

# Sugar and Organic Acid Variations in Commercial Cantaloupes and Their Inbred Parents

J.C. Beaulieu<sup>1</sup>, J.M. Lea, and G. Eggleston

United States Department of Agriculture, Agricultural Research Service, Southern Regional Research Center, 1100 Robert E. Lee Boulevard, New Orleans, LA 70124

Z. Peralta-Inga

Advanced Materials Research Institute, University of New Orleans, 2000 Lakeshore Drive, New Orleans, LA 70148

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**ABSTRACT.** Markedly higher average sucrose (58.1%) was recovered from mesocarp tissue of six orange-flesh cantaloupe (*Cucumis melo* L.) genotypes over three seasons compared to glucose (17.5%) and fructose (25.6%). A significant decrease in sucrose concentration was observed in the fall for all six genotypes, and the glucose (21.2%) and fructose (33.5%) ratios were also higher in the fall; markedly different than the spring fruit averages. The female inbreds had significantly ( $P = 0.05$ ) lower glucose, fructose, sucrose, and total sugars than the commercial hybrids. Compared to the male and female inbreds, commercial hybrids had significantly ( $P = 0.05$ ) higher concentrations of fructose, sucrose and total sugars, but not glucose. Two refractometric digital measures of °Brix (°Brix-At and °Brix-II) in homogenized slurries were positively correlated ( $r = 0.914$ ;  $P \leq 0.001$ ), and were also correlated with total sugars ( $r \geq 0.839$ ) and sucrose ( $r \geq 0.752$ ). °Brix of cubes (°Brix-cube) was significantly correlated with sucrose and total sugars ( $r \geq 0.627$ ). Total sugar was positively correlated with sucrose ( $r = 0.843$ ;  $P \leq 0.001$ ). Eastern-type U.S. melons had significantly ( $P = 0.05$ ) higher °Brix-cube and °Brix-At compared to U.S. western shipper-types. Female inbreds were significantly ( $P = 0.05$ ) lower in mean °Brix (all three measures) compared to the hybrids and male inbreds, and female inbreds had higher pH than the male inbreds. Western shippers had significantly ( $P = 0.05$ ) higher pH compared to eastern genotypes. The predominant organic acid in all six genotypes was succinic acid, generally followed by oxalic, citric/isocitric, then malic acid. Succinic acid recovery was significantly higher in all six genotypes harvested in the fall, compared to spring. Eastern genotypes had significantly ( $P = 0.05$ ) lower organic acids compared to western genotypes. Results indicate that maternal inheritance appears to confer lower sugar accumulating capacity and higher pH, which, is associated with vacuolar acid invertase (AI) and hexose balance. Breeding programs should focus on hybrid vigor derived through accentuating homozygous female inbreds with lower pH and higher capacity for sucrose accumulation, as well as morphological and agronomic traits often carried in the female line.

Cantaloupe and other melons (*Cucumis melo*) are highly regarded for their unique flavor and high sugar levels are often the determinant of quality (Yamaguchi et al., 1977). Sucrose is the dominant sugar that accumulates in most desert melons (Bianco and Pratt, 1977; Lester and Dunlap, 1985; Lingle and Dunlap, 1987; Stepansky et al., 1999), and fruit accumulate sucrose massively during the final stages of maturation (Cohen and Hicks, 1986; Hayata et al., 2000; Hubbard et al., 1989; Lester, 1998; Lester et al., 2001; McCollum et al., 1988; Wang et al., 1996). Increases in sucrose are accompanied by a dramatic decrease in acid invertase (AI) and neutral invertase activities and increases in sucrose phosphate synthase (SPS) and sucrose synthase (SSYN) activities (Hubbard et al., 1989; Lester et al., 2001; Lingle and Dunlap, 1987; Ranwala et al., 1991; Schaffer et al., 1987; Stepansky et al., 1999). High SPS activity is considered the determining factor limiting the ultimate sucrose concentration per melon cultivar

(Hubbard et al., 1989), and high sucrose accumulation has been attributed to a single recessive gene designated *suc* (Burger et al., 2002). Lesser quantities of fructose and glucose generally accumulate during development, and they decrease or remain unchanged during ripening.

