Postharvest Application of Calcium and Magnesium to Honeydew and Netted Muskmelons: Effects on Tissue Ion Concentrations, Quality, and Senescence

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ADDITIONAL INDEX WORDS. *Cucumis melo*, Inodorus Group, Reticulatus Group, disease, firmness, histology, plasma membrane

ABSTRACT. Muskmelon senescence is directly associated with a decline in hypodermal mesocarp membrane integrity and its Ca concentration, but infusing Ca into melons has been a problem. Fully ripened and abscised hybrid honeydew [*Cucumis melo* L. (Inodorus Group) 'Honey Brew'] and netted muskmelon [*Cucumis melo* L. (Reticulatus Group) 'Explorer'] fruit were submerged (dipped) 20 min at 25 ± 3 °C in a solution containing a Ca-chelate, a Mg-chelate, a combination of both chelates, or no mineral chelate. Following 10 or 24 days of cold storage (4 °C for 'Explorer' and 10 °C for 'Honey Brew'), fruit were analyzed for mineral content and various senescence-related parameters. Abscised 'Honey Brew' fruit dipped in either Ca-chelate or (Ca+Mg)-chelate and abscised 'Explorer' fruit dipped in (Ca+Mg)-chelate, followed by 10 days cold storage, had hypodermal mesocarp Ca concentrations of at least 6.0 mg·g⁻¹ dry weight. Maintaining hypodermal mesocarp tissue Ca concentrations at this level during postharvest storage, especially for fully ripe 'Honey Brew' fruit, maintained membrane integrity and fruit firmness, and extended storage life 2.4-fold (i.e., to 24 days). The senescence regulatory effect of postharvest Ca-chelate treatments on abscised 'Explorer' was highly variable, compared to 'Honey Brew', which appeared to be due to the surface netting interfering with movement of Ca into the hypodermal mesocarp. Thus, postharvest Ca-chelate application to abscised 'Honey Brew' fruit could delay fruit senescence in commercial storage, and open up new markets for fully ripened honeydew melons.

Maintenance of cellular integrity in the hypodermal mesocarp tissue is critical for regulating postharvest senescence in fruit of netted muskmelon [*Cucumis melo* (Reticulatus Group)] and honeydew [*Cucumis melo* (Inodorus Group)] (Lester and Stein, 1993; Lester, 1998b). Cellular integrity is determined, in part, by tissue Ca levels; Ca is believed to be involved as an antiripening, antisenesescence agent in fruit (Ferguson, 1984; Poovaiah et al., 1988), and as an ion necessary for cellular signal transduction (Poovaiah and Reddy, 1993).

Calcium concentration within the edible mesocarp of netted muskmelon remains constant or declines slightly throughout fruit growth and maturation (Bernadac et al., 1996). In the rind-associated hypodermal mesocarp tissue, in particular, Ca concentration increases throughout muskmelon development, then declines sharply following maturity and during postharvest senescence (Bernadac et al., 1996). Senescence can be delayed in netted muskmelon by incubating hypodermal mesocarp disks in isotonic CaCl₂ solution, which retards plasma membrane lipid degradation and maintains osmotic tonicity of the cells (Lester, 1996).

If muskmelon Ca concentration could be increased in vivo, especially in the hypodermal mesocarp tissue, whole fruit post-

harvest senescence could be delayed. However, applications of Ca fertilizer to soil may be of questionable value. For example, Ca soil enrichments at the time of planting (280 to 1120 kg·ha⁻¹) had no effect on Ca concentration in the rind tissue of watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nak.] (Scott et al., 1993). Instead, supplemental Ca may need to be applied to the muskmelon surface. Recent studies on sour cherry (*Prunus cerasus* L.) (Anderson and Campbell, 1995); kiwifruit (*Actinidia deliciosa* C.S. Liang & A.R. Ferguson) (Gerasopoulos et al., 1996); mushrooms (*Agaricus bisporus* L.) (Miklus and Beelman, 1996); and strawberries (*Fragaria × ananassa* Duchesne) (Garcia et al., 1996), showed that applications of Ca immediately before, or just after harvest, maintained cell turgor, plasma membrane integrity, fruit firmness, and extended storage life.

The effectiveness of postharvest application of Ca to whole netted or honeydew muskmelons has not been reported. Therefore, the objectives of this study were first, to establish the developmental profile of Ca in honeydew mesocarp tissues during growth, maturation and postharvest senescence, for comparison with netted muskmelons (Bernadac et al., 1996). The second objective was to evaluate and contrast the tissue ion concentrations, surface characteristics, and mesocarp physicochemical characteristics of postharvest netted and honeydew muskmelons after exposure to Ca- and/or Mg-chelate dip solutions, and following commercial storage for 10 or 24 d.

