Plasma Membrane Physicochemical Changes during Maturation and Postharvest Storage of Muskmelon Fruit

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Additional index words. *Cucumis melo*, ATPase, electrolyte leakage, fatty acids, free sterols, phospholipids

Abstract. Changes in the physical and chemical properties of the plasma membrane from hypodermal mesocarp tissue of netted muskmelon (*Cucumis melo* L. var. *reticulatus* Naud.) fruit were compared in relation to the permeability changes of the same tissue during fruit maturation and storage at 4 or 24°C. As muskmelon fruit progress from immaturity to maturity, and with storage of mature fruit at 4 or 24°C, increased permeability of the hypodermal-mesocarp tissue occurs coincident with an increase in the saturation index of the plasma membrane phospholipids. Buoyant density of the plasma membrane from hypodermal mesocarp tissue increased from 1.13 to 1.14 g·cm⁻³ during fruit maturation. Vanadate-sensitive ATPase (EC 3.6.1.35) activity was highest in mature fruit at harvest. After 10 days of storage, vanadate-sensitive ATPase activity was much lower in fruit kept at 24°C than in those kept at 4°C. The decrease in vanadate-sensitive ATPase activity in fruit stored at 24°C was correlated with increased hypodermal-mesocarp membrane permeability. We suggest that biochemical changes affecting the lipid matrix of the plasma membrane influence fruit membrane permeability and possibly muskmelon storage life.

Green, full-slip netted muskmelon fruit have a storage life, limited by the rate of softening and senescence, of less than 14 days, depending on the cultivar and temperature. In netted muskmelon, softening and senescence have not been associated with cell wall breakdown (Lester and Dunlap, 1985; McCollum et al., 1989) but rather with loss of hypodermal-mesocarp membrane integrity (Lester, 1988). Loss of hypodermal-mesocarp tissue membrane integrity during fruit senescence has been attributed to lipoygenase (EC 1.13.11.12 activity, which results in perturbation to microsomal membranes (Lester, 1990b). Therefore, netted muskmelon fruit softening and senescence may be regulated by membrane integrity. In apple fruit (*Malus domestica* Borkh.), changes in the integrity of microsomal membranes are correlated with increased concentrations of membrane free sterols and increased saturation indices of phospholipid fatty acids (Lurie and Ben-Arie, 1983). In bell pepper fruits (*Capsicum annuum* L.), loss of microsomal membrane integrity has been demonstrated by increased leakage of electrolytes and a correlation with increased total free sterols (Lurie and Ben-Yehoshua, 1986). Whitaker (1991) showed that the level of total free sterols and phospholipids in bell pepper fruit can either decrease, increase, or remain unchanged with maturation and senescence, depending on the microsomal membrane. Plant plasma membranes have a higher free sterol concentration than other cell membranes. The composition of free sterols and their interaction with adjacent phospholipids can restrict the motion of acyl chains, resulting in decreased membrane fluidity and increased electrolyte leakage (Douglas and Walker, 1984). In *Citrus* roots, an inverse relationship exists between plasma membrane fluidity and plasma membrane ATPase activity (Douglas and Walker, 1984). Therefore, characterization of the protein, total free sterols, total phospholipids, phospholipid fatty acid saturation index, and ATPase enzyme activity of muskmelon hypodermal-mesocarp tissue plasma membranes may help eluci-

date the role of the lipid matrix on plasma membrane integrity in netted muskmelon fruit.

This paper describes the lipid matrix composition of the hypodermal-mesocarp tissue plasma membranes from ripening muskmelon fruit and compares it with changes in membrane permeability, density, and ATPase activity.

Materials and Methods

Plant material. Fruit of 'Explorer' muskmelon with a mean weight of 1.5 kg and free of defects were harvested just after sunrise from a commercial field located near Weslaco, Texas. Fruit classified as immature were ~25 to 30 days postpollination, based on external characteristics (Lester and Dunlap, 1985). Fruit classified as mature were green, full-slip. Fruit were washed with distilled water and either processed immediately or placed in ventilated, waxed muskmelon-shipping boxes for 10 days in rooms at 4 ± 1°C with 85% ± 5% RH or 24 ± 2°C with 85% ± 5% RH. Storage experiments were performed with mature fruit only.