Consumer acceptance of melons is driven most often by sweetness (Bianco and Pratt, 1977) and also by an acceptable aroma bouquet or presence of volatiles. Sucrose concentration was correlated to total sugar concentration in many *C. melo* cultivars that accumulate sucrose (Stepansky et al., 1999). However, soluble solids (SS) are only partially correlated with sweetness (Mutton et al., 1981; Yamaguchi et al., 1977) and high SS alone did not appear to adequately define good melon quality (Aulenbach and Worthington, 1974; Currence and Larson, 1941; Mutton et al., 1981). °Brix (SS) is unfortunately often the only reliable, easily measured quality assessment for determination of optimum melon harvest. Other non-destructive harvest indicators such as ground color change, degree of netting and dehiscence (slip) can fluctuate due to cultivar, local environmental variation and season. Choice of cultivar, overall intrinsic harvest quality, and maintenance of that quality will ultimately determine consumer acceptance of the marketed melon.

Unlike amino acids that contribute significantly as precursors to acceptable melon flavor (Wyllie et al., 1995, 1996), little or no work exists reporting the relative contribution of organic acids to overall cantaloupe quality. The major organic acids (citric, malic

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<sup>1</sup>Research plant physiologist; to whom correspondence should be addressed; e-mail beaulieu@.srrc.ars.usda.gov.

and succinic) recovered in many *C. melo* fruit have been reported (Chachin and Iwata, 1988; Hashinaga et al., 1984; Lamikanra et al., 2000; Pratt, 1971; Wang et al., 1996). The predominant organic acid seems to vary as fruit develop and mature on the vine (Chachin and Iwata, 1988; Wang et al., 1996), and possibly based on whether or not the melon flesh is green (*inodorus* or *cantalupensis*) versus orange (*cantalupensis* or *reticulatus*). Yet, no correlation or significance was attributed to differences in pH, acidity or organic acids at harvest in orange-fleshed 'Mission' cantaloupe (Lamikanra et al., 2000).

Although sucrose metabolism in melons has been well documented and characterized, information regarding genetic control of sucrose, organic acid accumulation and volatile genesis in specific cultivars, as related to male or female inbred parental lines or typical production region and season, is limited. Beaulieu and Grimm (2001) attempted to find volatile markers in eastern and western U.S. cantaloupes, yet additional physiological quality markers are required as breeding tools. Limited linkage studies exist for *C. melo* (Pitrat, 1991) and most published work has focused on insect or disease resistance. As a consequence, this study was undertaken to analyze simple mono- and disaccharides and organic acids in two commercially available cantaloupe cultivars and their inbred males and females, to determine if differences in these quality parameters could be correlated with, or ascribed to parental lineage or melon type and growing region.

## Materials and Methods

**PLANT MATERIAL.** Orange-flesh cantaloupes (*Cucumis melo* var. *reticulatus* Naud.) were grown on commercial raised beds with furrow irrigation. Fruit from 'Athena' (a larger eastern-type melon with less flesh firmness and shorter shelf life) and 'Sol Real' (a typical western shipper) and both homozygous inbred parental breeding lines (genotypes designated only as male and female for proprietary reasons) were harvested in Spring 2000 (June, Dome, Ariz.), Fall 2000 (August/September, Woodland, Calif.) and in Spring 2001 (June, Yuma, Ariz.). Fields were monitored daily as commercial maturity approached (ground color change, full netting and the initiation of dehiscence), and only three-quarters slip, ripe fruit were harvested, field hydrocooled, packed carefully with styrofoam beads and air freighted overnight to the Southern Regional Research Center (SRRC) in New Orleans, La. Over the three seasons, the six genotypes were harvested within 5 d, shipped and held at 4 °C for up to 2 d before sample preparation on a single day.

**SAMPLE HOMOGENATES.** Whole fruit were sanitized in 100  $\mu\text{L}\cdot\text{L}^{-1}$  bleach (5.25% NaClO) and uniformly peeled with a Muro CP-44 melon peeler (Tokyo, Japan), stem and blossom portions ( $\approx 2$  to 3 cm) were cut off and each melon was sliced once longitudinally, seeds were removed, and the seed cavity was cleaned by removal of 1 to 2 mm tissue with a sharp knife. Mesocarp cubes were rapidly juiced ( $\approx 15$  s) into a slurry with a Braun MP80 juicer (Germany). Slurries were prepared in triplicate, each from 300 g of randomized cubes from a representative pool of five to six fruit per genotype. Decanted juice (without foam) was stored at  $-80$  °C until analysis.

