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Antioxidant, Sugar, Mineral, and Phytonutrient Concentrations across Edible Fruit Tissues of Orange-Fleshed Honeydew Melon (*Cucumis melo* L.)

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Orange-fleshed, non-netted honeydew (*Cucumis melo* L.) is a relatively new melon in the marketplace and has shown a lot of potential as an alternative to netted muskmelons (cantaloupes), which are often prone to surface contamination by enteric bacteria. Orange-fleshed honeydew is a cross between orange-fleshed cantaloupe and non-netted, green-fleshed honeydew. This glasshouse study investigated the nutritional profile (phytonutrient and sugar contents) in different tissues of mature orange-fleshed honeydew melon fruit. The equatorial mesocarp of ripe fruit was segmented into hypodermal (subpeel), outer, middle, and inner (near the seed cavity) tissues and then assayed for total sugars, mineral nutrients, phytonutrients, total proteins, and enzymatic antioxidant activities. The concentrations of soluble solids, sucrose, total sugars, β-carotene, and 5-methyltetrahydrofolic acid increased in an inward direction from the subpeel mesocarp tissues toward the seed cavity. The activities of ascorbate peroxidase, catalase, and superoxide dismutase also increased in an inward direction. The concentrations of calcium, iron, magnesium, manganese, and sodium all decreased in the inward direction. When expressed on a dry weight basis, the concentrations of ascorbic acid, boron, copper, fructose, glucose, phosphorus, potassium, and zinc were higher in the subpeel region compared to the inner mesocarp tissues, but the reverse was true when data were expressed on a fresh weight basis. These data reveal that there is considerable variation in sugars, minerals, and phytonutrients across the mesocarp regions and that expressing the data on a fresh or dry weight basis can alter interpretations of the nutritional significance and health benefits of fruit. The data also confirm that orange-fleshed honeydew melon can be a rich source of many human health-related nutrients.

KEYWORDS: Ascorbate peroxidase; ascorbic acid; β-carotene; boron; calcium; catalase; copper; Cucurbitaceae; 5-methyltetrahydrofolic acid; fructose; glucose; iron; manganese; magnesium; phosphorus; potassium; sodium; sucrose; superoxide dismutase; zinc

INTRODUCTION

Muskmelons (*Cucumis melo* L.), including the netted varieties such as cantaloupes (*C. melo* Reticulatus Group) and smooth-skinned varieties such as honeydew melons (*C. melo* Inodorus Group), are rich sources of many minerals and health-promoting nutrients. Consumer preference for these fruits is determined largely by sweetness (sugar content), flavor/aroma, texture, and, more recently, their reputation as rich sources of human health-benefiting phytonutrients. Over the past 40 years, investigations into melon fruit growth, development, maturity, and senescence have chronicled changes in vitamin, mineral, carbohydrate, protein, and textural qualities as the fruit matures. Treatments regulating these quality attributes have resulted in improved commercial handling guidelines (*1–4*).

It is now well established that melon fruit soluble solids concentrations (SSC) and calcium (Ca) vary significantly among different mesocarp regions. For instance, soluble solids can range from 6.0% near the hypodermal (subpeel) region to 22.8% near the seed cavity (*5*). Hence, the USDA Agricultural Marketing Service (*6, 7*) has established 9% SSC as the minimum for marketability, and a specific region of mesocarp tissue for assaying SSC (i.e., a 1 cm^3 cube from the equatorial region, ~1 cm in from the peel). In contrast to SSC, Ca mesocarp concentrations tend to be highest in the subpeel region and lowest near the seed cavity. A minimum Ca concentration (~6 mg g of dry wt^{-1}) is required to maintain melon plasma membrane integrity for prolonged fruit firmness and shelf life (*8, 9*). Other compounds, in addition to SSC and Ca, are important indicators of melon fruit market quality (sugars, dry weight, and protein), human nutritional value (vitamins and minerals), and postharvest shelf life (antioxidants and minerals),

