Physicochemical Characterization of Hybrid Honey Dew Muskmelon Fruit (*Cucumis melo* L. var. *inodorus* Naud.) following Maturation, Abscission, and Postharvest Storage

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ADDITIONAL INDEX WORDS. ATPase, firmness, lipoxygenase, moisture content, plasma membrane, phospholipids, soluble solids concentration, sterols, sugars, volume

ABSTRACT. Hybrid honey dew muskmelon (*Cucumis melo* L. var. *inodorus* Naud.) fruit physiological maturity, the period of maximized or greatest compositional changes, occurs by 40 days after anthesis (DAA). Fruit maturity was determined by major changes in quality attributes: glucose, fructose, sucrose, and moisture content, firmness, mass, volume, and hypodermal-mesocarp plasma membrane specific H⁺-ATPase (E.C. 3.6.1.3) activity. Fruit ripening occurs by 50 DAA, as determined by additional changes in the mentioned quality attributes, and by fruit abscission at 50 DAA. Fruit senescence begins with decreases in almost all quality attributes, H⁺-ATPase activity, protein content, by the largest increase in the total free sterol : total phospholipid (FS:PL) ratio, and in hypodermal-mesocarp lipoxygenase (E.C. 1.13.11.12) activity. Physicochemical profiles of hybrid honey dew muskmelon fruit during growth and maturation should be useful to schedule commercial harvest of mature fruit, which is necessary for maximum honey dew fruit quality, extended shelf-life, and enhanced consumer satisfaction.

Hybrid honey dew, nonnetted, green-fleshed muskmelon (*Cucumis melo* var. *inodorus*) fruit within the last 10 years have replaced most of the open-pollinated (OP) varieties produced for shipment from Texas and are 100% of the production in Mexico and Central America. Ten years ago, hybrid netted, orange-fleshed muskmelon (*Cucumis melo* L. var. *reticulatus* Naud.) fruit were introduced into these same regions and have essentially replaced all OP production (D. Wolff, personal communication). The reasons hybrid *C. melo* cultivars are replacing OP cultivars are protection of proprietary lines, uniformity of fruit set and size, hybrid vigor, higher yield, and earliness of maturity. Within the last 10 years most melon fruit physicochemical studies have been on netted muskmelon hybrids. These physicochemical studies have aided in understanding netted muskmelon fruit quality changes during growth, postharvest handling, and storage and the biochemical factors associated with regulating senescence (Seymour and McGlasson, 1993). In OP honey dew fruit, Pratt et al. (1977) have reported some quality (number of flowers and fruit per plant, fruit mass, volume, and total soluble solids), ethylene, and respiration changes during growth, development, and ripening. But almost no chemical or biochemical (enzymes activities, free sugar content, lipids changes, or membrane composition) data have been reported for either OP or hybrid cultivars throughout fruit growth, maturation, and senescence. Therefore, the purpose of this study is to document developmental changes in hybrid honey dew 'Honey Brew' fruit, using methods that describe hybrid netted muskmelon quality attributes for establishing appropriate harvest and postharvest handling criteria and biochemical attributes for regulating postharvest shelf life or senescence.

Received for publication 18 Feb. 1997. Accepted for publication 23 May 1997. Aleena Tarshis Moreno is gratefully acknowledged for muskmelon fruit production. Use of company or product names by the U.S. Dept. of Agriculture does not imply approval or recommendation of the product to the exclusion of others which 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.

Materials and Methods

PLANT MATERIAL. 'Honey Brew' hybrid honey dew, nonnetted, green-fleshed muskmelons were grown in a greenhouse. Flowers were hand-pollinated, and one fruit per plant was allowed to develop. Fruit were harvested at 20, 30, and 40 d after anthesis (DAA) and at abscission ≈50 DAA. Fruit harvested at 50 DAA, held at 10 °C and 90% relative humidity (RH) for 7 d then at 24 °C and 86% RH for 3 d are referred to as 60 DAA. All data are combined from two separate growings, ≈4 months apart, with fruit as replications and 23 fruit total per assay except H⁺-ATPase activity, where N = 6.