**SUGAR AND ORGANIC ACID ANALYSIS.** Soluble solids of homogenized slurries were measured using a hand held electronic refractometer ( $^{\circ}\text{Brix-At}$  = Atago, PR101, Japan) before freezing, and an Index Instrument (TCR 15-30, England =  $^{\circ}\text{Brix-II}$ ) temperature controlled refractometer, accurate to  $\pm 0.01$   $^{\circ}\text{Brix}$ , after thawing.  $^{\circ}\text{Brix}$  were also measured with the Atago PR101 from hand-ex-

pressed juice of freshly cut individual cubes ( $^{\circ}\text{Brix-cube}$ ) of each genotype ( $n = 15$ ). Freshly prepared slurry pH was also measured (Cole Parmer 5986-50, Vernon Hills, Ill.) in triplicate.

Determination of sucrose, fructose and glucose in melon juice by gas chromatograph (GC) was based on the International Commission for Uniform Methods in Sugar Analysis (ICUMSA) method GS7/4-22 (1998), with modifications (Eggleston et al., 2002). Samples (5  $\mu\text{L}$ ) were derivatized with 0.5 mL oximation reagent (dimethyl amino ethanol added to a solution of hydroxylamine hydrochloride and pyridine) with constant mixing and heating (10 min at 80 °C), followed by silylation with 0.45 mL of hexamethyldisilazane with gentle swirling and then addition of 50  $\mu\text{L}$  trifluoroacetic acid. Heating blocks were used to heat the derivatized samples at 80 °C for 10 min and samples remained overnight to allow precipitate to settle before transferring supernatant into the GC sample vials. Simultaneous determination of sugars and organic acids ( $n = 3$ ) in homogenized slurries was performed by the modified ICUMSA method, similar to (Morvai and Molnár-Perl, 1992) but, GC conditions varied considerably because further separation of sugars was required for increased accuracy. Separation occurred on a DB-5 capillary column (5%-Phenyl-methyl polysiloxane, 30 m  $\times$  0.25 mm (internal diam.), column film thickness was 0.1  $\mu\text{m}$ ) with a Hewlett Packard 5890A GC, equipped with a flame ionization detector. Operation conditions were injection port 300 °C; detector 310 °C; oven started at 100 °C for 3 min then was programmed at 5  $^{\circ}/\text{min}$  until 150 °C, then 10  $^{\circ}/\text{min}$  until 300 °C, remaining at 300 °C for 10 min. Head pressure was 145 kPa with a 25:1 split ratio; sample volume was 1  $\mu\text{L}$ . Trehalose dihydrate (Alltech) was the internal standard for sucrose, and methyl- $\alpha$ -D-glucopyranoside (Sigma) the internal standard for glucose and fructose. Organic acid standards were silylated and run with the same GC-FID method to determine their RT and authenticity. Methyl- $\alpha$ -D-glucopyranoside (Sigma) was the internal standard employed for the organic acid analysis. All GC sugar and organic acid data are expressed on a percentage (w/w) basis.

**STATISTICAL DESIGN AND ANALYSIS.** The experiment was set up as a completely randomized design with a two-way treatment structure consisting of six genotype levels ('Athena', 'Athena'-male, 'Athena'-female, 'Sol Real', 'Sol Real'-male and 'Sol Real'-female) and three season levels (Spring 2000, Fall 2000, and Spring 2001). An analysis of variance (ANOVA) was performed (S-PLUS 2000, Version 6, Professional Release 1, Insightful Corp., Seattle, Wash.). Main or interaction effects that resulted in a statistically significant F value ( $P < 0.05$ ) were evaluated using Tukey's multiple comparison procedure; conducted at the 0.05 significance level. Fructose, glucose, sucrose, total sugars, malic, oxalic, succinic and citric/isocitric acids had treatment combinations with three replications, with the exception of the treatment combination 'Athena'-female/Spring 2000, which had no measurements. For the physiological measurements ( $^{\circ}\text{Brix-At}$ ,  $^{\circ}\text{Brix-II}$ ,  $^{\circ}\text{Brix-cube}$ , and pH), each of the 18 treatment combinations for  $^{\circ}\text{Brix-At}$  and pH had three replications.  $^{\circ}\text{Brix-II}$  had nine replications and  $^{\circ}\text{Brix-cube}$  had 15 replications, with the exception of the treatment combination 'Athena'-female/Spring 2000, which had no measurements.

## Results and Discussion

**GLUCOSE, FRUCTOSE, SUCROSE AND TOTAL SUGARS.** In a survey of many cultivars of ripe orange fleshed cantaloupe mesocarp, we found an average distribution of approximately 19.6% glucose, 23.7% fructose and 57.1% sucrose (Cohen and Hicks, 1986; Lester and Dunlap, 1985; Mizuno et al., 1971; Stepansky et al., 1999; Wang et

Table 1. Genotype × season mean comparisons for sugars quantified with gas chromatography from two hybrid cantaloupe cultivars ('Athena' and 'Sol Real') and their inbred parents in three growing seasons (n = 3).