Electrolyte leakage. Electrolyte leakage was determined on 20 hypodermal mesocarp disks (10 mm wide × 1 mm thick), according to a procedure that substitutes the hypotonic bathing solution with a 0.35 M mannitol solution (Lester and Bruton, 1986). Total electrolytes were determined in the same solution after freezing of the disks at -20°C for 18 h then rewarming to 21°C. Leakage data were expressed as a percentage of the conductivity of total tissue electrolytes.

Isolation of microsomes. All procedures were conducted at 2 to 4°C on hypodermal-mesocarp tissue and were a modification of those described in Lester (1990a). Two to 3-mm thick strips of exposed hypodermal-mesocarp tissue were prepared with a vegetable peeler. The epidermal and lenticular tissues were removed, with a vegetable peeler, from the entire fruit excluding the ground spot, stem, and blossom ends. Hypodermal-mesocarp tissue (150

Received for publication 18 Feb. 1992. Accepted for publication 1 Oct. 1992. 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.

Abbreviations: DTT, dithiothreitol; FAME, fatty acid methyl ester; NL, neutral lipids; PL, polar lipids; PVPP, polyvinylpyrrolidone.

g) from three fruit was macerated with a commercial food processor in 250 ml of homogenizing buffer consisting of: 0.5 M sucrose, 50 mM Hepes-KOH, 5 mM ascorbic acid, 5 mM EDTA, 5 mM dithiothreitol (DTT), and 0.6% (w/v) polyvinylpyrrolidone (PVPP), pH 7.5. Ascorbic acid and PVPP were added just before homogenizing. Hypodermal-mesocarp tissue was macerated in the homogenizing medium for 5 min, placed under vacuum for 5 min, then macerated an additional 5 min. The material was transferred to a 1-liter plastic beaker and homogenized for 30 sec at medium speed using a Polytron homogenizer (Brinkman Instruments, Wesbury, N.Y.) equipped with a PTA-50 generating probe. The homogenate was squeezed through two layers of Masslin Towel (Chicopee, New Brunswick, N.J.) and centrifuged at $14,000\times g$ for 20 min at 2°C. A microsomal pellet was obtained from the $14,000\times g$ supernatant by centrifugation at $100,000\times g$ for 30 min. This pellet was homogenized for 10 sec at low speed using a Polytron homogenizer equipped with a 20TS generating probe in 10 ml of suspension buffer consisting of: 0.33 M sucrose, 3 mM KCl, and 5 mM potassium phosphate, pH 7.8. The homogenized suspension (9 g) was added to 27 g aqueous polymer buffer containing 6.2% (w/w) dextran T500 and 6.2% (w/w) PEG 3400. The phase system was mixed well and maintained on ice for 1 h. Afterward, phase separation was accelerated by centrifugation in a swinging bucket rotor at $1500\times g$ for 3 min. Upper and lower layers were separated, and the lower phases were washed with equal volumes of 6.2% (w/w) PEG 3400 fresh upper phase. The upper phases were washed with equal volumes of fresh 6.2% dextran T500 lower phase until a chlorophyll-free preparation was achieved (about two washes). The upper phases were pooled, and a plasma membrane-enriched pellet was recovered by centrifugation at $100,000\times g$ for 30 min. Individual pellets were suspended in 2 ml of storage buffer: 20% sucrose (w/w), 40 mM Tris-MES, 1 mM EDTA, and 1 mM DTT, pH 8.0 and were either kept on ice for immediate use or pelleted, flushed with N_2 , and stored at -80°C .

