Evaluation of Divergent C. melo Germplasm Fruits for Antioxidant Content

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Keywords: ascorbic acid, β-carotene, bioactives, human nutrition, oxidative stress, phenolics, postharvest quality

Abstract

Consumption of netted muskmelons (Cucumis melo L. Reticulatus group) has been associated with health concerns due to pathogenic bacteria attaching to inaccessitable sites on the netted rind. The purpose of this study was to compare 1) the enzymic and non-enzymic antioxidant capacity between representative cultivars of netted muskmelon (‘Cruiser’) and both green- (‘Honey Brew’) and orange- (‘Orange Dew’) fleshed honey dew muskmelons over a storage period of 17 days and 2) levels of non-nutrient phytochemicals between these genotypes in consideration of ultimately substituting netted orange-fleshed with non-netted orange-fleshed muskmelon. Melons were harvested in Texas in 2004. Fruit were analyzed immediately (day 0) or stored simulating retail conditions for 7 or 14 days at 7°C and 95% ±2% RH plus 3 days at 21°C. Both non-netted honey dew exhibited similar and less lipid peroxidation, and hence postharvest senescence, during the 17 days storage period than the netted muskmelon ‘Cruiser’. In comparison with ‘Cruiser’, ‘Orange Dew’ exhibited higher concentrations of 13-carotene and phenolics and, with few exceptions, higher activities of the antioxidant enzymes ascorbate peroxidase (AsPX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), catalase (CAT), guaiacol peroxidase (POX), and superoxide dismutase (SOD). Higher AsPX, POX, and SOD activities in both ‘Orange Dew’ and ‘Honey Brew’ appear to confer a greater resistance to lipid peroxidation in these muskmelon genotypes than in the netted ‘Cruiser’. ‘Orange Dew’ also appears to be a healthier food choice not only due to its lack of a netted rind which could potentially harbour human illness-related pathogens, but also that it is superior to both ‘Cruiser’ and ‘Honey Brew’ in overall beta-carotene and phenolic levels.

INTRODUCTION

Netted muskmelon (Cucumis melo L. Reticulatus group) has consistently tested positive for Salmonella (Castillo et al., 2004), Shigella (U.S. Food and Drug Administration, 2003) and Escherichia coli O157:H7 (Del Rosario and Beuchat, 1995), and has been associated with large outbreaks of human illness (more than 25,000 individual cases since 1990) in the US and Canada (Castillo et al., 2004), and in 2001 and 2002 two cases of human death (Canadian Food Inspection Agency, 2003). Survival of human illness pathogens on netted muskmelon following surface pasteurization (Ukuku, 2004) compared to non-netted honey dew melon (Cucumis melo L. Inodorus group) is attributed to inaccessible sites on the rind such as the netting (Beuchat and Scouten, 2004). Replacing netted, orange-fleshed muskmelon with a suitably phytounutrient dense non-netted, orange-fleshed type such as orange-fleshed honey dew, would greatly reduce the incidence of food borne illness associated with netted fruit.

Little is known regarding the human health-promoting phytochemicals or the antioxidant capacity of orange-fleshed honey dew melons. Moreover, enzymatic antioxidant capacity in orange-fleshed honey dew muskmelon associated with postharvest storage life has not been addressed. It is unknown if non-netted orange-fleshed honey dew
muskmelon cultivars have a postharvest storage life similar to those of green-fleshed honey dew or orange-fleshed netted muskmelons.

Oxidative stress has been associated with postharvest quality losses of fresh fruits and vegetables (Hodges et al., 2004). Active oxygen species (AOS) such as superoxide (O$_2^-$) and the hydroxyl radical (OH) play significant roles in lipid peroxidation, polysaccharide cleavage, and in both nucleic acid and protein degradation (Hodges, 2003). In addition, human epidemiological evidence has linked intake of fruits and vegetables with lower incidences of cancer, cardiovascular disease, immune system decline, and certain neurological disorders (Huang et al., 2005), all of which have been associated with oxidative damage (Hodges and Kalt, 2003); it is the antioxidative capacity in fruits and vegetables that has been identified as a major mechanism contributing to human health maintenance (Hodges and Kalt, 2003; Vinson et al., 2001).

Enzymic and non-enzymic antioxidants represent an important mechanism in AOS scavenging (for reviews, see Lurie, 2003). Superoxide dismutase (SOD; EC 1.15.1.1) catalyzes the dismutation of O$_2^-$ to H$_2$O$_2$. Both catalase (CAT; EC 1.11.1.6) and ascorbate peroxidase (AsPX; EC 1.11.1.11) eliminate H$_2$O$_2$, with ascorbate (vitamin C) being oxidized during the latter reaction catalyzed by AsPX. Ascorbate is re-reduced directly through activities of monodehydroascorbate reductase (MDHAR; EC 1.6.5.4) and dehydroascorbate reductase (DHAR; EC 1.8.5.4). Peroxidases (POX; EC 1.11.1.7) also convert H$_2$O$_2$ to HO, though they can form O$_2$ and H$_2$O$_2$ in a complex reaction involving NADH oxidation.