Genotype	Glucose	Fructose	Sucrose	Total
Spring 2000				
Athena	0.893 de <sup>z</sup>	1.220 b	5.292 i	7.408 k
Male	0.911 e	1.183 b	4.448 gh	6.542 ghi
Female	---	---	---	---
Sol Real	1.079 g	1.419 c	4.527 gh	7.025 jk
Male	1.080 g	1.478 cd	4.444 gh	7.002 ijk
Female	1.005 f	1.426 c	3.419 e	5.850 def
Fall 2000				
Athena	0.876 d	1.620 e	3.077 d	5.573 d
Male	1.152 i	1.589 e	2.202 a	4.948 bc
Female	0.758 c	1.344 c	2.232 a	4.335 a
Sol Real	1.277 j	1.950 g	2.599 b	5.826 de
Male	1.484 m	2.204 h	2.728 bc	6.416 fgh
Female	1.004 f	1.562 de	1.983 a	4.549 ab
Spring 2001				
Athena	1.144 hi	1.552 de	4.309 g	7.004 ijk
Male	1.349 k	1.431 c	3.026 cd	5.806 d
Female	0.587 b	0.807 a	4.734 h	6.128 efg
Sol Real	1.117 h	1.625 e	3.924 f	6.666 hij
Male	0.528 a	1.185 b	4.057 f	5.771 d
Female	1.403 l	1.745 f	2.181 a	5.327 cd
SE	0.0074	0.026	0.091	0.152

<sup>z</sup>Means separated by different letters within columns are significantly different ( $P < 0.05$ ) according to Tukey's multiple comparison procedure with an experiment-wise error rate at 0.05.

al., 1996). Relatively low levels of sucrose in a few orange-fleshed cantaloupes have also been reported (Lamikanra et al., 2000; Lingle and Dunlap, 1987; Stepansky et al., 1999) and those data were, therefore, not included in the aforementioned summations. Across the three seasons in this study, we determined that the average percent sucrose recovery (58.1%) from mesocarp tissue was always markedly higher than glucose (17.5%) and fructose (25.6%). These results are congruent with most of the literature indicating higher sucrose accumulation than fructose and glucose in many sweet melon cultivars (Bianco and Pratt, 1977; Cohen and Hicks, 1986; Hayata et al., 2000; Lester and Dunlap, 1985; McCollum et al., 1988; Stepansky et al., 1999; Wang et al., 1996).

Unlike cantaloupes grown in southern Texas that had very similar spring versus fall sugar profiles (Lester et al., 2001), there was a dramatic decrease in fall sucrose concentrations in all six genotypes grown in the Central Valley of California (Table 1). This may not be surprising since the circadian rhythm controlling the activity of SPS and carbon metabolism in tomato cells is delayed by chilling treatments (Jones et al., 1998). Presumably, the night temperatures in southern Texas were not low enough in the fall to negatively affect sucrose accumulation. In contrast, the average glucose (21.2%) and fructose (33.5%) ratios were higher in the fall, and markedly different than the spring fruit averages (Table 1). Average glucose and fructose only comprised 15.4% and 20.9%, respectively, of the total sugars in the six genotypes over both spring seasons. Explanations for this may be that there were cooler overall and night temperatures and shorter daylength resulting in lower SPS or, that there was higher AI activity in the fall fruit, or there was poor translocation of photoassimilate to the ripening fruit as suspected in a previous study (Mitchell and Madore, 1992). Similarly, inconsistent sugar accumulation patterns were found in

Table 2. Sugar mean comparisons based on sex (commercial cultivar versus male versus female) and melon type (eastern versus western) using Tukey's multiple comparison procedure for two hybrid cantaloupe cultivars ('Athena' and 'Sol Real') and their inbred parents grown in three seasons.

Category	Glucose	Fructose	Sucrose	Total
Sex				
Hybrid cultivar (n = 18) <sup>z</sup>	1.064 by	1.564 a	3.955 a	6.584 a
Female (n = 15)	0.951 c	1.377 c	2.910 c	5.238 c
Male (n = 18)	1.084 a	1.512 b	3.485 b	6.081 b
SE (18,18)	0.0030	0.010	0.037	0.062
SE (15,18)	0.0031	0.011	0.039	0.065
Type				
Eastern (n = 18)	1.054 a	1.433 b	3.727 a	6.214 a
Western (n = 33)	1.029 b	1.522 a	3.348 b	5.900 b
SE	0.003	0.009	0.032	0.054

<sup>z</sup>Replications used per comparison are indicated in parenthesis.