Materials and Methods

PLANT MATERIAL. 'Honey Brew' hybrid honeydew (nonnetted, green-fleshed) and 'Explorer' hybrid (netted, orange-fleshed) muskmelon plants were grown in a greenhouse as described

Received for publication 15 Dec. 1998. Accepted for publication 30 Apr. 1999. Aleena Tarshis Moreno is gratefully acknowledged for muskmelon production. This research was funded by the USDA-ARS under CRIS no. 6204-43000-007-00D to G.E.L. and under cooperative agreement no. 58-6250-1-003 to M.A.G. Use of company or product names by the USDA does not imply approval or recommendation of the product to the exclusion of others that also may be suitable. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

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previously (Lester, 1998a). Flowers were hand-pollinated, and one fruit per plant was allowed to develop.

CHELATE TREATMENTS. Fruit designated for postharvest dip treatments were harvested at abscission, just after sunrise, and were assayed immediately, or were submerged (dipped) for 20 min in one of the following solutions at the same temperatures as that of the harvested fruit: tap water, Ca-chelate (0.08 M Ca-metalosate), Mg-chelate (0.08 M Mg-metalosate), or a mixture of the Ca-chelate and the Mg-chelate (0.08 M Ca-metalosate and 0.08 M Mg-metalosate), which will be referred to as (Ca+Mg)-chelate. These chelates are amino acid based and are proprietary formulations of Albion Laboratories, Clearfield, Utah. All chelate solutions were adjusted to pH 7 with citric acid. Following the dip, all fruit were air dried for 20 min before commercial storage conditions (4 °C and 90% ± 5% relative humidity (RH) for 'Explorer' or 10 °C and 90% ± 5% RH for 'Honey Brew') for 7 or 21 d. All fruit were stored an additional 3 d at 21 °C and 86% RH (10 d or 24 d total storage) before retrieval for various analyses.

FRUIT SURFACE CHARACTERISTICS. Following storage, all fruit were rated for surface darkening, diseased areas, and stem-end mold, as described by Carter (1981) and Lester (1989). Data are presented as the percentage of all fruit affected within a treatment.

DETERMINATION OF FRUIT WEIGHT LOSS, FIRMNESS, AND ELECTROLYTE LEAKAGE. Weight loss was calculated as the percent difference in weight for each fruit from the day of harvest to 10 or 24 d storage. Firmness of whole-fruit equatorial hypodermal mesocarp tissue, less the epidermis, was measured using a 4.5 cm × 3 mm diameter V-tip gauge (Chatillon force gauge; Lindy Electric, Glen Head, N.Y.) and expressed as mean force in Newtons (N). Electrolyte leakage was determined on three hypodermal mesocarp disks (10 mm diameter × 1 mm thick) from three different locations along the fruit equatorial circumference as described previously (Lester and Stein, 1993).

ISOLATION OF PLASMA MEMBRANE VESICLES FOR ANALYSIS OF LIPID, PROTEIN, AND H⁺-ATPASE ACTIVITY. Plasma membranes were isolated from the equatorial region of hypodermal mesocarp tissues as described previously (Lester and Stein, 1993). Lipids were extracted from plasma membrane pellet preparations and total free sterols and total phospholipids were measured as described previously (Lester and Stein, 1993). Protein content was determined by the method of Bradford (1976). Vanadate sensitive H⁺-ATPase activity was assayed using procedures of Lester and Stein (1993), and inorganic phosphate was determined as a measure of H⁺-ATPase activity by the method of Peterson (1978).

LIPXYGENASE ACTIVITY ASSAY. Lipoxxygenase activity in hypodermal mesocarp tissues was determined as reported previously by Lester (1998b). One unit of lipoxxygenase activity was equal to 0.001 absorbance units according to Holman (1955). Protein content of the mesocarp extract was determined by the method of Bradford (1976).

CALCIUM AND MAGNESIUM ANALYSES. A 17-mm-diameter cork borer was used to cut core segments from harvested melons. Cores were taken from the equatorial plane of the fruit. Mineral concentrations were determined on hypodermal mesocarp, middle mesocarp, and inner mesocarp tissue sections cut from the cores. The hypodermal mesocarp section (5 mm thick) was taken from 5 mm under the epidermis, the inner mesocarp section (10 mm thick) was taken from the portion of the mesocarp closest to the seed cavity, and the middle mesocarp section (10 mm thick) was taken from the center of the mesocarp. All sections were weighed

immediately to determine fresh weights, and were then dried at 70 °C for 48 h for dry weight determinations. Tissue sections were wet digested and the digestate resuspended as described in Grusak (1994). Total Ca or Mg content per gram dry weight was determined using atomic absorption spectrophotometry (model 2100; Perkin Elmer, Norwalk, Conn.). In one study, 'Honey Brew' mesocarp tissue was assayed for Ca concentration at 20, 30, 40, 50 (abscission), and 60 d (abscission plus 10 d storage at 10 °C and 90% ± 5% RH) postanthesis. Calcium and Mg concentrations also were determined on mesocarp samples taken from fruit at abscission, or following tap water or mineral chelate treatments and postharvest storage.