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Table 1. Dry Weight, Protein, and Soluble Solids Concentration Gradients in Different Mesocarp Tissues of Mature Orange-Fleshed Honeydew Melon Fruit[a]

		protein		
mesocarp tissue	dry wt (%)	dry wt (mg/g)	fresh wt (mg/g)	SSC (%)
hypodermal	4.7 d	8.1 a	0.4 c	4.1 d
outer	5.6 c	7.4 a	0.4 c	6.3 c
middle	9.0 b	4.3 b	0.5 b	8.6 b
inner	12.1 a	4.4 b	0.6 a	13.3 a

[a] Means within a column followed by the same letter are not significantly different at $P \leq 0.05$.

but no known attempt has been made to determine their mesocarp tissue concentration profiles in melon mesocarp.

Smooth-skinned, orange-fleshed honeydew melons are relatively new in the fresh produce industry, a cross between netted, orange-fleshed muskmelon (cantaloupe) and smooth-skinned honeydew melons; they are a viable alternative to netted cantaloupes, which are often prone to surface contamination by enteric bacteria. However, the retail industry has not fully embraced this melon, in part, due to lack of information about its market quality characteristics. Also, a complete profile of the quality traits, which are important for retail (e.g., sugar content, postharvest shelf life) and human nutrition (e.g., mineral, vitamin, and phytonutrient contents), of orange-fleshed honeydew melon has not been investigated.

In this glasshouse study, we characterized the nutritional profile (phytonutrient and sugar contents) in different tissues of mature orange-fleshed honeydew melon fruit. A thorough profiling of the aforementioned quality trait changes across fruit tissues is needed to establish physiologically based postharvest treatment guidelines for this unique melon.

MATERIALS AND METHODS

Plant Material and Growing Conditions. Non-netted, orange-fleshed honeydew melon 'Orange Dew' (Shamrock Seed Co., Inc., Salinas, CA) was grown in a glasshouse following the procedures previously described by Lester et al. (*10*). Briefly, plants were grown in 15 L black plastic pots containing commercial potting medium [Sunshine mix 2 (Sun Gro Horticulture, Bellevue, WA)] at the U.S. Department of Agriculture, Agricultural Research Service, Kika de la Garza Subtropical Agricultural Research Center, Weslaco, TX (latitutde 26° 10′ N, longitude 97° 58′ W, elevation 21 m). Following germination (7 days after planting), seedlings were thinned to one per pot and trained vertically, using twine attached to glasshouse fixtures. Mutual shading among plants was minimized by placing pots at least 45 cm apart. Plants were watered at least once per day, using an automatic drip irrigation system, and fertigated twice per week with a complete water-soluble fertilizer as previously described in Lester et al. (*10*). Natural sunlight was supplemented with 400 W high-pressure sodium-vapor lamps. The average daily photosynthetic photon flux (PPF) at the canopy level was 20.7 ± 0.7 mol m^{-2}. Cumulative PPF for the entire growth period was 1674 mol m^{-2}. Average day/night temperatures were 35.9 ± 0.7/24.8 ± 0.3 °C, whereas average day/night relative humidity values were 42.0 ± 1.4/74.3 ± 1.1%. Flowers were hand-pollinated, and only one fruit per plant was allowed to develop. Mature fully abscised (slipped) fruit were harvested at 8:00 a.m. each day.

Immediately after harvest all fruits were washed with distilled water, and the epidermis (peel) was removed with a vegetable peeler. The two polar ends (totaling two-thirds of the fruit) were removed and discarded. Wedges of the remaining equatorial region edible mesocarp tissue were carefully subsectioned, starting from the outermost tissues, as hypodermal mesocarp (or subpeel mesocarp, 2 mm thick), outer mesocarp (8 mm thick), middle mesocarp (variable thickness of remaining tissue), and inner mesocarp (closest to the seed cavity, 1 cm thick). The subsections were homogenized separately in a food processor (Quick 'N Easy; Black and Decker, Towson, MD) using 3−5 s pulses. Homogenized tissue subsections were immediately assayed fresh for SSC using a temperature-corrected, digital refractometer (Reichert Scientific Instruments, Buffalo, NY). The remaining samples were frozen in liquid nitrogen and then either stored at −80 °C for antioxidant enzyme activities, ascorbic acid, 5-methyltetrahydrofolic acid (folic acid), and protein content or lyophilized for dry weight, β-carotene, minerals, and free sugar analyses.