Isopycnic sucrose density gradient. Upper phase plasma membrane-enriched pellets suspended in 2 ml of storage buffer were layered on a 36-ml linear sucrose gradient of 20% to 45% (w/w) sucrose in 40 mM Tris-MES, 1 mM EDTA, and 1 mM DTT, pH 8.0, and then centrifuged at $75,000\times g$ for 15 h at 2°C. After centrifugation, 1.5 ml fractions were collected from the bottom of the gradient with a proportioning pump at a flow rate of $0.5\text{ ml}\cdot\text{min}^{-1}$ at 4°C. Density of the fractions was determined using an automatic temperature adjusting Abbe Mark II refractometer (Riechart Scientific Instruments, Buffalo, N.Y.).

Protein and enzyme analysis. Protein content of the density fractions was determined by the method of Bradford (1976). Vanadate sensitive ATPase was assayed at pH 6.5 and 38°C, according to the modified method of Hodges et al. (1972). Reaction mixtures contained 1 ml of 3 mM Tris-ATP, 4 mM MgSO_4 , 33 mM Tris-MES, 50 mM KNO_3 , and 1 mM DTT with or without 1 mM Na_3VO_4 . ATPase enzyme assays were stopped by adding 2 ml of 2 N HCl containing 1.25% (w/v) ammonium molybdate. Inorganic phosphate was determined as a measure of ATPase activity by the method described by Peterson (1978).

Total free sterol and total phospholipid determination. Lipids were extracted from plasma membrane preparations using the procedure of Bligh and Dryer (1959). The solvent extract was evaporated to dryness with N_2 and then redissolved in 1 ml of hexane. Polar lipids (PL) were separated from neutral lipids (NL) by passing the extract through a Sep-Pak silica gel cartridge (Waters Assoc., Milford, Mass.) as described in Forney (1990). The extract was allowed to enter the silica, and the neutral lipids were eluted with 5 ml of chloroform. Steryl glycosides were eluted

with 10 ml of acetone. PL were eluted with 10 ml of chloroform:methanol (1:1, v/v) followed by 10 ml of methanol. The collected fractions were reduced to 2.0 ml under a stream of N_2 and stored at 0 to 4°C until analyzed.

The sterols were analyzed by gas chromatography and detected by use of flame ionization. Separation of sterols from other compounds was made on a DB 1701 capillary column (15 m $\times$ 0.245 mm) programmed at a rate of 4°C/min from 200 to 275°C, according to Brown and DuPont (1989). The carrier gas was helium. A Hewlett-Packard 3696 series II integrator was used to identify and quantitate the sterol peaks, against sitosterol using the external standard program. A plant sterol mixture of brassicasterol, campesterol, stigmasterol, and sitosterol (Sigma Chemical Co. St. Louis) was initially used for the co-chromatography of free sterols in the NL fraction.

Phospholipids present in the polar lipid fraction were quantified by the method of Ames (1966) for inorganic phosphate.

Fatty acid methyl esters. Fatty acid methyl esters (FAMES) were analyzed by capillary gas-liquid chromatography immediately following transesterification of the polar fractions with 0.5 N sodium methoxide at 80°C for 20 min, according to Uemura and Yoshida (1984). The FAME solution was reduced to dryness with N_2 , and the residue redissolved in 0.1 ml of hexane for gas chromatography. FAMES were separated on a Hewlett-Packard fused silica esterified polyethylene glycol column (30 m $\times$ 0.53 cm) programmed from 50 to 170°C at 3°C/min, followed by a rate of 1°C/min from 170 to 200°C. The splitless injection mode was used. The injection port was at 225°C and the detector at 250°C, according to Robacker and Guerra (1989). FAMES were detected with flame ionization, and a Shimadzu chromatographic C-RIB was used for peak area determination. Identification and quantification of individual FAMES were made using known standards and the external standard program.

Statistics. Analysis of variance was used to evaluate maturity and storage differences for the physiological data (SAS, 1988). Duncan's multiple range test ($P \leq 0.05$) was used to discern between maturation and storage classifications when F values were significant.

