiration and degreening (Paul *et al*., 2012). Ethylene influences color development by the degradation of chlorophyll while increasing carotenoid synthesis (Ladaniya, 2008). During fruit maturation, the peel color shows yellowing, changing color color from green to yellow (Paull and Duarte, 2012).

The yellowing and granulation which could be directly and indirectly associated to the action of ethylene, lower fruit quality, thus influencing fruit marketability. To lessen the adverse effects of ethylene, 1-methylcyclopropene (1-MCP) has been shown as an effective inhibitor of ethylene to improve postharvest quality of horticultural crops. 1-MCP acts by binding on ethylene receptors which block the ethylene (Tassoni *et al.*, 2006). 1-MCP delays the action of ethylene, which initiates changes in texture, softening, color, and other processes involved in fruit ripening and senescence (Saltveit, 1999).

Earlier studies have reported the effects of 1-MCP on the postharvest quality of citrus fruit. McCollum and Maul (2007) reported that the concentration of equal to or greater than 75 nL•L^{-1} inhibited ethylene-induced degreening in 'Marsh' grapefruit but 1-MCP at concentrations greater than 150 nL•L^{-1} did not further inhibit degreening. In clementine *(Citrus reticulata)*, the concentrations of 1-MCP ranging from 1500 to 2500 nL•L^{-1} effectively prolonged the fruit

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shelf-life, reduced changes in skin color, maintained fruit firmness, and resulted in high acid content and low TSS/acid ratio. At lower concentrations of 250 or 500 nL•L^{-1}, 1-MCP delayed yellowing in lime (Win *et al*., 2006). Ethylene-induced degreening was also delayed in 'Shamouti' oranges treated with 50 and 100 nL•L^{-1} 1-MCP (Porat *et al*., 1999).

An earlier study of Bayogan *et al.* (2015) has shown that the visual quality of 'Magallanes' pummelo was improved by 1-MCP at 500 nL•L^{-1} while higher concentrations at 2000 and 1500 nL•L^{-1} resulted in higher electrolyte leakage. This study took a closer look on rind yellowing, color change being an aspect of visual quality, at different 1-MCP concentrations (0, 50, 500 nL•L^{-1}) for eight hours. Granulation, which affects internal fruit quality, and some physico-chemical characteristics and electrolyte leakage of 'Magallanes' pummelo fruit treated were also evaluated.

Materials and Methods

At least one hundred eleven (111) newly-harvested pummelo (cv. 'Magallanes') fruit (180 days after anthesis) of uniform quality were obtained from Tugbok District, Davao City. Pummelo fruit were treated for eight hours with 1-methylcyclopropene (1-MCP at 0, 50, and 500 nL•L^{-1}) using an air-tight chamber. Fruit were stored in ambient conditions (26.39±0.66 ºC, 83.31±4.03 % RH) for 12 weeks. Ten fruit from each concentration were used as nondestructive samples for 12 weeks. Unique six pieces per treatment were destructively sampled every three weeks for physical, chemical and physiological attributes. Nine fruit were used for the initial data.

The physical data gathered included color (L^*, a^*, b^*, chroma, and hue angle using a CR 400 Minolta chromameter); weight loss; decay (1-no disease; 3-6-10% of the surface area affected and 5- >16% of the surface area affected); visual quality (9-8-excellent, field; 7-6-good, defects minor; 5-4-fair, defects moderate; 3-defects serious, limit of saleability; 2-poor; 1-non-edible under usual condition) (Kader et al., 1973); degree of shriveling (1-no shriveling; 2-1-10% of surface area shriveled; 3-11-20%; 5-31-40%; 7-51-60%; 9-71-80%); percentage and degree of granulation (1-no granulation; 3-11-25%; 4-26-50%; 5-51-75%) (Bayogan *et al.*, 2015). Percentage granulation indicates the number of segments per fruit exhibiting granulation whether slight or severe. The chemical and physiological data gathered included juice content, pH, total soluble solids (TSS) using a digital refractometer, titratable acidity (TA) by manual titration method, TSS:TA ratio, and electrolyte leakage. Electrolyte leakage was measured using the method of Suwapanich and Haesungchareon (2006) with modification. Ten grams of pulp were washed thrice with distilled water. The pulp was placed in 30 mL of 0.4 M mannitol solution. The prepared pulp solution was subjected to a shaker at 50 rpm for one hour in ambient temperature. Initial conductivity was measured after shaking of samples. Samples were then autoclaved for 15 minutes at 151^{o}C. Final conductivity was measured once cooled.