Antioxidant Enzyme Assays. The activities of ascorbate peroxidase (AsPX; EC 1.11.1.11), catalase (CAT; EC 1.11.1.6), and superoxide dismutase (SOD; EC 1.15.1.1) were assayed using 15 g tissue samples as described in Lester et al. (*11*). Enzyme activities were assayed spectrophotometrically at 25 °C using a UV−vis spectrophotometer (model Lambda 2, Perkin-Elmer, Uberlingen, Germany) equipped with a water bath (model FE-2, Haake Instruments, Berlin, Germany) for temperature control.

Carbohydrates. Fruit sugars (fructose, glucose, raffinose, stachyose, and sucrose) were extracted from 0.3 g of lyophilized tissue with ethanol (80%) at 90 °C and quantified by high-performance liquid chromatography (HPLC) as previously described by Lester et al. (*10*).

Dry Weight and Protein Determinations. Tissue dry weight was determined as a percentage of fresh tissue after lyophilization.

All protein concentrations were determined spectrophotometically at 595 nm using the Bio-Rad Protein Assay Dye Reagent Concentrate (Bio-Rad Laboratories, Hercules, CA) according to a method based on that of Bradford (*12*). Bovine γ-globulin (0.25−1.4 mg mL^{-1}) was used as a standard reference.

Minerals. Boron, calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc were extracted from 2 g of lyophilized tissue by ashing (3 h at 550 °C) in acid-washed porcelain crucibles. The cooled ash was dissolved in 2 mL of 1.0 M HCl, filtered (Whatman no. 1; Maidestone, U.K.), and brought up to 100 mL with HPLC water. Minerals were determined by atomic absorption/emission spectrophotometry (model 210 VGP; Buck Scientific, East Norwalk, CT) using the detection procedures and reference standards supplied with the instrument.

Vitamin Assays. Ascorbic acid and dehydroascorbate were extracted from 7.5 g of frozen (−80 °C) tissue and determined by spectrophotometry at 525 nm according to the procedure of Hodges et al. (*13*) and reported as total ascorbic acid. β-Carotene was extracted under low-light conditions from lyophilized tissue (0.020 g) and measured by UV−vis HPLC at 454 nm following the procedure of Lester et al. (*10*). External β-carotene and apo-8′-carotene (Sigma Chemical Co., St. Louis, MO) were used for standard curve/retention time determinations and as internal standard, respectively. Folic acid, as 5-methyltetrahydrofolate, was extracted from 7.5 g of frozen tissue and measured with a fluorescence HPLC with 290 nm excitation, 350 nm emission,

Table 2. Free Sugars (Fructose, Glucose, Sucrose) and Total Sugar Concentration Gradients in Different Mesocarp Tissues of Mature Orange-Fleshed Honeydew Melon Fruit[a]

	fructose (mg/g)		glucose (mg/g)		sucrose (mg/g)		total sugars (mg/g)	
mesocarp tissue	dry wt	fresh wt	dry wt	fresh wt	dry wt	fresh wt	dry wt	fresh wt
hypodermal	357 a	16.8 b	237 a	11.2 c	50 c	2.4 c	729 b	34.3 c
outer	253 b	13.9 c	184 b	10.1 c	88 c	5.0 c	581 c	32.1 c
middle	235 b	21.2 a	194 b	17.5 a	373 b	33.8 b	847 a	76.5 b
inner	146 c	17.5 b	117 c	14.0 b	516 a	62.6 a	814 ab	98.3 a

[a] Means within a column followed by the same letter are not significantly different at $P \leq 0.05$.