RADIOLABELED CALCIUM MOVEMENT INTO MESOCARP TISSUES. To assess the short-term movement of Ca into mesocarp tissues, 'Honey Brew' and 'Explorer' melons (harvested at abscission) were treated with an epidermal application of ⁴⁵Ca-labeled Ca-chelate (0.08 M Ca-metalosate; specific activity: 9.25 × 10⁸ Bq·mol⁻¹), and were stored for 24 h at 5 °C. The Ca-chelate and ⁴⁵Ca stock solution (⁴⁵CaCl₂; NEN Life Science Products, Boston, Mass.) were mixed together 24 h before the initiation of labeling. Radiolabeling was performed by first affixing a plastic collar (8 cm diameter), using softened lanolin paste, to form a well on the surface of each melon. Twenty-five milliliters of ⁴⁵Ca-chelate solution were applied to the well for 20 min at 22 °C; this solution was removed and the surface gently blotted. Labeled surfaces were allowed to air dry at 22 °C for 40 min; all fruit were then placed in a 5 °C cold room. Twenty-four hours after the initiation of labeling, a 7-mm-diameter cork borer was used to remove radial samples from the central portion of each labeled region. These were cut into the following segments: epidermis, hypodermal mesocarp, first centimeter of middle mesocarp, second centimeter of middle mesocarp, and the remaining middle to inner mesocarp (not including seeds). All segments were placed in scintillation vials with 0.5 mL 95% ethanol plus 0.5 mL 0.5% (w/v) sodium hypochlorite, and stored for 24 h. Ten milliliters of BCS liquid scintillation cocktail (Amersham Corp., Arlington Heights, Ill.) were added to each vial and radioactivity was determined 24 h later by coincidence counting using a liquid scintillation counter (model LS 5000TD; Beckman Instruments, Fullerton, Calif.). All sample counts were corrected for background counts and quenching. Data are presented for each segment as the percent of ⁴⁵Ca measured in the total core sample (i.e., ⁴⁵Ca adsorbed on the epidermal surface and absorbed into the core sample).

HISTOLOGY. Epidermal and hypodermal mesocarp tissue segments (10 × 5 × 2 mm) taken from the fruit equatorial region were fixed for at least 48 h in 1 formalin : 1 acetic acid : 18 alcohol (by volume) under a slight vacuum. After fixation, the segments were dehydrated in a tertiary butyl alcohol series, embedded in Paraplast (Oxford Labware, St. Louis, Mo.) (melting point 56 to 57 °C), sectioned serially on a rotary microtome at 12 µm thickness, affixed to slides using Haupt's adhesive, then infiltrated and stained with safranin and fast green, (Johanson, 1940). Photomicrographs were generated using a photomicroscope (Axioplan; Carl Zeiss Inc., Thornwood, N.Y.) equipped with an attached 35 mm camera.

STATISTICS. Analysis of variance was used to evaluate treatment differences for physicochemical and ion content data (SAS Inst., Cary, N.C.). Duncan's multiple range test (*P* ≤ 0.05) was used to discern between treatment classifications when F values were significant for main effects. Data are expressed as the average of five, single-fruit replications per cultivar per treatment.

Table 1. Calcium concentration of 'Honey Brew' honeydew mesocarp tissues 20 to 60 d after anthesis.

Days after anthesis	Mesocarp		
	Hypodermal	Middle	Inner
	Ca (mg·g⁻¹ dry wt)		
20	3.09 ± 0.27	2.38 ± 0.12	2.73 ± 0.12
30	4.60 ± 0.30	1.58 ± 0.30	1.10 ± 0.11
40	3.99 ± 0.36	0.29 ± 0.02	0.19 ± 0.01
50	3.88 ± 0.22	0.14 ± 0.01	0.14 ± 0.01
60 ^a	2.65 ± 0.27	0.20 ± 0.02	0.17 ± 0.01

^aSixty-day postanthesis fruit were abscised at 50 d, and then were stored 7 d at 10 °C plus 3 d at 21 °C. Data are expressed as means ±SD of 10 replications.

Results and Discussion

CALCIUM PROFILE STUDY. To develop a baseline of Ca accumulation in 'Honey Brew' honeydew, the Ca concentration was measured as fruit developed. Calcium concentration in middle and inner mesocarp tissues were similar on either a dry weight basis (Table 1) or a fresh weight basis (data not shown). Middle and inner mesocarp tissue Ca concentration was highest 20 d after anthesis (DAA), declined with age until 50 DAA, then increased slightly by 60 DAA. Hypodermal mesocarp Ca concentration showed the opposite trend in that it increased from 20 DAA to 50 DAA (abscission), then declined by 60 DAA. These mesocarp Ca concentration profiles in 'Honey Brew' are similar to those previously reported for netted muskmelons (Bernadac et al., 1996). 'Honey Brew' (Table 1) and netted muskmelon (Bernadac et al., 1996) hypodermal mesocarp tissue Ca concentration increased during fruit growth, peaked at fruit maturation, and then declined during postharvest storage. It is this postharvest decline in melon hypodermal mesocarp tissue that is of concern, because as previously reported (Lester, 1996), senescence can be retarded if postharvest muskmelon hypodermal mesocarp tissue Ca concentration can be maintained at an elevated level.