DETERMINATION OF FRUIT FIRMNESS, MOISTURE, SOLUBLE SOLIDS CONCENTRATION, SUGARS, AND MASS. Firmness of whole fruit equatorial hypodermal-mesocarp tissue, less the epidermis, was measured using a 4.5 × 3-mm diameter V-tip Chatillon Force gauge (Lindy Electric, Glen Head, N.Y.) and expressed as mean force Newtons (N). Moisture was determined on 10 g of lyophilized equatorial middle-mesocarp tissue. Soluble solids concentration, in juice expressed through a hand held garlic press from a 10-mm-diameter equatorial plug of mesocarp tissue, was determined using a temperature corrected, digital refractometer (Reichert Scientific Instruments, Buffalo, N.Y.). Fruit sugars were extracted from 0.3 g lyophilized equatorial middle-mesocarp tissue by stirring with 5 mL of water (Milli-Q; Millipore Corp., Bedford, Mass.) for 1 min at room temperature. The solution was filtered through filter paper (No. 1; Whatman, Maidstone, England) and the residue washed with additional 5 mL Milli-Q water. The filtrate was passed through a cartridge (C18 Sep-Pac; Waters Corp., Milford, Mass.) and diluted 1:4 (v/v) with Milli-Q water before determination of fructose, glucose, and sucrose using the high-performance liquid chromatography procedure previously described (Lester and Dunlap, 1985). Fruit volume was determined using the formula $[(C_L/6.28)^3 \times 4.19] + [(C_E/6.28)^3 \times 4.19]/2$ where C refers to the circumference and subscripts L and E refer to longitude and equator, respectively as previously published (Lester et al., 1994).

ISOLATION OF PLASMA MEMBRANE, H⁺-ATPase ACTIVITY, AND LIPID AND PROTEIN ANALYSES. Plasma membrane was isolated from equato-

Table 1. Changes in hybrid honey dew fruit volume, mass and mesocarp firmness (Newtons), percent moisture content, and soluble solids concentration 20 to 60 d after anthesis (DAA). Sixty-DAA fruit were fully abscised (50 DAA), held at 10 °C plus 90% relative humidity (RH) for 7 d then at 24 °C plus 86% RH for 3 d. Data are means of 23 fruit $\pm$ SD.

DAA	Vol (cm ³)	Mass (kg)	Firmness (N)	Moisture (%)	Soluble solids (%)
20	1167 $\pm$ 108	1.02 $\pm$ 0.09	24 $\pm$ 7	93.0 $\pm$ 1.9	4.1 $\pm$ 0.2
30	1970 $\pm$ 175	1.70 $\pm$ 0.14	31 $\pm$ 3	93.9 $\pm$ 0.4	6.3 $\pm$ 0.3
40	2144 $\pm$ 170	1.89 $\pm$ 0.14	23 $\pm$ 3	91.2 $\pm$ 0.8	9.3 $\pm$ 0.7
50	2220 $\pm$ 201	2.00 $\pm$ 0.15	14 $\pm$ 2	89.1 $\pm$ 0.8	12.0 $\pm$ 0.8
60	2140 $\pm$ 231	1.95 $\pm$ 0.25	6 $\pm$ 1	91.0 $\pm$ 0.7	11.1 $\pm$ 1.2