Table 3. Ascorbic Acid (AsA), β-Carotene, and 5-Methyltetrahydrofolic Acid (Folate) Concentration Gradients in Different Mesocarp Tissues of Mature Orange-Fleshed Honeydew Melon Fruit[a]

	AsA (mg/100 g)		β-carotene (mg/100 g)		folate (μg/100 g)	
mesocarp tissue	dry wt	fresh wt	dry wt	fresh wt	dry wt	fresh wt
hypodermal	148.5 ab	6.9 d	17.9 b	0.9 c	143.0 d	7.8 d
outer	158.5 a	8.8 c	26.9 a	1.6 b	196.0 c	11.5 c
middle	135.5 bc	12.2 b	21.5 ab	2.3 a	202.0 b	21.3 b
inner	127.8 c	15.4 a	21.4 ab	2.7 a	253.0 a	32.1 a

[a] Means within a column followed by the same letter are not significantly different at $P \leq 0.05$.

Table 4. Ascorbate Peroxidase (AsPX), Catalase (CAT), and Superoxide Dismutase (SOD) Activities in Different Mesocarp Tissues of Mature Orange-Fleshed Honeydew Melon Fruit[a]

	AsPX		CAT		SOD	
mesocarp tissue	specific activity[b]	total activity[c]	specific activity	total activity	specific activity	total activity
hypodermal	5.4 bc	3.4 bc	0.020 a	0.012 ab	0.034 a	0.016 b
outer	4.7 c	3.0 c	0.015 c	0.009 c	0.035 a	0.017 b
middle	5.5 b	3.7 b	0.016 bc	0.010 bc	0.034 a	0.019 b
inner	6.5 a	5.1 a	0.018 ab	0.014 a	0.035 a	0.025 a

[a] Means within a column followed by the same letter are not significantly different at $P \leq 0.05$. [b] Specific activities are as follows: AsPX, millimolar ascorbate oxidized per minute per milligram of protein; CAT, millimolar H_2O_2 decomposed per minute per milligram of protein; SOD, millimolar cytrochrome *c* conserved per minute per milligram of protein. [c] Total activities are as follows: AsPX, millimolar ascorbate oxidized per minute per gram of fresh weight; CAT, millimolar H_2O_2 decomposed per minute per gram of fresh weight; SOD, millimolar cytrochrome *c* conserved per minute per gram of fresh weight.

and a photomultiplier gain setting of 12 (*14*). An external folic acid standard (Sigma Chemical Co.) was used for standard curve and retention time determinations.

Statistical Analyses. Seventy-two plants with one melon fruit per plant were grown in a completely random design. Each of the 72 fruits (1 fruit = a replication) were analyzed, and data from mesocarp sections were subjected to analysis of variance using the general linear model (GLM) procedure of SAS (SAS Institute Inc., Cary, NC). Means from each mesocarp section were compared using the least-squares means (LSMEANS) procedures of SAS. Only significant $P \leq 0.05$ differences are discussed unless stated otherwise.

RESULTS AND DISCUSSION

Fruit tissue dry matter content increased in an inward direction from 4.7% in the hypodermal (subpeel) region to 12.1% in the inner mesocarp region near the seed cavity (**Table 1**). The same trend was observed for SSC, increasing from 4% in the hypodermal tissue to 13.3% in the inner mesocarp. This trend for SSC is consistent with previous findings (*1–4*). In contrast to the trends for dry matter content and SSC, protein concentrations, expressed on a dry weight basis, were highest in the outer hypodermal mesocarp and lowest in the inner mesocarp (**Table 1**). These trends may be due to differences in maturity stages among the mesocarp regions. Mesocarp tissues near the seed cavity are developmentally more mature and physiologically less active than tissues near the outer peel region (*15*). Mesocarp tissues near the seed cavity may thus serve as carbohydrates reserves (higher dry weight) for seed development, whereas the "younger" developing tissues near the peel are enzymatically active, requiring higher protein content for various physiological processes associated with postharvest shelf-life quality (*16, 17*). The aforementioned physiological basis for the observed trends would likely have been overlooked had protein concentrations been expressed solely on a fresh weight basis.