POSTHARVEST MINERAL CHELATE APPLICATION. At abscission (harvest), 'Honey Brew' and 'Explorer' hypodermal mesocarp tissue Ca concentrations were similar and as much as 44-fold higher than those of middle or inner mesocarp tissues (Table 2).

In water-treated fruit, 'Honey Brew' and 'Explorer' hypodermal mesocarp tissue Ca concentration declined 75% and 36%, respectively, following postharvest storage for 24 d. The middle and inner mesocarp tissue Ca concentration remained largely unchanged in water-treated fruit, except for the case of 'Explorer' inner mesocarp tissue, which had a 2.7-fold increase.

Treating 'Honey Brew' and 'Explorer' muskmelons with Ca-chelate or (Ca+Mg)-chelate solutions resulted in higher hypodermal mesocarp Ca concentrations after 10 d cold storage, relative to fruit treated with water or Mg-chelate solutions (Table 2). At this time, the Ca concentration in hypodermal mesocarp tissue of fruit treated with Ca-chelate or (Ca+Mg)-chelate was similar to that of fruit analyzed at harvest. Following 10 d cold storage, 'Honey Brew' hypodermal mesocarp tissue Ca concentration was significantly higher following Ca-chelate and (Ca+Mg)-chelate treatments, versus Mg-chelate and water treatments, but such was not the case for 'Explorer' fruit. Only the 'Explorer' fruit treated with (Ca+Mg)-chelate had significantly higher hypodermal mesocarp tissue Ca concentration versus all other 'Explorer' postharvest treatments. Following 24 d storage, hypodermal mesocarp tissue Ca concentrations were similar across most treatments for 'Honey Brew' and 'Explorer' although a trend toward higher Ca concentrations was evident for Ca-chelate and (Ca+Mg)-chelate treated fruit.

It is uncertain why the (Ca+Mg)-chelate treatment yielded the highest hypodermal mesocarp Ca concentration for 'Honey Brew' and 'Explorer' fruit, but a possible answer may be that the ion carrier contained a small amount of surfactant (Albion Labs, personal communication). Mason et al. (1974) clearly showed that submerging apple [*Malus sylvestris* (L.) Mill var. *domestica* (Borkh.) Mansf. 'Spartan'] in CaCl₂ with surfactants increased fruit Ca concentration up to 3 times as compared to CaCl₂ alone. In our study, the surfactant concentration of the (Ca+Mg)-chelate solution was 2-fold higher than that of the individual mineral chelate solutions.

Lack of an effect of the Ca-chelate treatment on hypodermal mesocarp Ca concentration in 'Explorer' fruit may be due to the presence of the epidermal lenticular tissue (netting). It is estimated (data not shown) that as much as 90% of the epidermis of netted muskmelons is covered with net. Photomicrographs of epidermal sections of netted muskmelon and honeydew fruit demonstrated that the epidermal region above the hypodermal

Table 2. Effect of postharvest application of Ca-chelate or Mg-chelate on Ca concentration of muskmelon mesocarp tissues. Abscised (harvested) fruit were dipped for 20 min in water, or a solution containing Ca-chelate (0.08 M), Mg-chelate (0.08 M), or a combination of each mineral chelate at a total concentration of 0.16 M [(Ca+Mg)-chelate]. Control fruit were analyzed at harvest; treated fruit were stored for 7 or 21 d at 10 °C, plus 3 d at 21 °C ('Honey Brew'), or 7 or 21 d at 4 °C, plus 3 d at 21 °C ('Explorer'). Tissue sectioning, processing, and Ca analyses were performed as described in the materials and methods.

Treatment	Mesocarp [Ca (mg·g ⁻¹ dry wt)]											
	Honey Brew						Explorer					
	Hypodermal	Middle	Inner	Hypodermal	Middle	Inner	Hypodermal	Middle	Inner	Hypodermal	Middle	Inner
	At harvest											
None	6.15 ± 1.71 ^a	0.22 ± 0.05	0.14 ± 0.03	6.57 ± 2.03	0.97 ± 0.38	0.25 ± 0.08						
	After postharvest storage (d)											
	10	24	10	24	10	24	10	24	10	24	10	24
Water	2.52 b ^y	1.51 b	0.22 a	0.14 b	0.12 a	0.17 a	4.88 b	4.23 a	0.88 b	0.64 b	0.73 a	0.68 a
Ca-chelate	6.23 a	3.26 a	0.25 a	0.26 a	0.10 a	0.08 b	5.19 b	5.54 a	1.27 a	1.15 a	0.69 a	0.70 a
Mg-chelate	3.43 b	3.00 ab	0.11 b	0.26 a	0.11 a	0.17 a	4.83 b	3.79 a	1.24 a	1.11 a	0.79 a	0.66 a
(Ca+Mg)-chelate	7.20 a	4.24 a	0.16 ab	0.16 b	0.14 a	0.16 a	6.95 a	5.19 a	1.22 a	0.95 ab	0.69 a	0.59 a
P ≤ 0.05	1.50	1.55	0.10	0.08	0.05	0.01	1.70	2.88	0.32	0.41	0.13	0.12

^aValues are means ± SD (n = 5).