The experiment followed a Completely Randomized Design (CRD) with six and ten replications per treatment for destructive and nondestructive tests, respectively. Least Significant Differences (LSD) at $P \leq 0.05$ was used to compare means of treatments.

Results and Discussion

Physical evaluation

Over the 12-week storage period, the L^*, a^*, b^*, chroma and hue values increased showing pummelo peel turning light green (a^* values) to yellow (b^*) (Figure 1a-1e). The increasing chroma values (Figure 1d) showed that the peel color became brighter, whereas, the increasing hue angle values (Figure 1e) approached 90o, an indication of the peel color getting close to yellow (McGuire, 1992). The pummelo rind color changed to yellow diminishing commercial value.

In West Indian lime peel, 1-MCP at 250 or 500 nL•L^{-1} slowed down the chlorophyllase and chlorophyll degrading peroxidase activities which delayed its yellowing (Win *et al.*, 2006). Though treatments which involve ethylene action blockage have been shown to inhibit yellowing in citrus fruits, yellowing of pummelo fruit was not influenced by 1-MCP, regardless of the concentration. Likewise, 1-MCP at 20, 100 and 200 nL•L^{-1} did not affect the color of 'Oroblanco', a pummelo – grapefruit hybrid (Porat *et al.*, 2001). This indicates that ethylene action on degreening may differ among citrus fruits.

Visual quality of 'Magallanes' pummelo steadily declined with storage (Figure 2a). At 6 and 9 WAT, 1-MCP treated pummelo fruit had better visual quality than untreated pummelos but this did not vary among 1-MCP concentrations. This study has confirmed that 1-MCP application in pummelo resulted in better visual quality as earlier reported in cured pummelo due to lower weight loss (Bayogan *et al.*, 2015). High water loss indicated by severe shriveling limits the marketability of fresh produce as it can adversely affect the weight and physical appearance (Nunes and Emond, 2007).

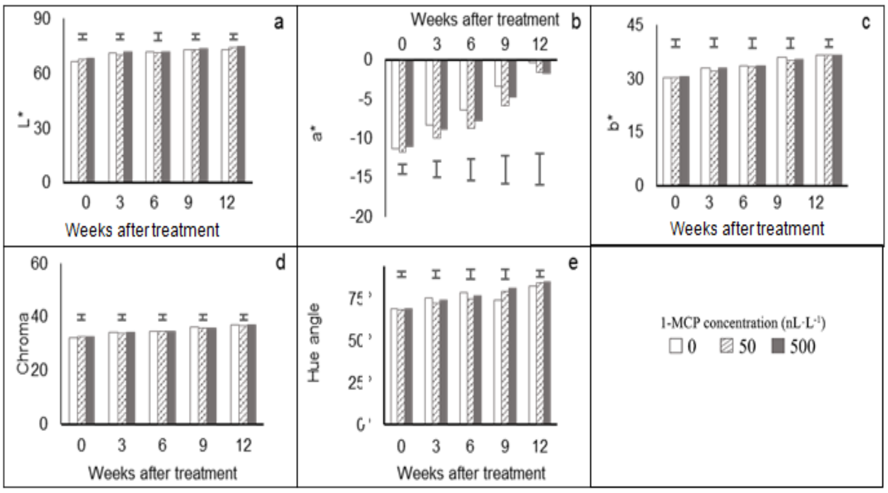

Figure 1. Peel color of 'Magallanes' pummelo stored at ambient conditions (26.39±0.66ºC, 83.31±4.03% RH) for 12 weeks as affected by 1-MCP concentration. LSD bars indicate significance at P≤0.05.

Application of 1-MCP reduced weight loss of pummelo but only at 6 weeks after treatment (WAT) (Figure 2b). Weight loss in 500 nL•L^{-1} 1-MCP-treated fruit tended to be lower at 9 and 12 WAT. Treatments varied on the degree of shriveling at 6 and 9 WAT with fruit treated with 500 nL•L^{-1} 1-MCP being less shriveled than the control, but not fruit treated with 50 nL•L^{-1} 1-MCP (Figure 2c). A slight reduction of weight loss was similarly observed in 'Ortanique' and 'Nova' mandarins treated with 0.5 µL•L^{-1} 1-MCP (Salvador *et al*., 2006). The increase in pummelo weight loss during storage was accompanied by shriveling of fruit. Shriveling is primarily caused by transpiration and weight loss in citrus fruits (Yahia, 2011).

