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1 **Multitrait analysis of fresh-cut cantaloupe melon enables discrimination between storage**  
2 **times and temperatures and identifies potential markers for quality assessments.**

3

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29 **Abbreviated running title:** Multi-trait analysis of post-harvest storage in fresh-cut melon.

30

31 **Abstract**

32 Fresh-cut cantaloupe melon is valued for its aroma but is highly perishable. Temperature of  
33 storage (typically 0 to 5 °C) is critical for maintaining fresh-cut melon quality, but often reaches  
34 10 °C during transportation and in retail outlets. A comparison amongst 0, 5 and 10 °C storage  
35 temperatures for fresh-cut melon over 14 days reveals that storage at 0 °C is optimal for  
36 avoiding increases in microbial load and loss of vitamin C especially at later time points.  
37 However, higher temperatures maintain better the balance of esters (acetate versus non-acetate)  
38 and phenolic content. The whole volatile organic compound (VOC) profile can be used to  
39 discriminate both time and temperature effects especially at earlier time points. Potential VOC  
40 markers for changes in vitamin C from day 0 to day 6 of storage (3-methyl butane nitrile) and  
41 temperature (limonene) are identified through a multi-trait analysis.

42 **143 words**

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44

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46 **Key words:** *Cucumis melo*, functional and nutritional quality, post-harvest storage, fresh-cut  
47 melon, volatile organic compounds.

48 **Chemical compounds:**

49 Catechin (PubChem CID: 9064), ethyl butanoate (PubChem CID: 7762), ethyl 2-methyl-  
50 butanoate (PubChem CID: 24020), ethyl acetate (PubChem CID: 8857), 3-  
51 methylbutanenitrile (PubChem CID: 12244), limonene (PubChem CID: 440917), eucalyptol  
52 (PubChem CID: 2758), (3E)-3-hexen-1-yl acetate (PubChem CID: 5363388), butyl acetate  
53 (PubChem CID: 31272), isobutyl acetate(PubChem CID: 8038 ).

54

## 55 **1. Introduction**

56 Melon (*Cucumis melo* L.) is a widely cultivated crop, consumed worldwide. *C. melo* var.  
57 *Cantaloupenensis* is a climacteric variety of melon, valued for its aroma and commonly used as  
58 fresh-cut fruit in fruit salads. However, fresh-cut melon has a very limited shelf-life.  
59 Temperature is the key factor limiting its post-harvest life affecting respiration rate and  
60 metabolic activity, microbial growth and water loss (Cantwell and Suslow, 1999). Microbial  
61 contamination is further enhanced by the high pH of melon flesh compared to other fruit  
62 (Soliva-Fortuny and Martín-Belloso, 2003). To retain cantaloupe melon quality including  
63 appearance, taste and aroma, and reduce the effects of wounding stress for the maximum time,  
64 fresh-cut melon is recommended to be stored at temperatures of 0 - 5 °C (Bett-Garber et al.,  
65 2011) although temperatures of up to 10 °C may be experienced during breaches of the cold  
66 chain, at the retail outlet or post-purchase.

67 Melon is valued for containing biologically active compounds, present in small quantities but  
68 with important effects on human health, including phenolic compounds, ascorbic acid (vitamin  
69 C) and  $\beta$ -carotene (Lester and Hodges, 2008). Although cantaloupe melon fruit does not rank  
70 particularly high in phenolic content (Fu et al., 2011), melons contain benzoic, vanillic, and  
71 *trans*-cinnamic acids (Kolayli et al., 2010), with reported health benefits (Williamson et al.,  
72 2005). However, both aromatic and nutritional quality of cantaloupe melon, including vitamin  
73 C is lost quickly during post-harvest storage (Beaulieu, 2006a), and even faster when processed  
74 (Lamikanra and Richard, 2002; Kalt, 2005).

75 Fresh melon aroma is composed of a large heterogeneous group of VOCs, with esters as  
76 the predominant chemical group, consisting of a wide range of acetate and non-acetate esters,  
77 but also alcohols, organic acids, aldehydes, ketones, terpenes, and sulphur compounds (El Hadi  
78 et al., 2013). The exact composition varies amongst cultivars (e.g. Amaro et al., 2012) with the  
79 proportion of acetate esters correlating positively with firmness across different cultivars

80 (Aubert and Bourger, 2004). Aroma profile is also affected by the stage of maturity at harvest  
81 (Beaulieu, 2006a).

