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Physiological, Metabolite and Volatile Analysis of Cut Size in Melon During Post-Harvest Storage

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Abstract

Melons are an important component of fresh fruit salads, however they suffer from limited shelf life. Processing of melon fruit for use in fruit salads induces a number of changes including alterations in colour and texture. In addition, respiration rate and ethylene production are affected. Processing also elicits changes in flavour (sweetness) and aroma (production of volatile organic compounds – VOCs), of critical importance to the consumer. Several parameters govern shelf life; temperature is a critical factor. In this study we tested whether cut size is another parameter that can affect quality indicators. Melon (*Cucumis melo* cv. arapaho) cubes of three sizes were stored at 4ºC and assessed for quality at five time-points over a 15 day period. We assessed a number of parameters including firmness, loss of fresh weight, respiration rate, antioxidant capacity, phenolic compounds and carotenoid content. In addition we measured VOC profiles to assess whether there were any qualitative changes associated with storage period and/or cut size. Fresh weight (FW) loss and respiration rate increased significantly with storage time and FW loss was affected by cut size. Total carotenoid levels remained stable during the storage time as did antioxidant capacity in all cut sizes. However, cinnamic acid levels tended to decrease in the last stage of the storage period and changes in β-carotene content correlated with cut size (though not significantly). We were also able to separate the VOC profiles from the different cut sizes indicating that VOCs may be useful markers as indicators of the effects of cut size and storage time on quality.

INTRODUCTION

Melons are an important component of fresh fruit salads, however they suffer from limited shelf life. This is a particular problem with the more aromatic cantaloupe-type (*Cantaloupensis*) cultivars (Sinha et al., 2012). Processing of melon fruit for use in fruit salads induces a number of changes including alterations in colour and texture (Portela and Cantwell, 1998). Importantly processing also elicits changes in flavour (sweetness) and aroma (production of volatile organic compounds – VOCs), of critical importance to the consumer (Beaulieu and Lancaster, 2007). Several parameters govern shelf life; among these temperature is a critical factor (Aguayo et al., 2004) and cut fruit is usually stored at low temperatures (around 4ºC; Lamikanra et al., 2000) while cut size represents a parameter that can be modulated.

Melons are prized for their characteristic aroma which is particularly associated with the climacteric aromatic varieties. The main volatiles produced by these varieties are esters, sulphur-containing aromatic compounds, short-chain alcohols and aldehydes, sesquiterpenes and norisoprenes (Beaulieu and Grimm, 2001). VOC profiles change with stage of maturity (Beaulieu and Grimm, 2001) and Beaulieu (2006) used solid phase micro extraction (SPME) followed by gas chromatography-mass spectrometry (GC-MS) to show that volatile profiles change during storage at 4ºC of 2.5 cm^3 cubes following blending. Furthermore changes associated with storage of similar-sized cut melon pieces affect sensory flavour (Bett-Garber et al., 2005).

The aim of our work presented here was to test the hypothesis that cut size affects physiological parameters and identify useful markers of changes in the fruit associated with deterioration of the product.

MATERIALS AND METHODS

Cucumis melo L. var arapaho (cantaloupe melon) was obtained commercially, and selected for uniformity and maturity based on ground colour appearance and waxy development at the stem end. Melons were cooled overnight to 5 °C, prior to processing, then washed in cold water, dipped in 100 µg/l of sodium hypochlorite solution for 2 min, rinsed with deionised water and allowed to drain. The skin was uniformly removed, the blossom and stem ends were discarded, placental tissue and seeds were removed and three cut types were prepared by hand with a sharp knife. Melons were excised into cubes of 10 mm x 10 mm (small), and two trapezoid- shaped-type sections of 15 × 15 mm (medium) and of 25 × 25 mm (large). Fresh-cut fruit prepared from numerous fruits were randomised before packaging in 1000 g polypropylene trays weighing ca. 150 g of fresh-cut melon. Samples were taken for analysis after 1, 4, 7, 10 and 14-15 days.

VOC Analyses

At each time point the tray was inserted into a multi-purpose roasting bag (25 cm x 38 cm, TJM Ltd) and opened. The bag was sealed and stored at 20 °C for 1 h to equilibrate the headspace before sampling 200 mL of headspace on SafeLok thermodesorption tubes (Tenax TA & Sulficarb, Markes International Ltd.) with an EasyVOC manual pump (Markes International Ltd.). Control samples were collected from empty bags on site, retention standards prepared by loading 1 µL C8-C20 alkane standard.