Ethylene production and respiration are two factors that are related to color change. These factors however were not determined in this study. In grapefruit, 1-MCP at 5 to 75 nL•L^{-1} delayed degreening but respiration increased with concentration while ethylene increased with concentration and treatment duration. In contrast, 1-MCP reduced yellowing and respiration rate in 'Tahiti' lime stored at low temperature (Jomori et al., 2003). Fruit treated with 500 nL•L^{-1} 1-MCP exhibited no decay throughout the storage period (Figure 2d) in contrast to samples treated with 50 nL•L^{-1}. 1-MCP prevented decay development in pummelo. In this study, 1-MCP at 500 nL•L^{-1} appear to be a concentration that can inhibit ethylene action on decay but not 50 nL•L^{-1}. Ethylene action on ripening, softening and senescence renders fruits vulnerable to pathogens. Inhibition of 1-MCP on decay development may be associated with the increase of enzymes involved in phenolics metabolism which contributes to fruits' resistance against pathogens (Zhang *et al*., 2012). On the other hand, application of 1-MCP in 'Shamouti' oranges increased decay development (Porat *et al*., 1999). Similarly, 1-MCP at 1 and 5 mg L^{-1} increased the development of decay in 'Fallglo' tangerine and white 'Marsh' grapefruit (Dou *et al*., 2005).

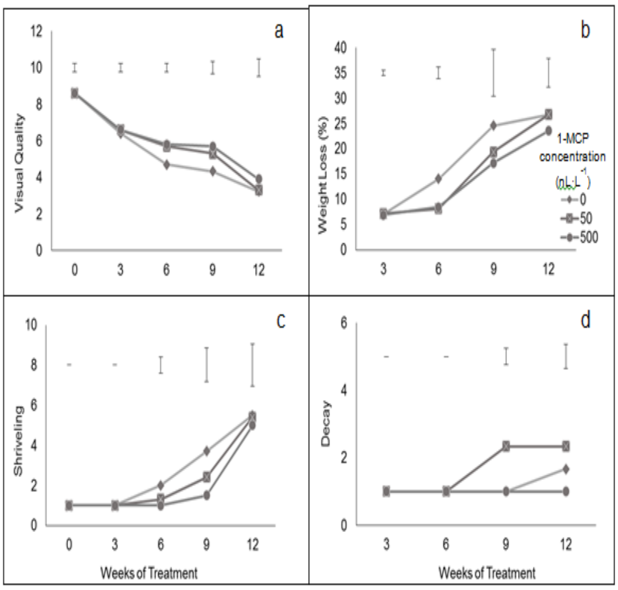

Figure 2. Some physical characteristics of 'Magallanes' pummelo stored at ambient conditions (26.39±0.66ºC, 83.31±4.03% RH) for 12 weeks as affected by 1-MCP concentration. LSD bars indicate significance at P≤0.05.

Granulation

Granulation in pummelo fruit increased with storage starting at 6 WAT when granulation was first observed (Figure 3). Overall, granulation in 'Magallanes' pummelo was not affected by 1-MCP when evaluated in terms of degree (Figure 3a) and percentage (Figure 3b) of granulation. In addition, the development of granulation more frequently occurred in the middle and stylar-end sections of the fruit, except much later in storage, when the granulation

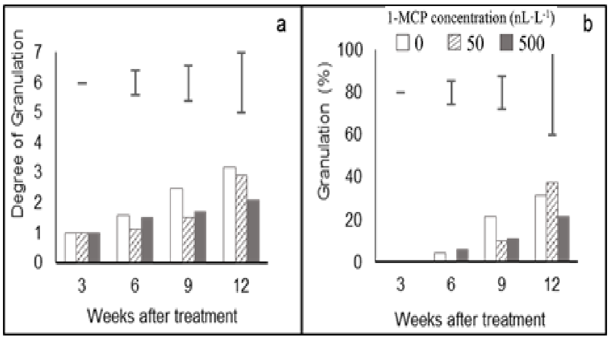

Figure 3. Granulation of ‘Magallanes’ pummelo stored at ambient conditions (26.39±0.66ºC, 283.31±4.03% RH) for 12 weeks as affected by 1-MCP concentration. LSD bars indicate significance at P≤0.05.