^yMean separation within columns by Duncan's multiple range test, P ≤ 0.05 (n = 5).

Fig. 1. Photomicrographs of longitudinal sections of epidermal and hypodermal tissues from the equatorial regions of (A) 40-d-old abscised netted muskmelons and (B) 50-d-old abscised honeydew melons. L = lenticel (netting), E = epidermis, HM = hypodermal mesocarp, CP = columnar phelloderm. Scale bars = 1mm.

mesocarp can be 5-fold thicker than the epidermis of honeydew fruit (Fig. 1). In addition to the net thickness, which created a greater distance for the Ca-chelate to travel to the hypodermal mesocarp, this netting mass is columnar phelloderm which is

derived from hypodermal mesocarp tissue (Lester, 1988). This netting may sequester Ca^{2+} and/or the Ca-chelate. To assess the effect of netting on the migration of Ca-chelate, ^{45}Ca -labeled chelate was applied to 'Honey Brew' and 'Explorer' epidermal tissues (Table 3). Within 24 h at 5 °C, 23% of the total labeled Ca present in the tissues of 'Honey Brew' fruit was measured in mesocarp tissues. However, for fruit regions of 'Explorer' with 100% netted epidermis, only 9% of the label was measured in the mesocarp tissues. Similar results were seen for 'Explorer' fruit regions with normal netting ($\approx 90\%$ covered), in which 11% of the label was measured in the mesocarp tissues. These results suggest that netting strongly impedes radial migration of applied Ca-chelate in muskmelons. Higher

concentrations of applied Ca-chelate may be needed for increased Ca penetration in netted melons.

Magnesium concentration at harvest was highest in the hypodermal region of the mesocarp in both muskmelon cultivars, but 'Honey Brew' hypodermal mesocarp Mg concentration was more than 2-fold higher that of 'Explorer' (Table 4). However middle mesocarp Mg concentration of 'Explorer' was about 2-fold higher than in 'Honey Brew'. 'Honey Brew' and 'Explorer' fruit treated with Mg-chelate or (Ca+Mg)-chelate did not contain

Table 3. Movement of labeled Ca into mesocarp tissues following a 20-min surface application of ^{45}Ca -chelate and storage of whole fruit at 5 °C for 24 h. Data are presented as a percent of the total ^{45}Ca recovered in muskmelon epidermal and mesocarp regions within a core sample.

Mesocarp tissues	Distance subepidermal (mm)	Total radioactivity (%)		
		Explorer (epidermal regions)		
		Honey Brew ^z	100% netting ^y	90% netting ^x
Hypodermal	0–2	10.9	3.1	5.7
Middle	2–12	8.9	3.8	3.4
Middle	12–22	2.5	1.4	1.0
Middle/inner	22+	1.1	0.8	0.8
Total	0–22+	23.4	9.1	10.9

^zHoney Brew melon epidermal tissue was not lenticulated (netted).

^yMuskmelon netting covered 100% of the epidermis.

^xMuskmelon netting covered >90% of the epidermis.

Table 4. Effect of postharvest application of Ca-chelate or Mg-chelate on Mg concentration of muskmelon mesocarp tissues. Treatments are described in Table 2.

Treatment	Mesocarp [Mg (mg·g ⁻¹ dry wt)]											
	Honey Brew						Explorer					
	Hypodermal		Middle		Inner	Hypodermal		Middle		Inner		
	At harvest											
None	6.27 ± 1.29 ^z		1.56 ± 0.25		0.78 ± 0.10		3.08 ± 0.41		3.09 ± 0.35		0.97 ± 0.19	
	After postharvest storage (d)											
	10	24	10	24	10	24	10	24	10	24	10	24
Water	5.00 ab ^y	4.01 a	0.88 b	0.64 b	0.73 a	0.68 a	4.35 a	5.66 a	2.78 a	3.08 ab	1.23 ab	1.32 a
Ca-chelate	3.75 b	4.88 a	1.27 a	1.14 a	0.69 a	0.70 a	5.13 a	6.09 a	2.32 a	3.48 a	1.33 a	1.16 a
Mg-chelate	6.79 a	4.90 a	1.24 a	1.11 ab	0.79 a	0.66 a	4.81 a	4.91 a	2.38 a	2.45 b	0.92 b	2.28 a
(Ca+Mg)-chelate	5.67 ab	6.43 a	1.22 a	0.95 ab	0.69 a	0.59 a	5.43 a	5.42 a	3.76 a	3.20 ab	0.98 b	1.46 a
<i>P</i> ≤ 0.05	1.79	2.52	0.15	0.49	0.10	0.12	1.78	1.55	1.45	0.75	0.32	1.40

^zValues are means ± SD (n = 5).