<sup>y</sup>Means separated by different letters, within columns within categories (sex or location), are significantly different ( $P < 0.05$ ) according to Tukey's multiple comparison procedure with an experiment-wise error rate at 0.05.

many cold-tolerant melon cultivars grown under two nighttime temperature regimes (Ventura and Mendlinger, 1999).

The sugars fructose, sucrose and glucose as well as the total of these sugars, all had significant main and interaction effects. The treatment combination 'Sol Real'-male/Fall 2000 had the highest concentration of fructose and glucose, which was significantly different ( $P \leq 0.001$ ) from all other interaction means (Table 1). The 'Athena'-female and 'Sol Real'-female inbreds generally had lower concentrations of glucose, sucrose and total sugars (Tables 1 and 2). When viewing hybrids versus their inbred pairs, hybrids had the highest or occasionally middle concentration of fructose, sucrose and total sugars over both years with one exception: fructose in 'Sol Real'/Spring 2000 (Table 1). The statistically significant genotype by season interaction effect for fructose, glucose, sucrose, and measures of °Brix, is an indication that the genotypes behaved differently from season to season. This is not surprising because markedly different sugar levels have been reported at different locations within a fruit (from stem to calyx) and across tissue types (i.e. placenta, inner pulp, middle pulp, outer pulp and pericarp) (Mizuno et al., 1971). Furthermore, environmental variation, especially water quality and availability (del Amor et al., 1999), can alter the expression of traits normally found in a given cultivar. Our replicates (n = 3) were each comprised from a pooled composite of five to six commercially ripe fruit of uniform maturity (three-quarters slip). One might have expected better statistical separation per genotype over season if greater replicates from discrete locations within individual fruit (of the same maturity) were used.

When genotype and season were ignored and mean comparisons were conducted based on the sex of the parent, female inbreds had significantly lower glucose, fructose, sucrose and total sugars than the hybrids, and the hybrid means were significantly higher than the male and female means for all sugars, except glucose (Table 2). When means comparisons were conducted based on horticultural type (eastern versus western U.S.), the eastern melons had significantly higher sugar concentrations than western shippers (Table 2). Assuming that an acceptable volatile profile is present and alike, the assertion that eastern fruit are superior, compared to western fruit, could be made based on the correlation of SS with sweetness and melon quality (Aulenbach and Worthington,

Table 3. Genotype × season mean comparisons for physiological data from two hybrid cantaloupe cultivars ('Athena' and 'Sol Real') and their inbred parents in three growing seasons.

Genotype	°Brix-cube	°Brix-At	°Brix-II	pH
Spring 2000				
Athena	12.167 g <sup>z</sup>	10.867 fg	10.071 j	6.120 c
Male	10.940 defg	9.900 def	9.093 g	6.053 a
Female	---	11.100 g	---	6.400 i
Sol Real	11.347 efg	10.767 fg	9.969 ij	6.173 d
Male	12.193 g	10.067 ef	9.720 hi	6.100 b
Female	9.307 bcd	8.767 bc	8.344 cd	6.513 k
Fall 2000				
Athena	10.727 defg	8.767 bc	8.297 cd	6.097 b
Male	8.300 ab	8.100 ab	7.427 b	6.293 f
Female	8.087 ab	7.333 a	6.727 a	6.130 c
Sol Real	9.027 bc	8.833 bc	8.563 de	6.290 f
Male	10.467 cdef	9.600 cde	9.100 g	6.230 e
Female	7.027 a	7.233 a	6.659 a	6.763 m
Spring, 2001				
Athena	10.827 defg	10.467 efg	10.111 j	6.343 h
Male	11.700 fg	10.067 ef	9.741 hi	6.177 d
Female	10.933 defg	9.000 bcd	8.682 ef	6.567 l
Sol Real	11.193 efg	9.467 cde	8.944 fg	6.387 i
Male	12.360 g	10.300 efg	9.522 h	6.323 g
Female	9.813 cde	8.933 bcd	8.139 c	6.497 j
SE	0.479	0.267	0.085	0.004
	(n=15) <sup>y</sup>	(n = 3)	(n = 9)	(n = 3)

<sup>z</sup>Means separated by different letters within column, are significantly different ( $P < 0.05$ ) according to Tukey's multiple comparison procedure with an experiment-wise error rate at 0.05.