82 Acetate esters such as 2-methylpropyl acetate, hexyl acetate, butyl acetate, 2-methylbutyl  
83 acetate, benzyl acetate and hexyl acetate were major components of the VOC profile when  
84 analysed from homogenised flesh after organic extraction (Aubert and Bourger, 2004) or by  
85 SPME (Beaulieu et al., 2006b). The abundance and proportion of each of the compounds found  
86 in the bouquet produces the characteristic cantaloupe melon aroma, with both abundance and  
87 odour activity value (OAV) of the compound being important for determining overall quality  
88 of the bouquet (El Hadi et al., 2013). However, the VOC profile reported also varies with the  
89 method of analysis: for example, extraction from homogenised melon flesh (Beaulieu, 2006b;  
90 Pang et al., 2012) found C6 and C9 aldehydes not found by others using headspace collection  
91 (Bauchot et al., 1998).

92 A compromise between sensorial and nutritional values is a major challenge as low  
93 storage temperatures may negatively affect the production of important biologically active  
94 compounds and VOCs. Storage temperature (5 °C or 10 °C) had little effect on total antioxidant  
95 activities in orange-fleshed honeydew, but ascorbate,  $\beta$ -carotene, and folic acid contents were  
96 differentially affected by storage temperature in different cultivars (Lester and Hodges, 2008).  
97 VOCs are also affected by storage time and temperature: at 4 °C the ratio of non-acetate to  
98 acetate esters rose with time (Beaulieu, 2006b) and it was hypothesised that some of the change  
99 may be due to degradation of metabolites during storage providing new substrates for the  
100 biosynthesis of non-acetate esters. VOCs can also provide easily measurable markers which  
101 reflect internal qualities or effects of storage and processing such as nutritional value and  
102 microbial growth (Spadafora et al., 2016).

103 In this study VOCs were analysed using passive headspace analysis by thermal  
104 desorption gas chromatography time of flight mass spectroscopy (TD-GC-TOF-MS) which

105 enables a rapid and non-destructive analysis of VOCs directly from the fresh-cut fruit without  
106 further processing. This enables a highly sensitive and representative profile of the aroma  
107 VOCs during post-harvest storage to be correlated with changes in physiological, biochemical  
108 and microbial status. We show that this methodology has excellent discriminating power at  
109 early time-points and between temperatures indicating that it has potential use for assessing  
110 breaches in the cold supply chain that may affect quality. Furthermore, a multi-trait analysis  
111 identifies correlations between VOCs and other metabolite content with potential for the  
112 development of diagnostic markers.

## 113 **2. Materials and methods**

### 114 *2.1 Raw material and fruit processing*

115 Orange fleshed cantaloupe melons (*Cucumis melo* var. *cantaloupensis* cv. Arapaho) were  
116 grown in the Santarém region in Portugal, using integrated farming with no specific  
117 supplementary irrigation or nutrition. Melons were harvested at commercial maturity (3/4 slip,  
118 peduncle almost abscised) and immediately transported to commercial fruit processing  
119 facilities where they were stored at low temperature (7 °C) until processing. Melons were  
120 inspected carefully for bruising and compression damage, and fruit with no visual defects and  
121 uniform in shape and size were selected. Fruits were washed in cold water, dipped in 100 µg·L<sup>-1</sup>  
122 sodium hypochlorite solution for 2 min, rinsed with deionised water and allowed to drain. In  
123 accordance with commercial methods, the skin was removed uniformly, the blossom and stem  
124 ends were discarded, the melons were then sliced open and placental tissue and seeds were  
125 removed. Trapezoidal pieces (approximately 2.5 x 3.5 cm<sup>2</sup>) were cut using a sharp knife. All  
126 cutting tools and containers were sanitized with 70 % ethanol and allowed to dry before use.

### 127 *2.2 Packaging and storage conditions*

128 Trapezoidal melon pieces prepared from numerous fruits were randomized before  
129 packaging. Fresh-cut melon (ca. 175 g) were placed in 500 g clamshells (in triplicate) with no

130 perforation, stored at 0, 5, or 10 °C. After 0, 2, 6, 9 and 14 days samples of melon pieces were  
131 removed from storage and divided randomly for the different analyses.

### 132 *2.3 Respiration rate and package CO<sub>2</sub> accumulation*

133 For respiration rate determination, a closed system method was used: fresh-cut melon  
134 pieces from each temperature treatment were weighed (ca. 50 g) and placed in 250 mL sealed  
135 glass jars, for 3 h. CO<sub>2</sub> production was determined using a CheckMate II, (PBI Dansensor,  
136 Ringsted, Denmark) by inserting a small needle into the glass jar or package headspace through  
137 a rubber septum, for respiration rate and package CO<sub>2</sub> accumulation, respectively.

