Application of 1-methylcyclopropene, calcium chloride and calcium amino acid chelate on fresh-cut cantaloupe muskmelon

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Abstract – The objective of this work was to determine the effects of postharvest application of 1-methylcyclopropene (1-MCP) and two calcium salts, applied individually or combined, on firmness and visual quality of fresh-cut muskmelon stored in air, for 18 days. Two sets of fruits, one of them exposed to 1-MCP at 300 nL L^{-1}, were cut into cubes, dipped in deionized water, or in 1% Ca solutions as $CaCl_2$, or in calcium amino acid chelate (Ca-chelate), placed in clamshell containers, and stored in air at 5±1°C and 90±5% RH, for 18 days. The assay was conducted using an entirely randomized design, with three replications, in a split plot array. Evaluation of visual appearance, color, flesh firmness, total soluble solids, titratable acidity, and pH was performed right after treatments, and every period of three days, up to eighteen days. Application of 1-MCP at 300 nL L^{-1}, calcium chloride or Ca-chelate, or the combination 1-MCP and calcium, preserved initial freshness and reduced softening of the samples. Ca-chelate synergistically enhanced the effect of 1-MCP on firmness after nine days of storage, while calcium chloride improved firmness of the samples throughout storage. Ca-chelate may serve as an alternative for shelf life extension of cantaloupe fresh-cut muskmelon.

Index terms: *Cucumis melo*, shelf life, firmness, quality, storage.

Aplicação de 1-metilciclopropeno, cloreto de cálcio e quelato amino-cálcico em melão cantaloupe minimamente processado

Resumo – O objetivo deste trabalho foi determinar o efeito da aplicação pós-colheita do 1-metilciclopropeno (1-MCP) e dois sais de cálcio, na firmeza e na qualidade visual do melão cantaloupe minimamente processado, armazenado por 18 dias; a aplicação foi feita individualmente ou de forma combinada. Dois grupos de frutos, um deles exposto ao 1-MCP (300 nL L^{-1}), foram cortados em cubos e imersos em água destilada ou em soluções com 1% de Ca, na forma de cloreto de cálcio ou de quelato amino-cálcico (Ca-quelado), colocados em embalagens de plástico e armazenados em atmosfera ambiente (5±1°C e 90±5% RH), por 18 dias. Avaliaram-se, imediatamente após a aplicação dos tratamentos e a cada três dias, até o décimo oitavo dia de armazenamento: aparência visual, coloração, firmeza da polpa, sólidos solúveis, acidez titulável e pH. As aplicações individuais ou combinadas de 300 nL L^{-1} 1-MCP, cloreto de cálcio ou de Ca-quelado preservaram a qualidade visual inicial e reduziram o amaciamento das amostras. A aplicação de Ca-quelado aumentou o efeito do 1-MCP sobre a firmeza, após nove dias de armazenamento, enquanto a imersão em cloreto de cálcio aumentou a firmeza das amostras, durante todo o período de armazenamento. O Ca-quelado pode ser utilizado como alternativa para a manutenção da firmeza do melão cantaloupe minimamente processado.

Termos para indexação: *Cucumis melo*, vida de prateleira, firmeza da polpa, qualidade, armazenamento.

Introduction

The variety of consumer trends and desires towards convenience, health and good taste have made fresh-cut products a growing segment of the horticultural industry. Consumers expect fresh-cut fruits to be visually acceptable and appealing. Nonetheless, the physical damages caused by preparation increase respiration rates and ethylene production (Rosen & Kader, 1989), with associated increase in rates of other biochemical reactions responsible for changes in color, flavor, and texture (Brecht, 1995). These unavoidable physical injuries cause undesirable physiological responses in the tissue. The cell wall and membrane establish a boundary

between the cell and its external environment, and serve as a barrier, which delimits compartments, wherein the chemical composition can differ from the surroundings and can be optimized for a particular activity. Not only wounding physically ruptures cellular membranes, but also affects their semipermeability, so that incompatible compounds can uncontrollably mix and produce unwanted reactions (Saltveit, 2003). As a result, the greatest hurdle to the commercial marketing of fresh-cut melon is its limited shelf life due to excessive tissue softening and lack of freshness. To minimize these effects, it is necessary to develop new technologies that, in conjunction with low temperature, extend shelf life of fresh-cut fruits.