<sup>y</sup>Replications used per comparison are indicated in parenthesis.

1974; Currence and Larson, 1941; Mutton et al., 1981; Yamaguchi et al., 1977). Many eastern-type melons have been bred with versatile European parental lines that allow tolerance to rainfall and humidity, and to confer higher disease resistance. Our results indicate that although the eastern inbreds tested had the highest sucrose and total sugar concentrations, these fruit generally had a shorter harvest window and postharvest fresh-cut shelf life (data not shown).

**PHYSIOLOGICAL QUALITY MEASUREMENTS.** °Brix-cube, °Brix-II, °Brix-At, and pH had significant main and interaction effects. Overall, the lowest values for °Brix-cube, °Brix-II, and °Brix-At were observed for melons harvested in fall, and the 'Sol Real'-female and 'Athena'-male and female consistently had the lowest values (Table 3). 'Athena' had significantly higher °Brix-II

compared to both parents in all three seasons, whereas the 'Sol Real'-male usually had the highest °Brix-II, except in Spring 2000 (Table 3).

On average, the °Brix slurry values (Atago and II) were 10% and 15% lower than those obtained from cubes (°Brix-cube), except for one occasion in Fall 2000 for the 'Sol Real'-female (Table 3). °Brix-II readings from frozen slurries were roughly 3% to 8% lower than freshly homogenized °Brix-At values; although these two measures were positively correlated ( $r = 0.914$ ;  $P \leq 0.001$ ). These differences could be based on the relative purity of hand expressed juice (cubes) versus tissue homogenates, temperature calibration during measurements (°Brix-II only), or due to sample freezing. °Brix-II and °Brix-At were also significantly correlated with total sugars ( $r \geq 0.839$ ;  $P \leq 0.001$ ) and sucrose ( $r \geq 0.752$ ;  $P \leq 0.001$ ), and °Brix-cube was also significantly correlated with sucrose and total sugars ( $r \geq 0.627$ ;  $P \leq 0.001$ ). Similar to observations by Cantwell and Portela (1997), we observed that roughly 66% to 71% of SS (total sugars/°Brix-II) was directly attributed to total sugars (glucose, fructose and sucrose), yet the relative range was depressed (55% to 62%) if the °Brix-cube measure of SS was used. Total sugars were also positively correlated with sucrose ( $r = 0.843$ ;  $P \leq 0.001$ ).

When means comparisons for the physiological data were conducted based on melon type (normal production region), the eastern melons displayed significantly higher ( $P < 0.05$ ) values for °Brix-cube and °Brix-At, compared to western shippers (Table 4). When genotype and season were ignored and mean comparisons conducted based on the sex of the parent, the female means were significantly lower ( $P < 0.05$ ) in °Brix (all three measures) compared to the hybrid and male means (Table 4). The female inbreds had significantly higher pH and the male inbreds had the lowest pH for each genotype in each season, except the 'Athena' group in Fall 2000 (Table 3). Ignoring genotype and season and conducting mean comparisons over three seasons based on the sex of the parent revealed that female inbreds had significantly higher ( $P < 0.05$ ) pH, male inbreds had the lowest pH and the hybrids were intermediate (Table 4). Mean comparisons conducted based on melon type also indicated that western shippers had significantly higher ( $P < 0.05$ ) pH compared to eastern genotypes (Table 4).

**ORGANIC ACIDS.** Using a simultaneous GC method for sugars and organic acids, oxalic, succinic, malic, citric and isocitric acids were the main organic acids recovered from the six cantaloupe genotypes. The method did not consistently or sufficiently separate citric acid from isocitric acid and these co-eluting peaks were therefore combined. The predominant organic acid in *C. melo* varies as fruit develop and mature on the vine (Chachin

Table 4. Physiological mean comparisons using Tukey's multiple comparison procedure for two hybrid cantaloupe cultivars ('Athena' and 'Sol Real'), the inbred parents, and melon types grown in three seasons.