^yMean separation within columns by Duncan's multiple range test, P ≤ 0.05 (n = 5).

Table 5. Surface characteristics of muskmelons at harvest or following mineral chelate treatments and storage. See Table 2 for treatment details.

Treatment	Storage (d)	Surface darkening		Diseased areas		Stem end mold
		Fruit affected ^z (%)	Severity ^y	Fruit affected (%)	No./ fruit	Fruit affected (%)
Honey Brew						
None	Harvest	0	0	0	0	0
Water	10	20	1	0	0	0
Ca-chelate	10	0	0	0	0	0
Mg-chelate	10	60	3	0	0	0
(Ca+Mg)-chelate	10	20	1	0	0	0
Water	24	60	5	80	2	0
Ca-chelate	24	0	0	0	0	20
Mg-chelate	24	40	3	40	1	40
(Ca+Mg)-chelate	24	0	0	0	0	0
Explorer						
None	Harvest	0	0	0	0	0
Water	10	0	0	0	0	0
Ca-chelate	10	0	0	0	0	20
Mg-chelate	10	0	0	0	0	0
(Ca+Mg)-chelate	10	0	0	0	0	0
Water	24	60	5	80	3	0
Ca-chelate	24	20	1	80	2	0
Mg-chelate	24	20	1	40	1	0
(Ca+Mg)-chelate	24	0	0	80	2	0

^z(n = 5).^ySeverity was rated on a scale 0 to 5 with: 0 = none, 1 = slight, 3 = medium, and 5 = severe (n = 5).

Table 6. Physical characteristics of muskmelons at abscission (harvest) or following mineral chelate treatments and storage. See Table 2 for treatment details.

Treatment	Storage (d)	Firmness (N)	Wt loss (%)	Electrolyte leakage (%) ^z
Honey Brew				
None	Harvest	21.8 ± 2.8 ^y	0.0	18.5 ± 2.9
Water	10	7.0 c ^x	1.9 a	23.4 a
Ca-chelate	10	18.5 a	1.0 b	15.4 b
Mg-chelate	10	14.3 b	1.2 b	18.2 b
(Ca+Mg)-chelate	10	16.8 ab	0.8 b	17.2 b
<i>P</i> ≤ 0.05		3.4	0.4	3.9
Water	24	5.6 b	2.6 a	27.6 ab
Ca-chelate	24	13.4 a	1.7 c	19.0 c
Mg-chelate	24	7.4 a	2.3 ab	32.2 a
(Ca+Mg)-chelate	24	12.6 a	1.9 bc	22.6 bc
<i>P</i> ≤ 0.05		3.2	0.4	5.8
Explorer				
None	Harvest	23.7 ± 2.3	0.0	18.5 ± 2.9
Water	10	9.1 c	6.8 a	31.4 a
Ca-chelate	10	9.5 c	4.0 b	26.8 b
Mg-chelate	10	13.7	b 3.8 b	24.0 b
(Ca+Mg)-chelate	10	19.3 a	3.2 b	22.8 b
<i>P</i> ≤ 0.05		1.8	0.8	4.1
Water	24	5.8 b	8.6 b	34.0 a
Ca-chelate	24	9.0 a	8.2 c	31.6 a
Mg-chelate	24	10.4 a	9.8 a	31.2 a
(Ca+Mg)-chelate	24	10.9 a	8.0 b	29.6 a
<i>P</i> ≤ 0.05		2.2	1.1	4.5

^zExpressed as a percentage of total electrolytes following freezing.^yValues are means ± SD (n = 5).^xMean separation within columns by Duncan's multiple range test, *P* ≤ 0.05 (n = 5).

higher Mg concentrations in hypodermal mesocarp relative to water-treated fruit (Table 4). Calcium-chelate-treated fruit of 'Honey Brew' showed a significantly lower Mg concentration in the hypodermal mesocarp following 10 d storage. Tyler and Lorenz (1963) compared Mg concentrations in four different muskmelon types (crenshaw, honeydew, netted muskmelon, and Persian), and found that fruit Mg concentration changed very little with age.

Surfaces of 'Honey Brew' and 'Explorer' fruit, regardless of treatment, were free of diseases following 10 d cold storage (Table 5). Surface darkening on 'Explorer' fruit stored 10 d was nonevident. On Mg-chelate-treated 'Honey Brew', surface darkening was moderate at 10 d and on all other treated 'Honey Brew' fruit, it was none to slight.

After being stored 24 d, 60% of water-treated fruit of 'Explorer' and 'Honey Brew' had severely darkened surfaces. Only Ca-chelate and (Ca+Mg)-chelate treated 'Honey Brew' and (Ca+Mg)-chelate treated 'Explorer' fruit had no surface darkening at this time. These fruit had higher Ca concentration in the hypodermal mesocarp 14 d earlier (Table 2), suggesting that incipient darkening of muskmelon fruit surfaces is associated with a localized, declining Ca concentration.