rial region hypodermal mesocarp tissues as previously described (Lester and Stein, 1993). All procedures were conducted at 2 to 4 °C. Hypodermal-mesocarp tissue (150 g) from three fruit was frozen in liquid nitrogen, pulverized to small (2 to 4 mm) pieces, then thawed in 250 mL of homogenizing buffer (0.5 M sucrose, 50 mM Hepes-KOH, 5 mM ascorbic acid, 5 mM ethylenediaminetetraacetic acid (EDTA), 5 mM ethyleneglycol-bis (aminoethylether) tetraacetic acid (EGTA), 5 mM dithiothreitol (DTT), and 0.6% (m/v) polyvinyl-pyrrolidone (PVPP), pH 7.8. The tissue was homogenized for 1 min at medium speed using a homogenizer (Polytron; Brinkman Instruments, Westbury, N.Y.) equipped with a PTA-50 generating probe. The homogenate was placed under vacuum for 10 min, then squeezed through two layers of Masslinn towel (Chicopee, New Brunswick, N.J.) and centrifuged at 14,000g_n for 20 min at 2 °C. A plasma membrane pellet was collected as previously described (Lester and Stein, 1993). Protein content was determined by the method of Bradford (1976). Vanadate sensitive H⁺-ATPase activity was assayed as previously described (Lester and Stein, 1993) and inorganic phosphorous was determined as a measure of H⁺-ATPase activity by the method of Peterson (1978).

LIPXYGENASE ACTIVITY ASSAY. Lipoxxygenase activity in both hypodermal- and middle-mesocarp tissues was determined following the modified procedure of Wang and Hildebrand (1987). All procedures were conducted at 2 to 4 °C. Mesocarp tissue (1 g) was frozen in liquid nitrogen, pulverized to a fine powder, and thawed in 2 mL extraction buffer (0.05 M Tris-HCl (pH 7.8), 1 mM PMSF dissolved in 1 mL EtOH, 2 mM DTT, 9 mM octylthio-glucopyranoside (OTGP), and 2% PVPP (m/v). Tissue was homogenized for 1 min at medium speed using a Polytron homogenizer equipped with a PTA-20TS generating probe. The homogenate was vacuum filtered through one layer of Masslinn towel, then centrifuged at 15,000g_n for 5 min at 4 °C. Lipoxxygenase activity, was determined following linoleic or oleic (control) oxidation. Lipoxxygenase specifically catalyzes the oxidation of *cis,cis*-1,4-pentadiene structures unique to unsaturated fatty acids linoleic, linolenic, and arachidonic (Eskin et al., 1977). One hundred microliters of commercial grade linoleic or oleic acid was dissolved in 0.4 mL absolute EtOH. Four hundred microliters of

the fatty acid solution were added to 0.6 mL of 1 M KOH, 50 mL Milli-Q water, and 100 μ L Tween 20 in a stoppered red glass flask, flushed with nitrogen, then mixed for 1 h at room temperature or until the fatty acid was emulsified. The substrate solution was prepared by combining emulsified fatty acid, with 0.2 M sodium phosphate buffer (pH 6.5) and Milli-Q water, 1:1:2 (v/v/v), respectively. The lipoxxygenase activity assay was initiated by combining 1.9 mL of substrate with 0.1 mL mesocarp extract and mixing for 10 min at 22 °C, while flushing continuously with air (300 mL·min⁻¹). The reaction was terminated by combining 1 mL of reaction solution with 6.2 mL absolute EtOH, then 2.8 mL Milli-Q water. Absorbance at 235 nm was recorded and enzyme activity was expressed as units of activity per milligram protein per minute. One unit of 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).

TOTAL FREE STEROL AND TOTAL PHOSPHOLIPID DETERMINATION. Lipids were extracted from plasma membrane pellet preparations as previously described (Lester and Stein, 1993).

STATISTICS. Analysis of variance was used to evaluate DAA differences for plasma membrane H⁺-ATPase activity and protein data (SAS Institute, Cary, N.C.). Duncan's multiple range test ($P \leq 0.05$) was used to discern between DAA classifications when F values were significant.