One-methylcyclopropene (1-MCP) has been known to extend postharvest life and quality of fruits by inhibiting ethylene action; and it is thought to occupy ethylene receptors, so this hormone cannot bind and elicit action (Blankenship & Dole, 2003). Studies on the application of 1-MCP on fresh-cut fruits have shown that it reduces tissue softening in pineapple (Budu & Joyce, 2003), muskmelon (Machado et al., 2004) and, most recently, in kiwifruit (Vilas-Boas & Kader, 2007).

Calcium has long been known for its firming effect on fruits, attributed to the stabilization of membrane system and the formation of calcium-pectates, which confer rigidity to the middle lamella and cell wall (Poovaiah, 1986). Calcium chloride has been used as firming agents for a wide variety of whole, peeled, and fresh-cut fruits and vegetables, including whole honeydew melons (Lester & Grusak, 1999) and fresh-cut melons (Luna-Guzmán & Barrett, 2000; Machado et al., 2008). However, Ca-chloride can cause a bitter aftertaste, depending on the applied concentration (Luna-Guzmán & Barrett, 2000), and this fact motivates researchers to study other Ca salts, such as calcium amino acid chelate, as an alternative to maintain firmness of fresh-cut melon. Calcium amino acid chelate formulations are nutritionally functional chelates for plant, animal, and human use (Albion Laboratories, 1998). A postharvest dip in a Ca amino acid chelate maintained firmness and more than doubled the shelf life of intact honeydew fruit (Lester & Grusak, 1999). All of Albion's Ca chelate formulations have FDA GRAS status, and the composing ingredients are certified by Kosher and Parve, Supervision of Teaneck, NJ (Albion Laboratories, 1998).

This study aimed to determine the effects of postharvest application of 1-MCP, Ca chloride, and Ca amino acid chelate, applied individually or combined, on firmness and visual quality of fresh-cut muskmelon stored in air for 18 days.

Materials and Methods

Muskmelons (*Cucumis melo* L.) hybrid Hy-Mark were harvested at commercial maturity, from exporting fields in Mossoró, RN, Brazil. The harvest was carried out early in the morning, 65 days after sowing. Fruit harvest was based on external appearance: background color, net development, fruit size, and abscission of the stem 3/4 slip as usual for commercial maturity. Upon harvesting, fruits were immediately transported in air-conditioned vehicle to the Laboratory of Postharvest Physiology and Technology, of Embrapa, in Fortaleza, CE, Brazil, where sorting, sanitation, and processing occurred at 10°C.

Just prior to fruit minimal processing, knives and spatulas used were well sharpened, working area was washed, and walls, tables, and water containers were rinsed with 500 mg L^{-1} chlorine solution. The equipment used for processing and the clamshell containers used to store samples were washed and sanitized through immersion in 300 mg L^{-1} chlorine solution for 30 min. Following sanitation, the working area was kept at 10°C up to the end of the minimal processing.

Upon arrival at the lab, fruits were sorted for ripeness stage, size, color, and freedom from defects. Only fruits scored as high quality were used. Fruit were initially washed with neutral soap and running water, immersed in 300 mg L^{-1} chlorine solution (10°C, pH 6.5) for 3 min, drained and air-dried over surface cleaned with 70% ethanol, covered with sterilized paper. Once dried, fruits were divided into two sets of 21 fruits each, placed in portable chambers, and one of the sets was exposed to 1-MCP at 300 nL L^{-1}. The 1-MCP was generated by mixing the SmartFresh powder (Rohm and Haas) with 0.14% of the active ingredient, and with distilled water at room temperature (20°C). Both sets were kept in chambers at 10±1°C (90±5% RH) for 12 hours. After that, the chambers were opened and each set of fruit was divided into three groups of seven fruits each, which were simultaneously cut into cubes and dipped in deionized water, Ca solutions containing 1% calcium as $CaCl_2$ (0.36 g $CaCl_2.2H_2O$ 100 mL^{-1}) or calcium amino acid chelate (0.55 g calcium amino acid chelate 100 mL^{-1}) (formulation #3515, 1.5%; Albion Laboratories, Clearfield, Utah), for three minutes.