A benefit of Ca and Ca+Mg treatments was observed for the physical characteristics of fruit firmness, weight loss, and hypodermal mesocarp electrolyte leakage depending on duration of storage (Table 6). 'Honey Brew' fruit from Ca-chelate and (Ca+Mg)-chelate treatments were the most firm following 10 d storage, had the lowest moisture loss and the least electrolyte leakage following 24 d storage, compared to water or Mg-chelate treated fruit. 'Explorer' fruit treated with (Ca+Mg)-chelate also

were the most firm (10 d storage), and had the least moisture loss (24 d storage). Peleg et al. (1993) reported that maintaining muskmelon firmness directly promotes longer fruit shelf life. The exact regulation of muskmelon firmness loss is unknown, but is thought to be associated with compositional changes in cell wall fractions (McCollum et al. 1989; Rose et al. 1998).

Our recent and current findings suggest that loss of muskmelon firmness is associated with changes in plasma membrane (PM) integrity as measured by electrolyte leakage, total free sterol to total phospholipid ratio (TFS:TPL), and H⁺-ATPase activity (Lester and Stein, 1993; Lester and Whitaker, 1996). In our current study, muskmelon hypodermal mesocarp PM integrity was beneficially affected by maintenance of a high tissue Ca concentration. 'Honey Brew' fruit, from Ca-chelate and (Ca+Mg)-chelate treatments, had the lowest TFS:TPL ratios following 24 d storage, and had the highest H⁺-ATPase activities at 10 and 24 d storage compared to water or Mg-chelate treated fruit (Tables 7 and 8). 'Explorer' fruit treated with (Ca+Mg)-chelate, versus the other treatments, also had the lowest (TFS:TPL) ratio (10 and 24 d storage) and the highest total protein content and H⁺-ATPase activities (10 d storage).

Calcium's beneficial effect on muskmelon hypodermal mesocarp PM integrity may be multifunctional. Calcium is known to regulate the expression and synthesis of proteins and enzymes (Poovaiah and Reddy, 1993), and to reduce catabolism of total phospholipids and delay an increase in TFS:TPL (Picchioni et al., 1996). In muskmelon PM, an increase in TFS:TPL is one of the most obvious changes which occurs during senescence, and is due to catabolism of phospholipids (Lester and Whitaker, 1996). Yermiyahu et al. (1994) described a direct Ca-related benefit to

Table 7. Total free sterol to protein ratio ($\mu\text{mol}\cdot\text{mg}^{-1}$), total phospholipid to protein ratio ($\mu\text{mol}\cdot\text{mg}^{-1}$) and total free sterol to total phospholipid mol ratio of hypodermal mesocarp plasma membrane fractions of muskmelons at abscission (harvest) or following mineral chelate treatments and storage. See Table 2 for treatment details.

Treatment	Storage (d)	Ratio		
		Total free sterol to protein	Total phospholipid to protein	Total free sterol to total phospholipid
Honey Brew				
None	Harvest	0.32 ± 0.04 ^z	0.68 ± 0.01	0.47 ^y
Water	10	0.46 ± 0.04	0.68 ± 0.03	0.67
Ca-chelate	10	0.44 ± 0.01	0.65 ± 0.05	0.68
Mg-chelate	10	0.43 ± 0.09	0.59 ± 0.04	0.73
(Ca+Mg)-chelate	10	0.39 ± 0.05	0.69 ± 0.04	0.56
Water	24	0.36 ± 0.04	0.45 ± 0.06	0.81
Ca-chelate	24	0.40 ± 0.05	0.57 ± 0.04	0.71
Mg-chelate	24	0.40 ± 0.04	0.44 ± 0.05	0.81
(Ca+Mg)-chelate	24	0.42 ± 0.05	0.58 ± 0.04	0.72
Explorer				
None	Harvest	0.35 ± 0.05	0.98 ± 0.04	0.36
Water	10	0.30 ± 0.06	0.34 ± 0.01	0.85
Ca-chelate	10	0.32 ± 0.06	0.49 ± 0.04	0.65
Mg-chelate	10	0.33 ± 0.03	0.53 ± 0.03	0.62
(Ca+Mg)-chelate	10	0.28 ± 0.01	0.57 ± 0.03	0.49
Water	24	0.25 ± 0.01	0.28 ± 0.03	0.89
Ca-chelate	24	0.24 ± 0.03	0.35 ± 0.03	0.68
Mg-chelate	24	0.29 ± 0.06	0.39 ± 0.03	0.74
(Ca+Mg)-chelate	24	0.26 ± 0.02	0.40 ± 0.02	0.65

^zValues are means $\pm$ SD (n = 5).

^yValues are (mean total free sterol : protein ratio) : (mean total phospholipid : protein ratio) ratios.

Table 8. Total and specific H⁺-ATPase activity, and protein content of hypodermal mesocarp plasma membrane and lipoxygenase (LOX) activity of the hypodermal mesocarp tissue of muskmelons at abscission (harvest) or following mineral chelate treatments and storage. See Table 2 for treatment details.