Not only can ascorbate reduce HO, in the AsPX catalyzed reaction described above, but this compound can also react directly with the AOS O$_2^-$, OH and singlet oxygen (O$_2^*$) (Hodges and Forney 2003), and may play a role in the regeneration of α-tocopherol (Horemans et al., 2000). One of the primary antioxidant functions of β-carotene, a lipid soluble antioxidant precursor to vitamin A, is to quench O$_2^*$ (Knox and Dodge, 1985). The phenolic class of phytochemicals, which include flavonoids, tannins, hydroxycinnamate derivatives, and lignin, also possess antioxidative properties due to their high reactivity as hydrogen or electron donors, their ability to chelate prooxidant transition metal ions such as Fe$^{3+}$ and Cu$^{2+}$, and their free radical chain-breaking function (Hodges and Kalt, 2003; Proestos et al., 2005). Studies have suggested that fruit extracts and/or isolated phenolics are associated with anti-cancer, anti-inflammatory, cardio-protective, and neuroprotective properties (Hodges and Kalt, 2003).

The purpose of this study was to compare ‘Orange Dew’, a representative orange-fleshed honey dew melon, with ‘Cruiser’, a netted orange-fleshed muskmelon, for 1) enzymatic and non-enzymatic (human nutritional) antioxidant capacities, 2) stability of antioxidant capacity over the commercial harvesting period, and 3) postharvest senescence (following 17 days of simulated commercial/retail storage). For comparative purposes, fruit of the green-fleshed honey dew muskmelon, ‘Honey Brew,’ were also included in this study.

**MATERIALS AND METHODS**

**Plant Material**

Fully abscised fruits of orange-fleshed, netted muskmelon (‘Cruiser’), orange-fleshed, non-netted honey dew (‘Orange Dew’), and green-fleshed, non-netted honey dew (‘Honey Brew’) melons, free of defects, were hand-harvested in 2004 from Starr Produce Co. (Rio Grande City, Texas). All fruits were collected from the field by 0900 HR. Fruits were washed in 0.02% sodium hypochlorite for 30 s, rinsed in tap water, and then randomized into lots for storage treatment. Fruit were assessed the day of harvest or after storage for 7 or 14 days at 7°C and 95 ±2% RH plus 3 days at 21°C to simulate commercial storage plus retail display temperatures.

All fruit were chilled to 4°C, washed with distilled water, the epidermis removed with a vegetable peeler, and the polar-ends (totaling 2/3 of the fruit) removed and discarded. Wedges of the remaining equatorial-region mesocarp tissue, devoid of seeds...
and integument tissue, were pureed in a food processor (Quick ‘N Easy, Black & Decker, Towson, Md.) using 3 to 5 s pulses. Tissue samples were assayed fresh for enzymes, frozen (liquid nitrogen, then stored at -80°C) for compounds and lyophilized (following freezing in liquid nitrogen) for total antioxidants.

**Metabolite Assays**

Ascorbic acid and dehydroascorbic acid were extracted from frozen tissue and determined according to the procedure of Hodges et al. (2001) and reported in this study as total ascorbate. 13-carotene was extracted under low light conditions from lyophilized tissue (0.020 g) using ice-cold heptane (1.0 ml) plus 0.5 ml internal standard [trans Apo-8'-carotene (40 μg·ml⁻¹) (Sigma Chemical Co. St. Louis, Mo.)] according to the modified procedure of Koch and Goldman (2004). Malondialdehyde (MDA) content was determined on 2.0 g of fresh tissue using the TBARS procedure of Hodges et al. (1999). Phenolic levels in 2.0 g of fresh tissue were assayed using the Folin-Ciocalteu method (Singleton and Rossi, 1965). Results are expressed as gallic acid equivalents (mg·g⁻¹ FW). Protein content was determined using the Bio-Rad assay (Bio-Rad Lab. Hercules, Calif.) based on the method of Bradford (1976).

**Enzyme Assays**

Activities of AsPX, CAT, DHAR, GR, MDHAR, POX, and SOD were analyzed in 15 g of fresh tissue as described in Lester et al. (2004).

**Total Antioxidant Assay**

Both lipophilic and hydrophilic antioxidants were analyzed using randomly methylated β-cyclodextrin (RMCD) as a solubility enhancer, 2,2'-azobis (2-amidino-propane) dihydrochloride (AAPH) as a peroxyl generator and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) as a standard according to Prior et al. (2003).