Fruits processing occurred first by removing blossom and stem ends and, then, seeds and placental tissue, leaving a wide ring, which was dipped into 50 mg L^{-1} chlorine solution (pH 7, 10ºC), for approximately 30 s and, then, by peeling and cutting the ring into cubes. The solutions were freshly prepared, immediately before use, in the same environmental conditions as the processing ones, with deionized water at 20ºC. Following the dipping, cubes correspondent to each treatment – control, Ca chloride dips, calcium amino acid chelate dips, exposure to 1-MCP, exposure to 1-MCP associated to Ca chloride dips, and exposure to 1-MCP associated to amino acid chelate dips – were evenly distributed into clamshell containers (10 cubes per container per replicate), and stored at 5±1ºC and 90±5% RH for 18 days.

Analyses were performed immediately after treatments and every three days, up to the eighteenth day of storage. Evaluations included overall visual quality (translucency and macroscopic decay), color, firmness, soluble solids, titratable acidity, and pH. All measurements were determined on three replicates, each one composed of 10 cubes, after samples were removed from cold storage and equilibrated to room temperature (20ºC). Visual appearance of each replicate was determined by three trained judges through the following visual scale: 5, excellent (freshly cut showing no translucency or macroscopic decay); 4, good (up to 10% of cubes per replicate showing slightly translucent corners and no macroscopic decay); 3, fair (up to 20% of cubes per replicate showing slightly translucent corners and no macroscopic decay); 2, poor (up to 50% of cubes per replicate showing translucent corners and no macroscopic decay); 1, very poor (over 50% of total cubes showing translucent corners and traces of macroscopic decay). A score of 3 was considered the limit of salability.

Color was determined using a Minolta Chroma Meter calibrated to a white porcelain reference plate. L*, a*, and b* values were scored from the side closer to the blossom end of the ten samples which composed each replicate. Results were expressed as L*, chroma $C^* = [(a^*)^2 + (b^*)^2]^{0.5}$, and hue angle $h_{ab} = \tan^{-1}[(b^*) \times (a^*)^{-1}]$ (McGuire, 1992). Firmness was determined on two sides of each cube using the TA.XT2 Texture Analyzer. During the evaluation test, a 6 mm diameter flat-head stainless steel cylindrical probe traveled approximately 30% of the height of the cube at 1 mm s^{-1}. Values were expressed as Newton (N). Soluble solids, pH and titratable acidity were determined from melon juice squeezed from cubes used to analyze color and firmness. Soluble solids were measured with a digital refractometer (0–45ºBrix) Palette 100, and expressed as Brix degree. Acidity was determined by titration with 0.1N NaOH, to the end point pH 8.2, and expressed as percentage of citric acid; pH was measured with a pH meter directly in the juice.

The assay was conducted using an entirely randomized model, with three replications, in a split plot array. The main plot was composed by the treatments, each consisting of seven fruits, which had been treated or not with 1-MCP, cut into 210 cubes, immersed or not in the correspondent calcium salt solution, and evenly distributed into 21 containers per treatment. The subplots included seven storage periods (0, 3, 6, 9, 12, 15, and 18 days). Each replication was expressed as the average of ten determinations. Analysis of variance was used to evaluate treatment differences (SISVAR 4.3), and Tukey's test or Kruskal-Wallis test, both at 5% probability, were used to discern between means and visual quality (%), respectively.