Treatment	Storage (d)	Total H ⁺ -ATPase activity [Pi (μmol·h ⁻¹)]	Specific H ⁺ -ATPase activity (μmol Pi/mg protein/h)	Total protein (mg/fraction)	LOX activity (mg protein/fraction)
Honey Brew					
None	Harvest	3.25 ± 0.30 ^z	4.92 ± 0.19	0.66 ± 0.04	77.6 ± 2.6
Water	10	1.90 d ^y	4.04 c	0.47 b	105.4 a
Ca-chelate	10	2.56 b	5.11 a	0.50 ab	72.2 b
Mg-chelate	10	2.09 c	4.36 b	0.48 b	111.0 a
(Ca+Mg)-chelate	10	2.68 a	4.87 a	0.55 a	74.5 c
<i>P</i> ≤ 0.05		0.10	0.23	0.05	5.6
Water	24	1.68 c	3.82 b	0.44 b	123.5 a
Ca-chelate	24	2.28 a	4.36 a	0.52 a	80.1 b
Mg-chelate	24	1.85 b	3.87 b	0.48 ab	130.2 a
(Ca+Mg)-chelate	24	2.27 a	4.37 a	0.52 a	84.7 b
<i>P</i> ≤ 0.05		0.09	0.23	0.07	7.9
Explorer					
None	Harvest	3.17 ± 0.10	7.55 ± 0.40	0.42 ± 0.20	68.0 ± 4.0
Water	10	2.66 b	5.65 d	0.47 a	108.7 a
Ca-chelate	10	2.14 d	5.94 c	0.36 b	90.0 b
Mg-chelate	10	2.84 a	6.30 b	0.36 b	86.1 b
(Ca+Mg)-chelate	10	2.84 a	6.92 a	0.41 a	71.5 c
<i>P</i> ≤ 0.05		0.11	0.23	0.08	7.8
Water	24	1.71 c	5.04 b	0.34 a	125.2 a
Ca-chelate	24	1.80 b	5.45 a	0.33 a	97.1 c
Mg-chelate	24	1.82 b	5.50 a	0.33 a	106.2 b
(Ca+Mg)-chelate	24	2.01 a	5.30 a	0.38 a	89.7 d
<i>P</i> ≤ 0.05		0.09	0.20	0.09	7.8

^zValues are means ± SD (n = 5).

^yMean separation within columns by Duncan's multiple range test, *P* ≤ 0.05 (n = 5).

muskmelon root PM phospholipids relative to Mg, by demonstrating that Ca serves as a specific electrostatic binder on the head group of negatively charged phospholipids, and that the phospholipid binding constant of Ca is 5.6-fold greater than that of Mg, thereby directly regulating melon PM permeability (i.e., integrity). Kinoshita et al. (1995) showed that cellular Ca concentration directly regulates PM H⁺-ATPase activity. In our study, muskmelons with elevated hypodermal mesocarp Ca concentration had lower TFS:TPL ratios and subsequently higher total protein content and H⁺-ATPase activity, which positively affected PM integrity.

An additional senescence-related assault on muskmelon PM integrity is lipid peroxidation by lipoxygenase (LOX). In netted and honeydew PM, lipid degradation via LOX activity is greatest in mature and postharvest fruit (Lacan and Baccou, 1998; Lester, 1990, 1998b). Ferguson (1984) reported that Ca directly influences membrane lipid peroxidation by lowering the concentration of free radicals. LOX activity (Table 8) was lower in fruit which had experienced the greatest Ca enrichment in hypodermal mesocarp tissues (Ca in 'Honey Brew' and Ca+Mg in 'Explorer' and 'Honey Brew'). This may have resulted from Ca indirectly interacting with LOX activity by disrupting free radical production, or by serving as a membrane protective barrier to disrupt lipid peroxidation by free radicals (Clarkson, 1984).

In summary, this study demonstrated that Ca concentration in muskmelon hypodermal mesocarp tissue is highest before abscission. Following 24 d postharvest storage, Ca concentration within the hypodermal mesocarp dropped 36% to 75%, depending on

muskmelon cultivar, and this loss in Ca was associated with heightened senescence as measured by surface darkening, diseased areas, decreased firmness, increased weight loss, and PM perturbation. The decline in the Ca concentration in the hypodermal mesocarp after harvest is due to Ca diffusing into the middle and inner layers of fruit and most likely repositing in the seeds. Treating fully abscised, greenhouse-grown, hybrid honeydew muskmelons with 0.08 M chelated Ca effectively maintained Ca concentration in the hypodermal mesocarp tissue at a level that extended postharvest storage. Inclusion of Ca-chelate dips with existing field harvesting operations could have a significant economic impact on the melon industry, by enabling shipment of high quality melons over greater distances, and thus to more markets. Additionally, new markets could be developed for very sweet, highly nutritious, vine ripened (abscised) honeydew fruit which, at present, have a limited postharvest storage life.

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