Results and Discussion

Major changes in almost all the quality attributes of hybrid honey dew melons indicate that these fruit attain physiological maturity, the period of maximized or greatest change in compositional attributes, at 40 DAA (Tables 1 and 2). Hybrid honey dew fruit become ripe after 40 DAA, which Pratt et al. (1977), and Sinnott (1945) determined as the same DAA period for maturity and ripening in OP honey dew fruit. Hybrid honey dew fruit obtained 97% of the fruit volume and 95% of the fruit mass at maturity (40 DAA). Fruit firmness and fruit moisture content reached a maximum between 30 and 40 DAA, then declined 56% and 3%, respectively, by abscission (50 DAA). Soluble solids

Table 2. Changes in hybrid honey dew middle-mesocarp sugar content 20 to 60 d after anthesis (DAA). Sixty-DAA fruit were fully abscised (50 DAA), held at 10 °C plus 90% relative humidity (RH) for 7 d then at 24 °C plus 86% RH for 3 d. Data are means of 23 fruit $\pm$ SD.

DAA	Fructose	Glucose	Sucrose	Total sugars	Total sugars
	(fresh mass basis) (mg·g ⁻¹)				(dry mass basis) (mg·g ⁻¹)
20	33.4 $\pm$ 4.1	30.5 $\pm$ 4.0	0.0 $\pm$ 0.0	63.9 $\pm$ 8.1	538.9 $\pm$ 23.2
30	33.8 $\pm$ 1.9	30.3 $\pm$ 1.9	1.7 $\pm$ 0.2	69.0 $\pm$ 3.9	645.6 $\pm$ 26.0
40	49.2 $\pm$ 1.2	40.4 $\pm$ 1.3	32.3 $\pm$ 3.0	121.9 $\pm$ 3.8	756.4 $\pm$ 26.1
50	45.7 $\pm$ 2.1	26.9 $\pm$ 1.9	43.6 $\pm$ 4.8	116.2 $\pm$ 5.8	790.0 $\pm$ 14.2
60	29.4 $\pm$ 1.8	15.9 $\pm$ 1.8	39.0 $\pm$ 9.1	85.6 $\pm$ 9.9	690.7 $\pm$ 24.6

Table 3. Changes in hybrid honey dew hypodermal-mesocarp plasma membrane vanadate-sensitive H⁺-ATPase activity, expressed as total inorganic phosphate (Pi) evolved or specific Pi evolved on a protein mass basis, protein content (fresh mass basis), and μmol total free sterols (FS) to μmol total phospholipids (PL) ratio 20 to 60 d after anthesis (DAA). Sixty-DAA fruit were fully abscised (50 DAA), held at 10 °C plus 90% relative humidity (RH) for 7 d then at 24 °C plus 86% RH for 3 d. Data are means of N = 6.

DAA	Total H ⁺ -ATPase (nmol Pi/h)	Specific H ⁺ -ATPase (nmol Pi/mg/h)	Total protein ($\mu\text{g}\cdot\text{g}^{-1}$)	Total FS: total PL
20	49.1	92.8	0.53	0.36
30	61.7	110.0	0.57	0.40
40	72.2	99.9	0.62	0.43
50	52.1	68.9	0.76	0.53
60	36.5	65.9	0.73	0.57
Duncan's $P \leq 0.05$	10.9	7.9	0.13	