Results and Discussion

All treatments positively affected samples visual quality, as shown by Kruskal-Wallis test (Table 1). While only 28% of control samples were scored as excellent, 38.1 and 33.1% of the samples were scored as excellent for the treatments 1-MCP or Ca chloride, respectively. Sixty-six percent of the samples treated with calcium amino acid chelate were scored as excellent, and only 9.5% as fair, which shows a remarkable effect of this treatment on the maintenance of sample freshness. Samples were scored as marketable up to the end of

Table 1. Percentage of sample visual quality, within each treatment, classified as excellent, good, and fair, by the Kruskal-Wallis test, at 5% probability, of muskmelon treated with 1-methylcyclopropene (1-MCP), minimally processed, dipped in 1% Ca as calcium chloride (Ca1) or calcium amino acid chelate (Ca2), and stored in air (5±1ºC, 90±5% RH), for 18 days.

Treatment	Visual quality (%)		
	Excellent	Good	Fair
Control	28.6	28.6	42.9
1% Ca as Ca1	33.3	38.1	28.6
1% Ca as Ca2	66.7	23.8	9.5
300 nL L^{-1} 1-MCP	38.1	28.6	33.3
1-MCP+Ca as Ca1	38.1	38.1	23.8
1-MCP+Ca as Ca 2	52.4	42.9	4.8

the experiment, for all treatments, and preserved initial visual quality up to the ninth day of storage, regardless of treatment. Thereafter, visual quality decreased with storage time, mostly due to a slight dryness of surface, instead of translucency or macroscopic decay, as expected.

Samples treated with calcium amino acid chelate, applied alone or after application of 1-MCP, presented the highest L values, and were slightly lighter than the other samples, but did not differ statistically from the control (Table 2). No tissue browning, as indicated by decreasing L values, was observed throughout storage, as L values increased from 63.14, at harvest time, to 65.1, at the end of the experiment. Yellow color of samples was kept stable throughout storage, but treated samples presented lower values of hue angle than the control, specially those treated with 1-MCP combined with calcium amino acid chelate (Table 2). The judges, however, did not notice the difference in hue angle, during evaluation of visual quality.

Table 2. Luminosity, hue angle, and pH of muskmelon treated with 1-methylcyclopropene (1-MCP), minimally processed, dipped in 1% Ca as calcium chloride (Ca1) or calcium amino acid chelate (Ca2), and stored in air (5±1ºC, 90±5% RH), for 18 days[(1)].

Treatment	Luminosity	Hue angle	pH
Control	65.02bc	67.24b	6.24b
1% Ca as Ca1	64.56abc	66.58ab	6.16ab
1% Ca as Ca2	65.17c	66.44ab	6.22b
300 nL L^{-1} 1-MCP	64.16ab	66.64ab	6.26b
1-MCP+Ca as Ca1	63.87a	66.27ab	6.11a
1-MCP+Ca as Ca 2	65.09c	66.03a	6.23b

[(1)]Means followed by equal letters, in the columns, do not differ by Tukey's test, at 5% probability.

Chromaticity of the samples was not significantly affected by the treatments throughout storage. Taken as a whole, treated samples presented a small decrease in chroma values, preserving most of the yellow purity at the end of the experiment, while control samples values decreased from 34.4, at processing, to 29.9 at the end of storage (Table 3). These results shows that the physical damage caused by processing did not cause surface darkness or loss of flesh yellow color during storage at 10ºC for 18 days.

The good maintenance of quality throughout storage in this study may be attributed to fruit initial quality and pre-cooling prior to processing (Saltveit, 2003), as well as to the sharpness of the cutting utensils used for processing, allied to a strict temperature control, once the response of the tissue is proportional to tissue wounding during processing operations (Saltveit, 2003). Similarly, Portela & Cantwell (2001) investigated the effect of severity on wounding at preparation of fresh-cut cantaloupe melon, and observed that samples taken with a sharp cutting borer preserved initial visual quality longer than those samples taken with a blunt borer. These findings, allied to the fact that translucency in fresh-cut melon is cultivar-dependent (Aguayo et al., 2004), may explain some of the discrepancies among reports on fresh-cut melon, regarding color and translucency development during storage. Arruda et al. (2003) observed no development of browning or translucency on cantaloupe fresh-cut melon stored under refrigeration, while Bai et al. (2003) observed that quality loss of fresh-cut honeydew melon was mostly due to the development of translucency.