Results and Discussion

Electrolyte leakage, assayed in mannitol osmoticum, was greater in mature than immature melons (Fig. 1). Increased electrolyte efflux with advancing melon maturity was previously observed when water was used as the bathing solution (Lester, 1988). No further increase in hypodermal-mesocarp tissue electrolyte leakage was observed after storage of mature melons for 10 days at 4°C; however, a significant increase in electrolyte leakage occurred following storage of mature melons for 10 days at 24°C. These data indicate that the changes in membrane permeability of mature muskmelon fruit occur with further ripening and likely are temperature and/or moisture loss dependent. Lurie and Ben-Arie (1983) revealed similar increases in permeability between preclimacteric (immature) and peak climacteric (mature) apple fruit. Although we did not determine respiration or ethylene production in our fruit, the muskmelon fruit ages (immature, mature) coincide approximately with preclimacteric and peak climacteric ripening stages, respectively (Lyons et al., 1962). Ripening-associated increased membrane permeability in apple fruit was coincident with increased membrane microviscosity (Lurie and Ben-Arie, 1983). Increased plasma membrane permeability and microviscosity of senescing rose [*Rosa* (hyb.) cv. Golden Wave] petals is likely due to increased lipase (EC 3.1.1.3)

Fig. 1. Efflux of electrolytes into 0.35 M mannitol solution from hypodermal mesocarp tissue from immature, mature (full-slip) at harvest, and mature muskmelon fruits stored 10 days at 4 or 24C.

Fig. 2. Vanadate-sensitive ATPase activity of linear sucrose gradient fractions of plasma membrane vesicles isolated from the hypodermal-mesocarp tissue of immature, mature (full-slip) at harvest, and mature muskmelon fruits stored 10 days at 4 or 24C.

activity, which alters the lipid matrix by increasing the free sterol to phospholipid ratio (Borochoy et al., 1978).

Plasma membrane density and vanadate-sensitive ATPase activity from muskmelon hypodermal-mesocarp tissue changed with ripening (Fig. 2). Vanadate-sensitive ATPase activity is a reliable marker for plasma membrane (Lester, 1990a; Olivari et al., 1991). When plasma membrane-enriched vesicles from different ripening stages of muskmelon fruit were separated on linear sucrose gradients, the banding pattern revealed an increase in density with maturity. These changes included an increase in protein concentration at 1.14 g·cm⁻³, relative to 1.13 g·cm⁻³ (Fig. 3) concurrent with the shift of peak vanadate-sensitive ATPase activity from 1.13 to 1.14 g·cm⁻³. The increase in plasma membrane density from 1.13 to 1.14 g·cm⁻³, which represents a shift from 30.0% to 32.2% sucrose concentration, occurred only in fruit passing from the

immature to the mature stage. An increase in plasma membrane density with fruit maturation has also been noted in avocado (*Persea americana* Mill.) fruit (Dallman et al., 1988). After muskmelon fruit have reached maturity and have apparently passed through their climacteric, there are no additional shifts in plasma membrane density. Vanadate-sensitive ATPase activity, however, changed during muskmelon fruit maturation and storage (Fig. 2). As muskmelon fruit passed from immature to the mature-green, full-slip stage, vanadate-sensitive ATPase peak activity increased 1.36-fold, but when mature fruits were kept for 10 days at 4 or 24C, ATPase activity decreased 1.18- and 2.10-fold, respectively. Poole et al. (1984) suggest that increases in plasma membrane density may be related to increased metabolic activity. Our finding of increased ATPase activity, coincident with increased plasma membrane density, corroborates this suggestion.

Fig. 3. Protein determination of linear sucrose gradient fractions of plasma membrane vesicles isolated from the hypodermal-mesocarp tissue of immature, mature (full-slip) at harvest, and mature fruits stored 10 days at 4 or 24C.

Table 1. Total free sterol lipids, total phospholipids, free sterol to phospholipid ratio, and saturated : unsaturated fatty acid ratio (percent : percent) of plasma membranes isolated from hypodermal-mesocarp tissue from immature and mature 'Explorer' muskmelon fruit. Mature fruit were assayed at time of harvest and after storage for 10 days at 4 or 24C.