### 138 *2.4 Colour and firmness*

139 Surface colour of the fresh-cut melon cubes was measured in the CIE L\*a\*b\* color space  
140 with a CR-400 colorimeter (Konica Minolta, Osaka, Japan), using the D65 illuminant and  
141 observer at 2°. Hue angle ( $h^\circ = \arctan(b^*/a^*)$ ) and chroma [ $C^* = (a^{*2} + b^{*2})^{1/2}$ ] were calculated  
142 from the primary a\* and b\* readings.

143 Firmness was measured with a TA-XT2 Plus texture analyzer (Stable Micro Systems,  
144 Godalming, UK) equipped with a 5 kg load cell. The force required to perforate the tissue to a  
145 depth of 5 mm with a cylindrical probe of 5 mm diameter at a speed of 1.5 mm s<sup>-1</sup> was  
146 registered.

147 One firmness and two colour measurements were taken from the lateral cut surface of  
148 each of three cubes from three replicated packages of each temperature treatment.

### 149 *2.5 Total phenolic compound content, catechin, and antioxidant activity*

150 Total phenolic compound content was determined according to Ferrante et al. (2004) and  
151 Spadafora et al. (2016) using about 2.5 g of fresh-cut melon tissue, homogenized with 10 mL  
152 methanol. Methanol extract (50 µL) was added to 50 µL Folin-Ciocalteu reagent and 1 mL of  
153 1 N sodium carbonate (Sigma-Aldrich), made up to 2.5 mL with deionized water. Samples  
154 were reacted in the dark for 30 min and absorbance measured at 765 nm. The total phenolic

155 content was calculated from a calibration curve, and the results expressed as mg of gallic acid  
156 equivalent per 100 g of fresh weight.

157 Profiles of individual phenolic compounds were determined by HPLC-DAD (Waters  
158 Series 600, Mildford MA, USA) exactly as described in Spadafora et al. (2016) using a reverse  
159 phase Symmetry® C18 column (250 x 4.6 mm i.d., 5 µm particle size and 125 Å pore size)  
160 with a guard column containing the same stationary phase (Symmetry® C18). Separation was  
161 carried out using solvent A – water, methanol and formic acid (92.5:5:2.5) – and solvent B –  
162 methanol and water (94:6) with a linear gradient of 0 – 10 % solvent B from 0 to 10 min at 0.5  
163 mL min<sup>-1</sup>, 10-30 % from 10 to 50 min at 0.65 mL min<sup>-1</sup>, 30-50 % for 50 to 70 min at 0.75 mL  
164 min<sup>-1</sup> and from 50 to 0 % from 70 to 80 min at 1 mL min<sup>-1</sup>. Injection volume was 20 µL.  
165 Detection was with a diode array detector (Waters, Massachusetts, EUA) at 200 to 600 nm in  
166 2 nm intervals. Retention times and compound spectra were analysed by comparison with pure  
167 standards; quantification was by calibration with catechin and absorbance at 280 nm and 320  
168 nm and expressed as µg g FW<sup>-1</sup>.

169 The ABTS ((2,2-azinobis(3-ethylbenzothiazoline-6-sulphonic) acid) diammonium salt)  
170 radical scavenging activity of methanol extracted samples was measured according to Gião et  
171 al. (2007). Total antioxidant activity was quantified by measuring absorbance at 734 nm with  
172 a UV mini 1240 spectrophotometer (Shimadzu, Tokyo, Japan), using a calibration curve of  
173 ascorbic acid (0.021-0.5 g L<sup>-1</sup>). Results are expressed as mg g FW<sup>-1</sup>.

#### 174 *2.6 Total carotenoid content and β-carotene*

175 Total carotenoid content was determined as previously described (Lavelli et al. 2008)  
176 with slight modifications. Fresh-cut melon samples (2.5 g) were homogenised with 10 mL of  
177 cooled ethanol using an Ultra-Turrax (IKA T18, Wilmington, NC, USA) before 10 mL of  
178 hexane were added. Following centrifugation (5000 g for 10 min at 4 °C) the hexane layer was  
179 transferred to a 20 ml volumetric flask. The residue, was re-extracted with 2.5 mL of saturated



180 sodium chloride solution and hexane (12 mL), and centrifuged as above, and the two hexane  
181 extracts were combined (made up to 20 ml with hexane). Saponification was according to  
182 Kimura et al. (1990). Hexane extract (15 mL) was added to 15 mL of 10 % methanolic  
183 potassium hydroxide in a sealed Pyrex bottle wrapped in aluminium foil to exclude light. The  
184 reaction was carried out for 16 h at room temperature, with gentle agitation. The mixture was  
185 then washed with 10% NaCl (50 mL) and then deionized water, until the pH of the rinse was  
186 neutral.  $\beta$ -Carotene was quantified by measuring absorbance at 454 nm as above using a  
187 calibration curve of pure  $\beta$ -Carotene standard (Extrasynthese, Lyon, France) and expressed as  
188  $\mu\text{g g}^{-1}$  FW.