Table 3. Chromaticity and titratable acidity of muskmelon treated with 1-methylcyclopropene (1-MCP), minimally processed, dipped in 1% Ca as calcium chloride (Ca1) or calcium amino acid chelate (Ca2), and stored in air (5±1ºC, 90±5% RH), for 18 days[(1)].

Treatment	Storage time (days)						
	0	3	6	9	12	15	18
				Chromaticity			
Control	34.40b	30.68a	29.45a	29.46a	32.99a	29.31a	29.90a
1% Ca as Ca1	31.20a	29.34a	29.97a	29.65a	31.35a	30.52a	30.23a
1% Ca as Ca2	31.81ab	29.51a	30.03a	29.49a	30.87a	30.18a	30.10a
300 nL L^{-1} 1-MCP	31.93ab	28.71a	30.02a	28.49a	31.39a	31.13a	30.23a
1-MCP+Ca as Ca1	30.12a	29.50a	30.54a	29.61a	31.54a	29.68a	31.19a
1-MCP+Ca as Ca 2	31.52a	29.96a	30.77a	30.61a	31.16a	29.41a	29.79a
				Titratable acidity			
Control	0.090a	0.113a	0.092a	0.113a	0.107a	0.127b	0.890a
1% Ca as Ca1	0.105a	0.103a	0.105a	0.122a	0.117a	0.110ab	0.096a
1% Ca as Ca2	0.094a	0.097a	0.097a	0.122a	0.110a	0.114b	0.088a
300 nL L^{-1}1-MCP	0.101a	0.104a	0.105a	0.138a	0.121a	0.104ab	0.096a
1-MCP+Ca as Ca1	0.110a	0.111a	0.098a	0.136a	0.114a	0.084a	0.084a
1-MCP+Ca as Ca 2	0.096a	0.112a	0.096a	0.128a	0.115a	0.110ab	0.099a

[(1)]Means followed by equal letters, in the columns, do not differ by Tukey's test, at 5% probability.

Samples treated with 1-MCP and both types of calcium, or the combination 1-MCP and calcium, softened at a slower rate than the control (Figure 1). While control samples lost 30.8% of the initial firmness, samples treated with 300 nL L^{-1} 1-MCP, or treated with calcium amino acid chelate, lost only 23% and 20%, respectively, at the end of the storage period. However, samples treated with 1-MCP associated with calcium amino acid chelate were firmer than those treated with 1-MCP or calcium amino acid chelate applied individually.

The effect of 1% calcium as Ca chloride on flesh firmness of fresh-cut muskmelon was remarkable. After fifteen days of storage, samples treated with 1% $CaCl_2$ alone, or in association with 1-MCP, were significantly firmer than samples of the other treatments.

Machado et al. (2004) reported that exposure of melons to 1-MCP, before processing, reduced firmness loss and maintained visual appearance in fresh-cut muskmelon. Similarly, Vilas-Boas & Kader (2007) studied the effect of 1-MCP applied before and after fruit processing, and observed that kiwifruit softened at slower rate, as compared to control samples. These authors also observed that a 2-min dip in 0.09 M (1%, w/v) $CaCl_2$ synergistically increased the effect of 1-MCP on firmness of fresh-cut kiwifruit slices. Aguayo et al. (2006) studied the combined effects of 1-MCP and $CaCl_2$ dips (1% for 2 min) and controlled atmosphere (3 kPa O_2 +10 kPa CO_2) on quality of fresh-cut strawberries, and observed that application of only 1-MCP, before and after cutting, did not have a significant effect on firmness and appearance quality during storage, for up to 12 days at 5°C, while exposure to 1-MCP combined with $CaCl_2$, plus controlled atmosphere, slowed down softening and deterioration rates.