All tubes were desorbed using a TD100 thermodesorption system (Markes International Ltd.) using the following settings for tube desorption: 10 min at 280 °C, trap flow of 40 ml/min and trap desorption and transfer: 40 °C/s to 300°C, split flow of 20 ml/min into GC (7890A; Agilent Technologies, Inc). Samples were separated over 60 m, 0.32 mm ID, 0.5 µm Rxi-5ms (Restek) at 2ml continuous flow helium using the following temperature program: Initial temperature 35 °C for 5 min, 5°C/min to 100 °C, then 15 °C to 250, final hold 5 min. Mass spectra were recorded from m/z 30 – 350 on a time-of-flight mass spectrometer (BenchTOF-dx, Almasco International).

Data from GC-MS measurements were deconvoluted and integrated with AMDIS (NIST) using a custom retention-indexed mass spectral library. Compounds, which were not abundant in all replicates of at least one single cut size, were excluded from statistical analyses as were compounds abundant in controls. Areas of remaining compounds were normalized within samples and standardised for each compound.

Physiological and Biochemical Analyses

1. Firmness, Respiration and Electrolyte Leakage. Firmness was assessed with a TA-XT2 Plus texture analyzer (Stable Micro 163 Systems, Surrey, UK) in 4 cubes (at 4 ºC) per treatment (maximum peak force required to drive a 5 mm flat head probe 5 mm into the cube with a travel speed of 1.5 mm/s).

Oxygen and CO_2 concentrations were monitored using a CheckMate II, (PBI Dansensor, Ringsted, Denmark). For respiration rate determination, a closed system method was used were melon cubes were weighed (ca. 70 g) and placed in 250 mL sealed glass jars, at 4º C for 3 h. CO_2production was determined inserting a small needle of CheckMate II, (PBI Dansensor, Ringsted, Denmark) into the glass jar headspace through a rubber septum.

Fresh-cut melon samples from three containers per cut-type were sampled on each day of storage. Disks (10 mm diameter and 5 mm thickness) were excised with a corkborer, rinsed with an isotonic mannitol solution and blot dried. Four disks (ca. 2.5 g) were immersed in 25 mL of 700 mM mannitol and incubated for 2 h at 20 ºC with agitation. Electrical conductivity of the incubation solution was measured with a Con 510 meter (Eutech Instruments, Nijkerk, The Netherlands) immediately after immersion of disks (EC_0) and after the 2 h incubation period (EC_f). After incubation, the solution containing the disks was frozen for 24 h then thawed and boiled for 30 min and cooled to room temperature before the measurement of total electrical conductivity (EC_t). Electrolyte leakage (EL) was expressed as percentage of total tissue electrolytes : $EL = (EC_f - EC_0)/(EC_t - EC_0) \times 100$

2. Total Antioxidant Capacity. Hydrophilic extracts were obtained, as described previously (Amaro et al., 2012), adding 25 mL of 88% methanol to 2,5 g fresh-cut melon samples. Total antioxidant capacity was measured using ABTS+ [(2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic) acid) diammonium salt] radical as described by Gião et al.(2007) measuring absorbance at 734 nm (UV mini 1240 spectrophotometer, Shimadzu, Tokyo, Japan) with ascorbic acid as standard.

3. Total Carotenoids and Beta Carotene Concentration. Total carotenoid content was determined essentially as previously described (Lavelli et al*.,* 2008*)*. Saponification was carried out as described by Kimura et al.(1990). β-Carotene was quantified by measuring absorbance at 454 nm. Carotenoid content was analyzed by HPLC as described previously (Oliveira et al., 2012). Three independent analyses were performed in each of the triplicate extracts obtained for each cut type.

4. Identification and Quantification of Specific Phenolic Compounds. Qualitative and quantitative profiles of phenolics were determined by HPLCDAD (Waters Series 600, Mildford MA, USA). Separation was performed in a reverse phase Symmetry® C18 column (250 x 4.6 mm i.d., 5 µm particle size and 125 Å pore size) with a guard column containing the same stationary phase (Symmetry® C18). Chromatographic separation of phenolic compounds was carried out with a solvent A formic acid, water and methanol (92.5:5:2.5) – and solvent B – methanol and water (94:6) – under the following conditions: linear gradient starting at 0 - 10% solvent B in 10 min at 0.5 mL/min, 10-30% in 40 min at 0.65 mL/min, 30-50% in 20 min at 0.75 mL/min and from 50 to 0% in 10 min at 1 mL/min. Injection volume was 20 µL. Detection was achieved by a diode array detector (Waters, Massachussets, EUA) at wavelengths ranging from 200 to 600 nm in 2 nm intervals. Absorbance was measured at 320 nm (cinnamic acids). Retention times and spectra of compounds were analyzed by comparison with pure standards and

quantification performed by the calibration curves of cinnamic acid and expressed as micrograms per gram of fresh biomass.