**Statistical Analyses**

Analysis of variance (ANOVA) using the general linear model procedures of SAS (SAS, Cary, N.C.) was conducted. Duncan’s multiple range tests (P≤0.05) were performed to evaluate the significance of differences between dependent variable means. Data are the average of five single-fruit replications per harvest.

**RESULTS**

**Metabolites**

No significant replicate effect was detected for any of the variables measured (data not shown). Although concentrations of MDA (nmol·g⁻¹ FW), the estimator of lipid peroxidation and indicator of senescence, increased in all cultivars during 17 days of storage, ‘Cruiser’ exhibited the highest level of lipid peroxidation while ‘Orange Dew’ and ‘Honey Brew’ exhibited the lowest level (Fig. 1A). Total ascorbate content (nmol·g⁻¹ FW) declined during storage in the non-netted orange-fleshed muskmelon, but not in the other melon cultivars (Fig. 1B). However, the only absolute difference between cultivars occurred on day 0 of storage, where ‘Honey Brew’ exhibited lower total ascorbate concentration.

β-carotene (nmol·g⁻¹ FW) was not detected in ‘Honey Brew’ (Fig. 1C). Levels of this lipophilic compound were higher in ‘Orange Dew’ compared to the netted melon ‘Cruiser’ on day 0 and throughout the storage period.

Phenolic levels, expressed as gallic acid equivalents (mg·g⁻¹ FW), increased in all cultivars during storage, but were highest on day 17 in non-netted ‘Orange Dew’ (Fig. 1D). No difference in phenolic concentrations between the three cultivars was observed on day 0.
Antioxidant Enzymes

Activities of AsPX (mmol·min⁻¹·g⁻¹ FW) were higher in ‘Orange Dew’ compared to ‘Cruiser’ on day 0 and throughout storage (Fig. 2A); both ‘Honey Brew’ and ‘Orange Dew’ exhibited significantly higher AsPX activities than ‘Cruiser’ on days 10 and 17. Activities of CAT (mmol·min⁻¹·g⁻¹ FW) were also significantly higher in ‘Orange Dew’ as compared to ‘Cruiser’ (Fig. 2B). There were no differences in activities of CAT between ‘Cruiser’ and ‘Honey Brew’ on days 10 and 17. Activities of this enzyme, although initially high, declined in ‘Honey Brew’ during the 17 day-storage period.

DHAR (mmol·min⁻¹·g⁻¹ FW) activities, similar to those of AsPX, were higher in ‘Orange Dew’ compared to ‘Cruiser’ on day 0 and during storage (Fig. 2C). DHAR activities of ‘Honey Brew’ and ‘Cruiser’ were not significantly different from one another.

Activities of MDHAR (mmol·min⁻¹·g⁻¹ FW) increased during storage for all cultivars (Fig. 2D). With the exception of day 17 fruits, ‘Orange Dew’ consistently exhibited the highest MDHAR activities.

As with ASPX, PDX activities (mmol·min⁻¹·g⁻¹ FW) were higher in ‘Orange Dew’ and ‘Honey Brew’ than ‘Cruiser’ fruit on all storage days (Fig. 2E).

SOD activities (mmol·min⁻¹·g⁻¹ FW) were higher in ‘Orange Dew’ than ‘Cruiser’ on days 10 and 17; no differences in activities of this enzyme were noted between ‘Orange Dew’ and ‘Honey Brew’ on these days (Fig. 2F).

Total Antioxidants

No overall change in total hydrophilic antioxidant levels (Trolox equivalents·g⁻¹ DW) within cultivars during storage were noted (Fig. 3A). No differences in total hydrophilic antioxidant content were observed between the three cultivars with the exception of day 17, where the water-soluble antioxidant activity of ‘Honey Brew’ was lower than that of the other two cultivars.

The only differences in lipophilic antioxidant activity (Trolox equivalents·g⁻¹ DW) between the cultivars occurred on day 10, where the lipid-soluble antioxidant activities of ‘Honey Brew’ and ‘Cruiser’ were less than that of ‘Orange Dew’ (Fig. 3B).

DISCUSSION

In comparison with the netted muskmelon ‘Cruiser’, the non-netted, orange-fleshed muskmelon ‘Orange Dew’ exhibited higher (~43%) concentrations of β-carotene and, with few exceptions, higher activities of the antioxidant enzymes AsPX, MDHAR, DHAR, CAT, POX, and SOD. These findings, observed on day 0 and following 17 days simulated commercial/retail storage, suggest that ‘Orange Dew’ has an inherently greater potential for antioxidant capacity than does the netted ‘Cruiser’. The lower levels of MDA, an estimator of lipid peroxidation (Hodges et al., 1999), in ‘Orange Dew’ also indicates that the greater antioxidant capacity of this cultivar had an effect on reducing AOS levels and, hence, oxidative stress, compared to ‘Cruiser’.