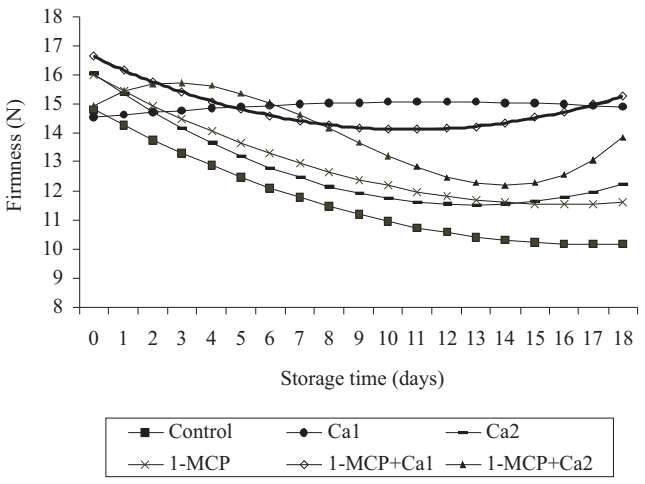

Figure1. Firmness (N) of muskmelon treated with 1-methylcyclopropene (1-MCP), minimally processed and dipped in 1% Ca as calcium chloride (Ca1) or calcium amino acid chelate (Ca2), and stored in air (5±1°C, 90±5% RH), for 18 days. Control = $0.0158x^2 - 0.5397x + 14.79$ ($R^2 = 0.9608$); Ca1 = $-0.004x^2 + 0.0934x + 14.522$ (nonsignificant, NS); Ca2 = $0.0273x^2 - 0.7017x + 16.028$ ($R^2 = 0.8364$); 1-MCP = $0.0174x^2 - 0.5552x + 15.995$ ($R^2 = 0.8646$); 1-MCP+Ca1 = $0.0218x^2 - 0.4669x + 16.612$ (NS); 1-MCP+Ca2 = $0.005x^3 - 0.1253x^2 + 0.5819x + 14.963$ ($R^2 = 0.8382$).

The firming effect of calcium chloride and calcium amino acid chelate dips on firmness of fresh-cut melon has been reported by Luna-Guzmán & Barrett (2000), and Machado et al. (2008). Reduction of tissue softening as a result of addition of calcium has been attributed to the crosslinking between the carboxyl groups of adjacent polyuronide chains and divalent calcium ions (Van-Buren, 1979), forming an "egg-box" model (Grant et al., 1973) known to confer rigidity to cell walls.

Soluble solids content average value was 9°Brix and did not change throughout storage. Stability of soluble solids content was reported for fresh-cut honeydew melon (Jenkins et al., 1998) and fresh-cut cantaloupe melon (Lamikanra et al., 2000) stored at low temperature.

Titratable acidity values were slightly higher on control samples. Those values, however, did not differ significantly, except for samples treated with 1-MCP combined with calcium amino acid chelate, after 15 days of storage (Table 3). Stability of organic acids, as observed in the present study, may indicate stability of metabolic reactions, such as respiration and production of volatiles under anaerobic conditions, once those acids may serve as substrate in such reactions.

Throughout storage, pH values were kept stable, but were affected by the treatments. Samples treated with 1-MCP combined with calcium chloride presented lower pH than the other treatments (Table 1). Stability of pH and soluble solids values were also observed by Bai et al. (2003) on fresh-cut honeydew melons harvested either on summer or winter.

Conclusions

1. The use of 1-methylcyclopropene at 300 nL L^{-1}, applied before processing, and 1% Ca as calcium chloride or calcium amino acid chelate, applied after processing, or the combination 1-MCP and calcium, preserves initial freshness and reduces softening of cantaloupe melon stored for 18 days in air (5±1°C, 90±5% RH).

2. Loss of freshness of fresh-cut muskmelon stored for 18 days in air (5±1ºC, 90±5% RH) may be prevented by application of 1% Ca as calcium amino acid chelate.

3. Softening of fresh-cut muskmelon stored for 18 days in air (5±1ºC, 90±5% RH) may be prevented by 1% Ca as calcium chloride dips.

4. Calcium amino acid chelate may serve as an alternative for shelf life extension of fresh-cut muskmelon.

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