Statistical Analyses

Physiological and metabolite results were statistically analysed using the Student's t-test using the statistical package GraphPad Prism, version 5.00 for Macintosh.

Differences between bouquets were assessed with PerMANOVA (function Adonis in vegan package) and canonical analysis of principle co-ordinates (function capscale in biodiversity package). Number of compounds was reduced using loadings of first six PCs from PCA and differences re-tested with the methods above. Data preparation was carried out in Excel, statistical calculation in R (R Development Core Team 2008)

RESULTS AND DISCUSSION

Changes in Physiological Parameters

Firmness declined very slowly during the sampling period though changes were not significant (Fig. 1). Respiration rate fell between day 1 and day 4 of storage but then rose steadily between 4 and 10 days ($p<0.05$). This confirms previous data (Aguayo, 2004) for several types of melon and may be due to tissue deterioration and increasing microbial load. Differences between the cut sizes were not statistically significant ($p>0.05$), although on day 1 there was a positive correlation between intensity of cutting and respiration rate so confirming previous reports (Aguayo et al*.,* 2004).

Electrolyte leakage remained constant until day 10 then increased by day 15 particularly in the small cut size ($p>0.05$). This relates to increased membrane damage and is in accordance with previous reports for melon (Portela and Cantwell (2001). However none of these parameters changed significantly amongst cut sizes. In contrast, fresh weight loss, which increased between day 7 and day 15 of the experiment showed a significant difference between cut sizes by day 15 ($p< 0.05$).

Metabolite Changes

Metabolite analyses were performed to identify potential markers indicating differences between the cut sizes (Fig. 1). Total carotenoids and antioxidant activity remained constant in all three cut sizes throughout the experiment. However cinnamic acid levels decreased between day 10 and day 14 in all three cut sizes (although changes were not significant). In contrast, β-carotene levels decreased more rapidly from the medium (M) and large (L) cut sizes compared to small (S) where relatively high levels were retained at days 10-14 (although changes were not significant). The concentration of β-carotene, the main carotenoid in orange-fleshed cantaloupes (Gil et al., 2006), usually increases during maturation and decreases during senescence (Yahia and Ornelas-Paz, 2010). Moreover, processing such as cutting affects carotenoid content and availability. Exposure of fruit tissues after cutting can lead to substantial losses of carotenoid content especially through oxidation (Yahia and Ornelas-Paz, 2010). Cinnamic acid and its derivates such as p-coumaric, ferulic, caffeic and chlorogenic acids have been reported in fruits such as cranberries, oranges and apples due to their natural antibacterial and antifungal activities (Sinha et al., 2012), being also associated with the fermentation of cabbage (Harbaum et al., 2008). This fact could explain the increase in the concentration of cinnamic acid at the day 10 of sampling.

Changes in VOC Profiles

A sub-set of 21 compounds (mostly esters) from an initial total of 114 identified from the NIST library using AMDIS preserved much of the discrimination observed in PerMANOVA ($P<0.001$) (Fig. 2). Significantly, small and large cut sizes were clearly discriminated on the basis of the 21 VOCs whereas the medium cut size overlapped both. Previous studies (e.g. Beaulieu, 2006) used SPME to collect headspace VOCs from blended material rather than intact pieces. The increased sensitivity of the TD-GC-MS used here may be a factor in allowing us to discriminate even small differences in wounding responses between the cut sizes.

CONCLUSIONS

Many physiological parameters were unaffected by cut size however fresh weight loss did vary with cut size. The most FW was lost from the medium cut size: this may be due to the uneven shape of the pieces resulting in differences in surface area to volume ratio. Levels of several metabolites were unaffected by cut size or storage in these experiments, indicating that the 4 ºC storage conditions were preserving levels of nutritionally-relevant compounds.

Cut size did affect VOC profiles indicating that the sensorial value of different cut sizes should be further investigated. The identity of the 21 VOCs that associate with cut size and storage are being verified by comparison to pure compounds and assessed for their use as marker indicators relating to storage parameters.