Category	°Brix-cube	°Brix At.	°Brix I.I.	pH
Sex				
Cultivar	10.881 (n = 90) a <sup>z</sup>	9.861 (n = 18) a	9.326 (n = 54) a	6.235 (n = 18) b
Female	9.033 (n = 75) b	8.278 (n = 18) b	7.710 (n = 45) c	6.478 (n = 18) a
Male	10.993 (n = 90) a	9.672 (n = 18) a	9.101 (n = 54) b	6.196 (n = 18) c
SE	0.196 (90/90)	0.109	0.035 (54/54)	0.002
SE	0.205 (75/90)	---	0.036 (45/54)	---
Type				
Eastern	10.777 (n = 90) a	9.694 (n = 18) a	9.123 (n = 54) a	6.181 (n = 18) b
Western	10.159 (n = 165) b	9.283 (n = 36) b	8.607 (n = 99) b	6.364 (n = 36) a
SE	0.172	0.094	0.031	0.001

<sup>z</sup>Means separated by different letters, within columns within categories (sex or type), are significantly different ( $P < 0.05$ ) according to Tukey's multiple comparison procedure with an experiment-wise error rate at 0.05. Replications used per comparison are indicated in parenthesis.

and Iwata, 1988; Wang et al., 1996) and is apparently based on whether or not the melon flesh is green (*inodorus* or *cantalupensis*) or orange (*cantalupensis* or *reticulatus*) (Chachin and Iwata, 1988; Hashinaga et al., 1984; Lamikanra et al., 2000; Wang et al., 1996). In our study, the predominant organic acid in all six orange-flesh genotypes was succinic (0.046%), generally followed by oxalic (0.026%), citric/isocitric (0.020%), then malic acid (0.016%). Aside from the 'Sol Real'-male in 2000, very low levels of malic acid were recovered. Overall, observations indicate that this simultaneous GC method may not be sensitive enough for detecting low level organic acids in native extracts. This observation was based on the small peak area for each organic acid, wide variation often observed within treatment triplicates, and because occasionally (four treatment combinations) no peaks were recovered for malic acid.

Nonetheless, the genotype  $\times$  season effect for succinic acid was statistically significant ( $P < 0.001$ ) and displayed an increasing trend from Spring to fall for all six genotypes. Genotype means for Spring harvests were all statistically equivalent at the  $P = 0.05$  level of significance and ranged from 0.013% to 0.034%, versus 0.075% to 0.122% for genotypes harvested in fall which, were statistically different from all Spring samples at the  $P = 0.05$  level. There was a statistically significant ( $P < 0.04$ ) genotype/season effect for both citric/isocitric acid and total organic acids. However, there were few clear trends for a given genotype over seasons in this study, except that 'Athena' in Spring 2000 had the lowest or next to lowest concentration of citric/isocitric, succinic and total organic acid. There were no statistically significant treatment effects for oxalic and malic acids. When means were evaluated by melon type, east versus west, only total organic acids had statistically significant ( $P < 0.05$ ) mean differences (east = 0.091%, west = 0.129%). When genotype and season were ignored and mean comparisons conducted based on the sex of the parental inbred, the citric/isocitric hybrid mean (0.028%) was statistically different ( $P < 0.05$ ) from the female (0.017%) and male means (0.013%); and the hybrid means (0.114%) and female means (0.119%) for the total organic acids were statistically different from the male mean (0.103%). There were no sex mean differences among the other organic acids. Overall, few consistent trends were observed over the two years for either set of hybrid cultivars along with their respective inbreds. However, these results might be a reflection of the method used, and may not correctly represent quantified organic acid concentrations. In six genotypes, organic acids did not significantly correlate with any sugar or °Brix measure, and therefore the acids do not appear to be good predictors of quality.

Sucrose accumulation in muskmelon vacuoles is driven by a mathematical difference between increasing SPS activity and the potential activity of the sucrose degrading enzyme AI (Hubbard et al., 1989). A stoichiometric relationship between low AI activity and increasing SPS activity are prerequisites for sucrose accumulation in cantaloupe (Lester et al., 2001). Similar enzymatic relationships have been reported for melons and tomatoes that accumulate sucrose. High sucrose accumulation in certain tomato fruit (i.e., *Lycopersicon chmielewskii*) appears to be controlled by a single recessive gene (*sucr*) located on chromosome 3 (Chetelat et al., 1993; Klann et al., 1993), which is associated with low AI protein. The trait of sucrose accumulation was determined by the AI gene at the molecular level (Harada et al., 1995), and the AI gene appears to be transcriptionally silent in sucrose accumulating tomato species (Klann et al., 1993). Recently, high sucrose accumulation in melon fruit was determined to be conferred by

a single recessive gene designated *suc* (Burger et al., 2002).