Fruit sugar (fructose, glucose, sucrose, and total sugars) concentrations differed in their mesocarp profiles (**Table 2**). Sucrose and total sugar gradient concentrations followed the same trends as dry matter content and SSC, increasing on both fresh and dry weight bases from the outer (subpeel) region to the inner mesocarp tissues near the seed cavity. Sucrose concentrations ranged from 10-fold (mg/g of dry wt) to 26-fold (mg/g of fresh wt) higher near the seed cavity compared to the subpeel mesocarp. Reducing sugar (fructose and glucose) concentrations (mg/g of dry wt), on the other hand, were higher in the outer (subpeel) tissues (2.4-fold higher for fructose and 2.1-fold higher for glucose) than in the inner mesocarp tissues near the seed cavity (**Table 2**). This trend is consistent with the earlier observation that outer tissues are metabolically active, with fructose and glucose derived from sucrose hydrolysis and serving as substrate for respiratory energy production to support growth and storage functions in the subpeel (i.e., hypodermal mesocarp) tissues (*10*). Sucrose concentrations were lowest in these outer tissues, which is consistent with previous findings (*18*) that soluble acid invertase (EC 3.2.1.26; which catalyzes the hydrolysis of sucrose into fructose and glucose) is found primarily in outer and hypodermal mesocarp melon fruit tissues. In contrast, ripened (middle and inner mesocarp) tissues accumulated sucrose to a much greater extent, due, in part, to lower soluble acid invertase activities in these primarily sucrose storage tissues.

Table 5. Concentration (Milligrams per Gram, Dry Weight Basis) of Boron, Calcium, Copper, Iron, Magnesium, Manganese, Phosphorus, Potassium, Sodium, and Zinc across Different Mesocarp Tissues of Mature Orange-Fleshed Honeydew Melon Fruit[a]

mesocarp tissue	B	Ca	Cu	Fe	Mg	Mn	P	K	Na	Zn
hypodermal	2.2 a	6.32 a	0.003 a	0.07 a	2.9 a	0.01 a	4.9 a	27.6 a	8.6 a	0.04 a
outer	1.8 b	0.32 b	0.003 a	0.04 b	1.6 b	0.006 b	3.7 b	29.2 a	7.2 b	0.03 b
middle	1.4 b	0.08 c	0.002 b	0.02 c	0.9 bc	0.003 c	3.0 c	21.6 b	2.6 c	0.02 c
inner	1.2 b	0.08 c	0.002 b	0.02 c	0.6 c	0.003 c	2.5 c	23.2 b	1.6 d	0.02 c

[a] Means within a column followed by the same letter are not significantly different at $P \leq 0.05$.

Table 6. Concentration (Fresh Weight Basis) of Boron, Calcium, Copper, Iron, Magnesium, Manganese, Phosphorus, Potassium, Sodium , and Zinc across Different Mesocarp Tissues of Mature Orange-Fleshed Honeydew Melon Fruit[a]

mesocarp tissue	B (mg/g)	Ca (mg/g)	Cu (μg/g)	Fe (μg/g)	Mg (mg/g)	Mn (μg/g)	P (mg/g)	K (mg/g)	Na (mg/g)	Zn (μg/g)
hypodermal	0.12 b	0.34 a	0.18 b	4.1 a	0.16 a	0.6 a	0.27 b	1.51 c	0.47 a	2.3 b
outer	0.11 b	0.018 b	0.19 b	2.4 c	0.10 b	0.3 b	0.22 c	1.71 c	0.42 a	1.9 c
middle	0.15 a	0.008 b	0.19 b	3.0 b	0.09 bc	0.4 b	0.32 a	2.28 b	0.28 b	2.3 b
inner	0.15 a	0.010 b	0.25 a	3.0 b	0.07 c	0.4 b	0.32 a	2.94 a	0.20 c	2.8 a

[a] Means within a column followed by the same letter are not significantly different at $P \leq 0.05$.