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Literature Cited

Aguayo, E., Escalona, V.H. and Rtés, F.A. 2004. Metabolic Behavior and Quality Changes of Whole and Fresh Processed Melon. Journal of Food Science. 69: SNQ148-SNQ155.

Amaro, A.L., Fundo, J.F., Oliveira, A., Beaulieu, J.C., Fernandez-Trujillo, J.P. and Almeida, D.P. 2012. 1-Methylcyclopropene effects on temporal changes of aroma volatiles and phytochemicals of fresh-cut cantaloupe. J Sci Food Agric. 93: 828-837

Beaulieu, J.C. 2006. Effect of cutting and storage on acetate and nonactetate esters in convenient, ready-to-eat fresh-cut melons and apples. HortScience 41: 65-73.

Beaulieu, J.C. and Grimm, C.C. 2001. Identification of volatile compounds in cantaloupe at various developmental stages using solid phase microextraction. J. Agric. Food Chem 49: 1345-1352.

Beaulieu, J.C. and Lancaster, V.A. 2007. Correlating Volatile Compounds, Sensory Attributes, and Quality Parameters in Stored Fresh-Cut Cantaloupe. J Agric Food Chem. 55, 9503-9513.

Bett-Garber, K.L., Lamikanra, O., Lester, G.E., Ingram, D.A. and Watson M.A. 2005. Influence of soil type and storage conditions on sensory qualities of fresh-cut cantaloupe (*Cucumis melo*). J. Sci. Food Agric. 85:825–830

Gião, M.S., González-Sanjosé, M.L., Rivero-Pérez, M.D., Pereira, C.I., Pintado, M.E. and Malcata, F.X. 2007. Infusions of Portuguese medicinal plants: Dependence of final antioxidant capacity and phenol content on extraction features. Journal of the Science of Food and Agriculture. 87, 2638-2647.

Gil, M.I., Aguayo, E. and Kader, A.A. 2006. Quality Changes and Nutrient Retention in Fresh-Cut versus Whole Fruits during Storage. J Agric Food Chem. 54: 4284-4296.

Harbaum, B., Hubbermann, E.M., Zhu, Z.J. and Schwarz, K. 2008. Impact of fermentation on phenolic compounds in leaves of pak choi (Brassica campestris L. ssp chinensis var. communis) and Chinese leaf mustard (Brassica juncea coss). J Agric Food Chem. 56: 148-157.

Kimura, M., Rodriguez-Amaya, D.B. and Godoy, H.T. 1990. Assessment of the saponification step in the quantitative determination of carotenoids and provitamins A. Food Chemistry. 35: 187-195.

Lamikanra, O., Chen, J.C., Banks, D. and Hunter, P.A. 2000. Biochemical and microbial changes during the storage of minimally processed cantaloupe. J Agric Food Chem. 48: 5955-5961.

Lavelli, V., Pompei, C. and Casadei, M.A. 2008. Optimization of Color and Antioxidant Activity of Peach and Nectarine Puree: Scale-Up Study from Pilot to Industrial Plant. J Agric Food Chem. 56: 7091-7099.

Oliveira, A., Pintado, M. and Almeida, D.P.F. 2012. Phytochemical composition and antioxidant activity of peach as affected by pasteurization and storage duration. Lwt-Food Science and Technology. 49: 202-207.

Portela, S.I. and Cantwell, M.I. 1998. Quality changes of minimally processed honeydew melons stored in air or controlled atmosphere. Postharvest Biology and Technology. 14: 351-357.

Portela, S.I. and Cantwell, M.I. 2001. Cutting blade sharpness affects appearance and other quality attributes of fresh-cut cantaloupe melon. Journal of Food Science. 66: 1265-1270.

R Development Core Team 2008. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org.

Singleton, V.L. and Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. american journal of enology and viticulture. 16, 144-158

Sinha, N.K., Sidhu, J.S., Barta, J., zsef, Wu, J.S.B. and Cano, M.P. 2012. Handbook of Fruits and Fruit Processing (2nd Edition): John Wiley & Sons.

Yahia, E.M. and Ornelas-Paz, J.d.J. 2010. Chemistry, stability and biological actions of carotenoids, lutein and lycopene. In *Fruit and vegetable phytochemicals: chemistry, nutritional value and stability* eds. De la Rosa, L., Alvarez-Parrilla, E. and Gonzalez-Aguilar, G. pp.177-222: Willey-Blackwell Publishing.