Breeders should subsequently attempt to down regulate AI, enhance SPS, or *suc*. Extracellular and vacuolar AIs have different pH optima and substrate specificities, depending on a single amino acid substitution; valine is found at the same position of vacuolar invertase whereas proline determines the more acidic pH optimum and the higher cleavage rate of raffinose in extracellular invertase (Goetz and Roitsch, 1999). Molecular approaches might be capable of down regulating AI in the higher pH environment of the vacuole via reducing the valine moieties in the amino acid sequence. However, manipulating the valine balance in order to favor the proline insertion in extracellular AI could negatively affect flavor because valine contributes significantly to acceptable melon volatile genesis (Wyllie et al., 1995). In tomato fruit, increased sucrose concentrations and reduced levels of AI were attained in transgenic plants expressing a constitutive antisense AI. However, even though phenotypically identical to wild-type plants, fruit were 30% smaller (Klann et al., 1996). Based on the backcrossing in melons and genetic approaches used in tomatoes, higher sucrose accumulation in melons may be better accomplished via increasing endogenous SPS activity, or by enhancing the *suc/suc* alleles.

In a survey of many diverse *C. melo* cultivars, the higher sugar accumulating genotypes had more basic flesh pH (> 6) than the low-sugar genotypes (Stepansky et al., 1999). The highest levels of malic and citric acids recovered from watermelon occurred in the more ripe heart and blossom ends of the fruit, yet those regions with the highest concentrations of organic acids were not significantly more acidic (Chisholm and Picha, 1986). Interestingly, in our study, female inbreds had significantly higher pHs compared to both the male inbreds and the commercial hybrids. The female inbreds also had significantly lower sugar levels (all measures) compared to the male inbreds and hybrids. Male inbreds generally had significantly more acidic pHs and lower sugar levels than the commercial cultivars, and significantly lower total organic acids compared to female inbreds and hybrids. Eastern melons had significantly higher sugar levels and more acidic pH than western shippers but lower organic acid levels. No direct measure of titratable acidity was performed because this measure is not commonly used for muskmelons. Nevertheless, it might be interesting to compare the significant pH and sugar trends observed to titratable acidity or specific isoforms of pH-dependent AI.

## Conclusions

Simple genetic control via a single recessive gene for high sucrose (*suc*) accumulation in *C. melo* was recently discovered, based on segregation patterns obtained from low and high sucrose melon cultivars (Burger et al., 2002). However, these cultivars actually belong to different melon subspecies (*flexuosus*  $\times$  *reticulatus*). When an intermediate and a high sucrose accumulating *reticulatus* subspecies were crossed, the segregation patterns were more complex. In a hybrid production field, an off-type can actually be the female parent. Therefore, seed companies try to breed the female to morphologically resemble the commercial hybrid. Subsequently, many specialty or unique horticultural traits are often placed in the male inbred to avoid losing proprietary secrets. Although a major gene appears to be controlling sucrose accumulation, the female inbred in highly related homozygous genotypes might be a limiting factor to the delivery of optimized horticultural quality. Our data suggests that in circumstances where reciprocal crosses yield extremely similar hybrids (resistance and

yield quality in addition to horticultural qualities) or where the male inbred confers most of the desired horticultural traits, more attention should be placed on careful selection of a female inbred with higher sugars and lower pHs. This could distribute improved sucrose accumulation to the commercial cultivar. Nonetheless, more commercial hybrids and parental inbreds need to be investigated to ascertain if these relationships are consistent.

Considering the main results of this work (chiefly that female inbreds had lower sugars and higher pHs than hybrids and male inbreds) and the fact that increased SPS activity is pivotal for sucrose accumulation (Hubbard et al., 1989; Lester et al., 2001), it would be logical to perform a comparative study of sucrose-related enzymes (AI, SPS) in commercial cultivars along with their inbred homozygous parents. We are unaware of any work relating these enzymes to maternal inheritance in cantaloupe. This suggests that a diallel or similar experiment could be informative by providing data on maternal effects, combining ability, measurements of additive or dominance effects, and epistatic interactions. If replicated in space and/or time, the magnitude of genotype × environment interactions on factors that contribute to cantaloupe quality could also be measured. Some studies, as outlined above, have indicated that non-sweet *C. melo* cultivars have reduced *suc* genetic potential or SPS activity and it would therefore be interesting to determine if female inbreds follow this trend, and can be screened to implicitly determine if desirable crosses can be based on specific enzymatic activity. Regarding season and temperature effects, it may also be useful to view differences in those enzymes known to be responsible for galactose metabolism and catabolism of raffinose oligosaccharides as they are phloem loaded into genetically related developing fruit.

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