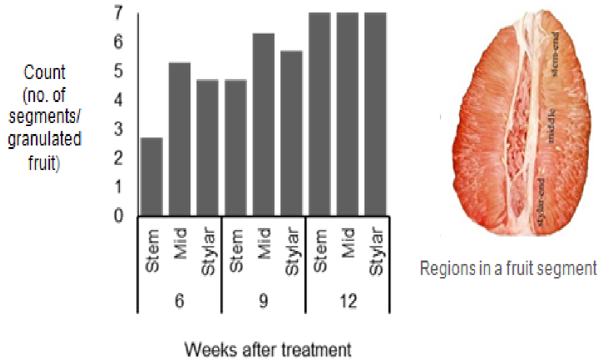

Figure 4. Parts of the ‘Magallanes’ pummelo fruit segment where granulation was observed over the 12-week storage period.

appeared in all three fruit sections (Figure 4).

Granulation development coincided with the reduction of sugars and acids in granulated juice vesicles (Figures 5b and 5e). This consequently resulted in reduced juice content in the later period of storage when granulation was observed in ‘Magallanes’ pummelo (Figure 5a). In the formation of granulated juice vesicles, substrates maybe used to support cell wall synthesis (Burns and Albrigo, 1997), thus, lowering sugar and acid levels, as observed in this study. Reduction of juice content in granulated citrus fruit is due to gel formation within the juice vesicles (Ladaniya, 2008; Ashebre, 2015). Higher incidence of granulation was observed in the stylar-end of large fruit which was also reported to have high alcohol-insoluble solids (Burns and Albrigo, 1997). A strong positive relationship of elevated respiration and ethylene production and the occurrence of granulation in ‘Kinnow’ mandarin was reported by Sharma *et al*. (2016).

Chemical evaluation

Juice content or extractable juice among treatments increased at 3 WAT but decreased later in storage (Figure 5a) as granulation developed. Juice content of pummelo fruit at all evaluation periods however did not vary. The synthesis of juice by juice sac cells during storage can cause an increase in juice content until fruit becomes fully ripe after which quality begin to decline (Alhassan and Mohammed, 2014).

The pH of 1-MCP treated pummelo fruit exhibiting non-granulated juice vesicles varied only at 9 WAT with the pH of 50 nL•L^{-1} lower than the control. On the other hand, granulated samples from control and 50 nL•L^{-1} exhibited higher pH relative to granulated 500 nL•L^{-1} fruit samples at 6 WAT. At 9 WAT, the pH from granulated samples of treated fruit was less acidic than those of the granulated control.

In contrast, Bayogan *et al.* (2015) earlier reported that 1-MCP did not influence pH of pummelo fruit because the pH (along with TSS and TA) of the granulated and non-granulated pulps were not determined separately. In the present study, the granulated samples exhibited higher pH than the non-granulated samples. Granulated juice vesicles was reported to contain 1.7 times the magnesium and more than twice the calcium concentration of normal vesicles, thereby increasing pH in granulated vesicles of citrus varieties (Burns and Albrigo, 1997).

Titratable acidity (TA) of samples from all treatments decreased then increased slightly until 9 WAT (Figure 5d). Treatments varied at 12 WAT wherein highest TA was observed in 500 nL•L^{-1} 1-MCP-treated fruit. Organic acids can be consumed as intermediate metabolites of the citric acid cycle during respiration, thus affecting TA with increasing storage duration (Rab *et al*., 2010).

The total soluble solids (TSS) of granulated juice vesicles were lower than the non-granulated samples (Figure 5e). In granulated juice vesicles, samples treated with 50 nL•L^{-1} had the highest TSS among treatments but this did not differ with 500 nL•L^{-1} at 12 WAT. Higher TSS in granulated control was observed at 6 WAT that varied in fruit treated with 50 and 500 nL•L^{-1} 1-MCP. TSS was similar in non-granulated juice vesicles. Lower TSS were observed in granulated vesicles because sugars along with acids are used in the process of cell wall thickening during granulation (Hofman, 2011). There was no difference among treatments except on the 12 WAT (Figure 5c) when TSS:TA ratio of fruit treated with 500 nL•L^{-1} was lower than the two other treatments. Compared to TSS, TSS:TA ratio provides a clear indication of fruit sweetness; the higher the TSS:TA ratio, the sweeter is the fruit (Ramful *et al*., 2011).