Presumably, higher activities of SOD in ‘Orange Dew’ led to greater O₂ dismutation and subsequent H₂O₂ production than in ‘Cruiser’ (for review of antioxidants, see Lurie, 2003). The higher activities of CAT, POX, and AsPX in ‘Orange Dew’, perhaps in response to the higher SOD activities, would give this cultivar an advantage as compared to ‘Cruiser’ in converting H₂O₂ from SOD activity as well as from other sources to H₂O. As ascorbate is oxidized to dehydroascorbate in the reaction catalyzed by AsPX, this would have necessitated the presence in ‘Orange Dew’ of higher activities of MDHAR and DHAR, which re-reduce dehydroascorbate back to ascorbate.

Both ‘Orange Dew’ and ‘Honey Brew’ exhibited lower lipid peroxidation levels, as estimated by MDA content, than ‘Cruiser’ during storage. However, only AsPX, POX, and SOD activities of ‘Honey Brew’ were similar to those of ‘Orange Dew’; activities of MDHAR, DHAR, and CAT, were, for the most part, no different than those of ‘Cruiser’. This suggests that the critical antioxidant enzymes for regulating postharvest-induced oxidative stress in non-netted muskmelon are AsPX, POX, and SOD, and that MDHAR, DHAR, and CAT play secondary roles. Other studies have also identified AsPX and SOD...
as enzymes playing an important role in postharvest oxidative stress of fruits and vegetables (e.g. Hodges et al., 2001; Jiménez et al., 2003). The similarities in lipid peroxidation during storage between the two non-netted honey dew (green- and orange-fleshed) cultivars suggests that β-carotene may play a less obvious role in limiting oxidative stress during postharvest melon storage.

In conclusion, 'Orange Dew' appears to be a healthier food choice not only due to its lack of a netted rind which could potentially harbour human illness-related pathogens, but also that it is superior to both 'Cruiser' and 'Honey Brew' in overall β-carotene and phenolic levels. Furthermore, both orange-fleshed ('Orange Dew') and green-fleshed ('Honey Brew') non-netted cultivars evinced less lipid peroxidation, and hence postharvest senescence, during the 17 days storage period than the netted muskmelon ‘Cruiser’.

**Literature Cited**


Fig. 1. Malondialdehyde (MDA) (A), total ascorbate (B), β-carotene (C), and phenolic acid contents (expressed as gallic acid equivalents) (D), in orange-fleshed cantaloupe cultivar Cruiser, green-fleshed honeydew cultivar Honey Brew and orange-fleshed honeydew cultivar Orange Dew fruits following 0, 10 (7 days at 10°C and 95% RH ±2% RH plus 3 days at 21°C) or 17 days (14 days at 10°C and 95% RH ±2% RH plus 3 days at 21°C) simulated commercial storage. Capital letters indicate significant differences (P<0.05) between cultivars within a storage day.
Fig. 2. Ascorbate peroxidase (ASPX) (A), catalase (CAT) (B), dehydroascorbate reductase (DHAR) (C), monodehydroascorbate reductase (MDHAR) (D), guaiacol peroxidase (POX) (E), and superoxide dismutase (SOD) (F), activities in orange-fleshed cantaloupe cultivar Cruiser, green-fleshed honeydew cultivar Honey Brew and orange-fleshed honeydew cultivar Orange Dew fruits following 0, 10 (7 days at 10°C and 95% RH ±2% RH plus 3 days at 21°C) or 17 days (14 days at 10°C and 95% RH ±2% RH plus 3 days at 21°C) simulated commercial storage. Capital letters indicate significant differences (P≤0.05) between cultivars within a storage day.

RESULTS AND DISCUSSION

Fig. 1 shows the changes of respiration rate after the harvest in onion bulbs. The respiration of bulb rapidly increased following the dormancy break. Commonly, the
Fig. 3. Total hydrophilic (A) and lipophilic (B) antioxidant activities (Trolox equivalents g⁻¹ DW) in orange-fleshed cantaloupe cultivar Cruiser, green-fleshed honeydew cultivar Honey Brew and orange-fleshed honeydew cultivar Orange Dew fruits following 0, 10 (7 days at 10°C and 95% RH ±2% RH plus 3 days at 21°C) or 17 days (14 days at 10°C and 95% RH ±2% RH plus 3 days at 21°C) simulated commercial storage. Capital letters indicate significant differences (P<0.05) between cultivars within a storage day.