Figures

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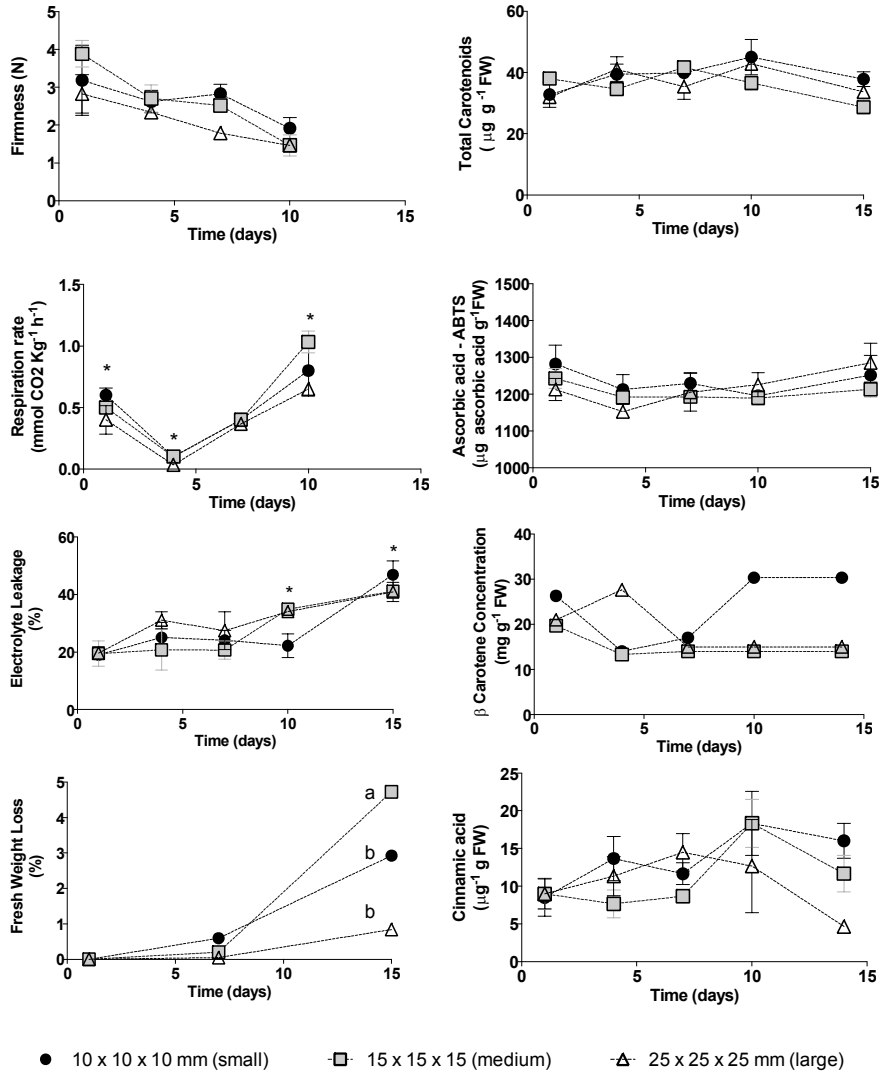

Fig. 1. Changes in physiological parameters and key metabolites of melon pieces in relation to cut size (small medium and large) over 15 days storage at 4ºC. (± SE, n=3). Letters indicate statistical differences between cut sizes and * between days (p<0.05)

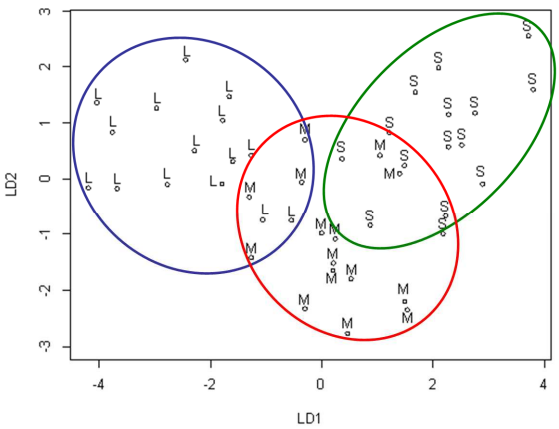

Fig. 2. Changes in VOC patterns of melon pieces in relation to cut size: large 25 x 25 x 25 mm (L), medium 15 x 15 x 15 mm (M), small 10 x 10 x 10 mm (S) over 15 days storage at 4°C. (± SE, n=3).