Fruit condition	Free sterols (μmol·mg ⁻¹ protein)	Phospholipids (μmol·mg ⁻¹ protein)	Free sterols : phospholipids	Fatty acids saturated : unsaturated
Immature ^z	0.31 a ^y	0.93 a	0.33	0.73
Mature	0.40 a	0.72 ab	0.55	0.81
10 days at 4C	0.34 a	0.69 ab	0.50	0.83
10 days at 24C	0.31 a	0.55 b	0.56	1.08

^zLipid composition from immature fruit was from plasma membrane isolated at a density of 1.13 g·cm⁻³. For mature and mature storage fruits, plasma membranes were isolated at a density of 1.14 g·cm⁻³.

^yMean separation within columns by Duncan's at *P* = 0.05 (*n* = 3).

Table 2. Percentage of fatty acids from total phospholipids of plasma membrane vesicles from hypodermal-mesocarp tissue of immature, mature 'Explorer' muskmelon fruit. Mature fruit were assayed at time of harvest and after storage for 10 days at 4 or 24C.

Fruit condition	Palmitic (16:0)	Steric (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
Immature ^z	35 c ^y	6 a	7 a	32 a	19 b
Mature	42 b	3 b	5 b	27 b	24 a
10 days at 4C	39 b	4 b	5 b	25 b	27 a
10 days at 24C	48 a	4 b	6 ab	18 c	25 a

^zFatty acids from immature fruits were from plasma membranes isolated at a density of 1.13 g·cm⁻³, for mature and mature stored fruits from plasma membranes isolated at 1.14 g·cm⁻³.

^yMean separation within columns by Duncan's at *P* = 0.05.

The composition of the lipid matrix was investigated as a possible cause of the shift in density of the plasma membrane during maturation (Table 1). Neither total free sterols nor total phospholipids per milligrams protein of the hypodermal-mesocarp tissue plasma membrane changed significantly during maturation. Also, between harvest and when mature fruit were kept for 10 days at 4 or 24C, no significant changes occurred in either the total free sterol or total phospholipid to milligrams protein ratios. Although total free sterol and total phospholipid to protein ratios may not be an index of the shift in density of muskmelon fruit hypodermal-

mesocarp plasma membrane, the changes in individual sterols or phospholipids may play a role in the buoyant density change. Alternatively, increases in fruit plasma membrane density with maturity may be due to changes in membrane carbohydrate : protein ratio (Dallman et al. 1988).

Changes in the lipid matrix coincided closely with increased muskmelon fruit membrane permeability. The ratio of total free sterols to total phospholipids generally increased with ripening (Table 1); the total phospholipid fatty acid saturation index also increased. The increase in free fatty acid saturation ratio during

fruit ripening was due to a significant increase in palmitic (C16:0) and a significant decrease in linoleic (C18:2) fatty acids (Table 2). Quinn (1981) stated that any biochemical process, such as an increase in the free sterol to phospholipid ratio and/or the increased saturation index of phospholipid fatty acids, is capable of altering membrane permeability. Knowles and Knowles (1989) found that with increasing storage time of potato (*Solanum tuberosum* L.) tubers, electrolyte leakage significantly increased and was highly negatively correlated with the unsaturation index of the fatty acids.

This study demonstrates that increased membrane permeability in muskmelon fruit coincides with an increase in the total free sterol : total phospholipid ratio and with an increase in the phospholipid fatty acid saturation index of the plasma membrane. We suggest that plasma membrane lipid changes may be responsible for the loss of muskmelon fruit membrane permeability. It is also clear from this study that the postharvest environment (storage at 4 vs. 24°C and vapor pressure difference $\approx$ 3.8-fold) affects mature muskmelon fruit membrane permeability. Further analysis of the plasma membrane should involve characterization of individual free sterols and the fatty acid composition of individual phospholipids.

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