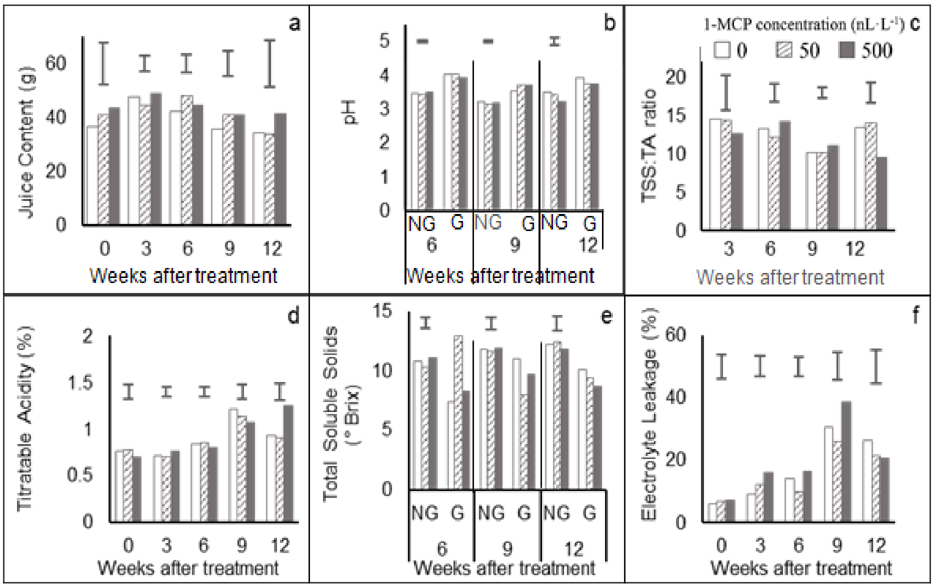

Figure 5. Chemical characteristics and electrolyte leakage of ‘Magallanes’ pummelo stored at ambient conditions (26.39±0.66ºC, 83.31±4.03% RH) for 12 weeks as affected by 1-MCP concentration. LSD bars indicate significance at P≤0.05. NG- non-granulated, G- granulated.

Physiological evaluation

Electrolyte leakage of granulated and non-granulated vesicles was not determined separately due to the limited amount of granulated vesicles. Electrolyte leakage was similar in all treatments during storage (Figure 5f) except at 9 WAT. Senescence is characterized by membrane deterioration resulting from breakdown of membrane lipids and proteins (Pometto *et al.*, 2005). It is associated with an increased leakage of ions (Whitlow *et al*., 1992). Electrolyte leakage measures the membrane permeability to ions but this technique does not measure membrane stability directly (Huang, 2006).

Conclusion

Application of 1-MCP at 50 and 500 nL•L^{-1} did not influence the yellowing and granulation of ‘Magallanes’ pummelo fruit. Similarly, juice content, and electrolyte leakage of the pummelo did not vary among concentrations. However, pH, TSS, TA, TSS:TA, visual quality, weight loss, degree of shrivelling and decay were affected by 1-MCP. Weight loss was significantly lower in 50 nL•L^{-1} 1-MCP at 6 WAT. Treated fruit had better visual quality than control fruit because of lesser shriveling. Treated fruit exhibited highest %TA and lowest TSS:TA ratio only at 12 WAT. At 6 WAT, fruit segments with granulation in 50 and 500 nL•L^{-1} 1-MCP had lower pH than the granulated control. Overall, granulated samples exhibited higher pH and lower TSS than the non-granulated samples in each treatment. Granulation was most frequently observed to start in the middle and stylar end of the fruit segment. 1-MCP did not affect yellowing and granulation but it maintained visual quality of ‘Magallanes’ pummelo fruit for 3 weeks longer than untreated fruit.

Acknowledgment

The authors thank the Department of Agriculture-Bureau of Agricultural Research for the research funds.

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