concentration (SSC) reached 78% of its maximum at 40 DAA, coincident with the greatest qualitative and quantitative changes in fruit sugars. Hybrid honey dew fruit sugars before 30 DAA are solely fructose and glucose (Table 2), which are required for fruit growth and development, and are the products of sucrose hydrolysis by acid invertase (Hubbard et al., 1990; Lingle and Dunlap, 1987; McCollum et al., 1988; Schaffer et al., 1987). Sucrose accumulation is regulated by sucrose phosphate synthase (Hubbard et al., 1990). At 40 DAA, sucrose content is 19 times greater than at 30 DAA and fructose and glucose content are 30% and 25%, respectively, indicating that acid invertase and sucrose phosphate synthase are active during this period of maturation. At 40 DAA, total sugar, on a fresh mass basis, nearly doubles. At 50 DAA there is an additional 25% increase in sucrose content, but fructose and glucose decline 7% and 33%, respectively, indicating a near or complete loss in acid invertase, and heightened sucrose phosphate synthase activities, which is the normal metabolic change in these enzymes in melon fruit (Schaffer et al., 1987). However, this metabolic change leaves total fruit sugars, on a fresh and dry mass basis, between 40 and 50 DAA relatively unchanged. Pratt et al., (1977) indirectly demonstrated the same nonsignificant change in total sugar content in OP honey dew fruit by showing no significant change in percentage SSC between maturity classes 1 and 2, which corresponds to 40 DAA and 50 DAA. This nonsignificant increase in total sugar content between 40 and 50 DAA (abscission) is regulated by several factors like an active loss in fruit moisture content (Lee, 1986), which is evident in the loss of honey dew melon fruit moisture content after 30 DAA. Plus, it is regulated by heightened fruit cellular phosphorylation activity (Schaffer et al., 1996), H⁺-ATPase activity, which is greatest at 40 DAA in hybrid honey dew fruit, then declines (Table 3), and by source supply and translocation (Hughes et al., 1983; Schaffer et al., 1996).

Hypodermal-mesocarp phosphorylation activity, indirectly measured by Vanadate-sensitive total H⁺-ATPase activity, in-

creases in hybrid honey dew fruit until 40 DAA (Table 3). H⁺-ATPase activity, a reliable marker of the muskmelon fruit plasma membrane (Lester and Stein, 1993), also increases in netted muskmelon fruit during physiological maturation, and this increased activity is associated with increased plasma membrane (PM) density and fruit metabolic activity. A decline in H⁺-ATPase activity, is directly associated with a loss of netted muskmelon fruit PM integrity and function (Lester and Whitaker, 1996). Following hybrid honey dew fruit maturation, a decline in fruit metabolism occurs during fruit ripening (40 to 50 DAA), as seen by little or no increase in most quality attributes (Tables 1 and 2), and by a decline in H⁺-ATPase specific and total activities, 31% and 28%, respectively (Table 3). An additional 30% decline in total H⁺-ATPase activity occurs after 10 d of postharvest storage (60 DAA), along with a decline in nearly every quality attribute. Hybrid honey dew fruit senescence begins just prior to fruit abscission (50 DAA) and continues throughout postharvest storage (Tables 3 and 4).

A key parameter of netted (Lester and Stein, 1993) and nonnetted (Lacan and Baccou, 1996) muskmelon fruit senescence, is a decline in PM integrity. Integrity of OP honey dew and orange-flesh muskmelon PM is directly associated with its selective permeability as measured by electrolyte leakage (Lacan and Baccou, 1996; Lester, 1988; Lester and Stein, 1993). Loss of selective permeability in melon fruit occurs with the onset of ripening, and is initially greatest in endo-mesocarp tissue at 30 DAA, then becomes progressively greater in middle- and hypodermal-mesocarp tissues following fruit ripening and fruit abscission, respectively. Demonstrating that melon fruit ripen and senesce from the seed cavity out toward the epidermis. Leshem (1992) reported that any biochemical process, such as an increase in free sterol to phospholipid ratio, is capable of altering membrane selective permeability.

Increased free sterols (FS) in PM are reported to reduce H⁺-ATPase activity (Sandstrom and Cleland, 1989). In hybrid honey dew fruit the PM total free sterols (FS) : total phospholipids (PL)

Table 4. Changes in hybrid honey dew hypodermal- and middle-mesocarp lipoxygenase (LOX) activity (expressed on a total protein mass basis) 20 to 60 d after anthesis (DAA). Sixty-DAA fruit were fully abscised (50 DAA), held at 10 °C plus 90% relative humidity (RH) for 7 d then at 24°C plus 86% RH for 3 d. Data are means of 23 fruit $\pm$ SD.