β-Carotene, ascorbic acid, and folic acid concentration profiles also varied across the mesocarp regions (**Table 3**). Folic acid and β-carotene concentrations (both dry and fresh weight bases) increased from subpeel to inner tissues. β-Carotene was expected to be highly concentrated near the seed cavity versus subpeel due to chromophore conversion in ripening tissues (*19*). Processes involved in folic acid biosynthesis, translocation, and storage in plants have not been fully elucidated (*20*); however, this vital phytonutrient has been observed to accumulate in mature plant tissues (*21, 22*), which is consistent with data of the current study. Folic acid concentrations were nearly 1.8 times higher (dry weight basis) and 4.1 times higher (fresh weight basis) in the inner, more mature storage mesocarp tissues near the seed cavity than in the outer immature subpeel tissues. Total ascorbic acid (AsA) concentrations (dry weight basis) were higher in the outer subpeel tissues than in the inner mesocarp tissues near the seed cavity. The higher concentration of AsA is perhaps associated with an increased antioxidant capacity requirement in this physiologically active region of the fruit. This trend was reversed when data were expressed on a fresh weight basis, possibly reflecting a dilution effect of this water-soluble compound.

Total enzyme activities of AsPX, CAT, and SOD increased inward from the outer subpeel tissues to the inner mesocarp tissues near the seed cavity (**Table 4**). Enzymatic antioxidants function to scavenge/neutralize reactive active oxygen species (*11*). Plant tissues with higher antioxidant capacities are better able to resist oxidative stress and, thereby, retard senescence (*23*). The higher activity of these antioxidant enzymes in the inner mesocarp tissues may be associated with ripening and seed maturation processes that generate reactive active oxygen species.

Mineral nutrient (B, Ca, Cu, Fe, Mg, Mn, P, K, Na, and Zn) concentrations (dry weight basis) were higher in the outer subpeel mesocarp tissues than in the inner mesocarp tissues near the seed cavity (**Table 5**). Metabolically active tissues such as the subpeel mesocarp require a greater supply of minerals (e.g., Ca, Mg, Zn) for cellular maintenance (e.g., membrane integrity), promoting postharvest longevity. When the data were expressed on a fresh weight basis, the above trend was conserved only for Ca, Fe, Mg, Mn, and Na concentrations and was reversed for B, Cu, P, K, and Zn concentrations (**Table 6**). The physiological significance of this reversed trend is unclear. Nonetheless, our results demonstrate that when mineral nutrient concentrations were expressed on a dry weight basis, their distribution profiles across various fruit tissues were consistent with their physiological functions and tissue requirements. On the other hand, when mineral concentrations were expressed on a fresh weight basis, interpretations of the physiological significance were less discerning.

In conclusion, our results demonstrate that when fruits or fleshy roots and tubers are sampled for analyses, it is important to be cognizant of the tissue profiles of the nutrient or mineral being investigated and to establish a uniform and consistent sampling scheme for all replicates. This avoids confounding interpretations of pre- and postharvest treatment effects due to variations in tissue concentrations of the nutrient or chemical under investigation. This study also revealed that reporting data on a fresh or dry weight basis can have implications for interpreting the physiological significance of any observed changes. The hypodermal mesocarp Ca concentrations measured in the present study were only slightly higher than the recommended minimum levels required for melon plasma membrane maintenance (*8, 9*), suggesting that postharvest Ca treatment may be needed for this cultivar of orange-fleshed honeydew melon. The relatively high activities of the antichilling enzymes [CAT and SOD (*24*)] measured in the present study indicate that this orange-flesh honeydew could safely be stored/transported at 4 °C compared to the recommended commercial storage temperatures of 7−10 °C for honeydew melon. This could potentially enable transport to distant markets without compromising shelf life and quality due to dehydration or chilling injury (*25, 26*).

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