DAA	Units ² of LOX activity/mg/min			
	Hypodermal-mesocarp		Middle-mesocarp	
	Linoleic	Oleic	Linoleic	Oleic
20	47.0 $\pm$ 4.1	36.2 $\pm$ 3.5	57.7 $\pm$ 3.6	33.1 $\pm$ 6.7
30	62.0 $\pm$ 9.3	31.0 $\pm$ 6.2	86.9 $\pm$ 8.5	36.2 $\pm$ 5.7
40	87.3 $\pm$ 13.9	33.1 $\pm$ 4.3	72.2 $\pm$ 8.7	26.4 $\pm$ 2.4
50	156.0 $\pm$ 12.9	51.1 $\pm$ 16.0	71.8 $\pm$ 1.6	22.5 $\pm$ 2.0
60	173.0 $\pm$ 15.6	55.5 $\pm$ 16.7	95.7 $\pm$ 22.1	31.7 $\pm$ 7.3

²A unit of LOX activity = 0.001 absorbance units.

ratio greatly increased after 40 DAA, which coincided with a significant decrease in H⁺-ATPase activity (Table 3). In orange-fleshed muskmelon PM, an increase in the FS:PL ratio occurs along with increased membrane permeability (electrolyte leakage) during fruit ripening and senescence, resulting in reduced fruit shelf life (Lacan and Baccou, 1996). An increase in lipoxygenase (LOX) activity (Table 4) was coincident with this lipid biophysical change in hybrid honey dew PM and the resulting loss in membrane integrity.

LOX, in tomato (*Lycopersicon esculentum* Mill.) fruit is localized in the plasma and tonoplast membranes and is shown to catabolize phospholipid derived free fatty acids (Droillard et al., 1993). Hybrid honey dew fruit LOX activity requires linoleic acid as a substrate, typical of all LOXs (Siedow, 1991), and has only one activity peak, at pH 6.8, and is temperature dependent, i.e., higher activities occur with higher temperatures (data not shown). LOX in hybrid honey dew fruit middle-mesocarp tissue reached peak activity at 30 DAA then declined with fruit ripening. Riley, et al. (1996) showed in tomato pericarp tissue, LOX peak activity occurs with fruit maturity then declines with ripening. In orange-fleshed muskmelon fruit LOX activity occurs only in hypodermal-mesocarp tissues, the tissue immediately under the epidermis, and increases with ripening and senescence (Lester, 1990). In hybrid honey dew, LOX activity occurred in both the hypodermal- and middle-mesocarp tissues, but with much different activity profiles (Table 4). The difference in LOX activities between hypodermal- and middle-mesocarp tissues may be due to differences in development and in tissue water deficit. Maccarrone et al. (1995) has reported that LOX expression in plants is regulated throughout development in response to wounding and water loss. Hybrid honey dew fruit lost moisture following maturation (Table 1) presumably through the epidermis; thus, it is possible high LOX activity in the adjacent hypodermal-mesocarp during ripening and postharvest storage could be influenced by tissue water loss. In orange-fleshed muskmelon fruit (Lester, 1990) increasing LOX activity is associated with a concomitant decrease in antioxidant activity, an inhibitor of LOX activity (BenAziz et al., 1968). Whatever the cause of high LOX activity in ripe and senescing hypodermal- mesocarp tissue, regulation of LOX activity in melon appears to be important in controlling fruit maturation and senescence.

Hybrid honey dew 'Honey Brew', although very similar in some physical and quality attributes to OP cultivars (Pratt et al., 1977), has distinct advantages. Hybrid fruit have uniform flowering, fruit set, and abscise at 50 DAA, which ensures field uniformity of fruit maturation and harvesting. Pratt et al. (1977) stated that enhanced honey dew fruit commercial quality is realized only when fruit were harvested as mature as possible. Hybrid Honey Brew fruit maturation and ripening stages are well defined with the present data. This should aide in better scheduling, from anthesis, and the harvest of mature and abscised fruit for maximum quality. Also, senescence associated enzymes, previously undocumented in honey dew fruit, are highlighted, which may eventually be regulated for improved shelf life of mature and abscised fruit.

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