189 Carotenoid content was also analyzed by HPLC after drying the extract under a stream  
190 of nitrogen and resuspension in 1ml of eluent using a Vydac 201TP54 C18 column (250 mm  
191  $\times$  4.6 mm), equipped with a C18 pre-column. Carotenoids were eluted using acetonitrile,  
192 methanol, dichloromethane, hexane and ammonium acetate (55:22:11.5:11.5:0.02) under  
193 isocratic conditions at 1.0 mL min<sup>-1</sup> flow rate over 20 min, at 25 °C. Injection volume was 40  
194  $\mu\text{L}$  and the detector was set at 454 nm.  $\beta$ -Carotene was quantified using a calibration curve as  
195 above.

196 Three independent analyses were performed in each of the triplicate extracts for each  
197 treatment.

### 198 *2.7 Identification and quantification of ascorbic acid (AA)*

199 Qualitative and quantitative profile of ascorbic acid (AA) was determined by HPLC after  
200 derivatization of DHAA into the fluorophore 3-(1,2-dihydroxyethyl)furo[3,4-b]quinoxaline-1-  
201 one (DFQ), with 1,2-phenylenediamine dihydrochloride (OPDA) according to Zapata and  
202 Duforur (1992). Fresh melon pieces (3 g) were ground in liquid nitrogen and homogenized  
203 with 6 mL of methanol–water (5:95) containing citric acid (21.0 g·L<sup>-1</sup>) and EDTA (0.5 g·L<sup>-1</sup>;  
204 Martínez- Sánchez et al. 2008). Freshly prepared OPDA solution (250  $\mu\text{L}$ ) was added to melon

205 extract (750  $\mu\text{L}$ ). Samples (20  $\mu\text{L}$ ) reacted for 37 min at room temperature in the dark and  
206 analysed by HPLC-DAD (Waters Series 600, Mildford MA, USA). Separation was performed  
207 in a reverse phase Symmetry® C18 column (250 x 4.6 mm i.d., 5  $\mu\text{m}$  particle size and 125  $\text{\AA}$   
208 pore size) with a guard column containing the same stationary phase (Symmetry® C18). The  
209 mobile phase was MeOH/H<sub>2</sub>O (5:95 v/v) containing 5 mM cetrimide and 50 mM NaH<sub>2</sub>PO<sub>4</sub> at  
210 pH 4.5. The flow rate was 0.9 mL min<sup>-1</sup>. Detection was achieved by a diode array detector  
211 (Waters, Mildford MA, EUA) at 348 nm for DAA and 261 nm for AA. Retention times and  
212 compound spectra were analysed by comparison to pure standards; quantification was  
213 performed using calibration curves of ascorbic acid (AA) and expressed as mg g<sup>-1</sup> FW. Three  
214 independent analyses were performed in each of the triplicate extracts obtained for each  
215 treatment.

## 216 *2.8 Collection and analysis of VOCs*

217 At each time point melon flesh cubes (175 g) were placed in a multipurpose roasting bag  
218 (25 cm x 38 cm, TJM Ltd). The bag was sealed around a capped 1.5 mL Eppendorf tube (with  
219 its bottom cut off), using an elastic band, to ensure a tight seal. The headspace was equilibrated  
220 at 20 °C for 1 h and then headspace samples (200 mL) were collected with an EasyVOC manual  
221 pump (Markes International Ltd.) onto SafeLok thermal desorption tubes (Tenax TA &  
222 Sulficarb, Markes International Ltd.). The sampling end of the tube was inserted tightly into  
223 the Eppendorf tube, that was tightly sealed to the bag, and the pump was connected to the other  
224 end of the tube. As controls, samples were also collected from empty bags on site. Retention  
225 standards were prepared by loading 1  $\mu\text{L}$  C8-C20 alkane standard (Sigma Aldrich) onto a  
226 separate TD collection tube. Three biological replicates were collected for each time point from  
227 separate samples of melon cubes and was performed at Universidade Católica Portuguesa.  
228 Tubes were transported to Cardiff University by courier and desorbed on a TD100 thermal  
229 desorption system (Markes International Ltd.) as follows: 10 min at 280 °C, with a trap flow

230 of 40 mL min<sup>-1</sup> and for trap desorption and transfer: 40 °C s<sup>-1</sup> to 300 °C, split flow of 40 mL  
231 min<sup>-1</sup> with a split ratio of 11:1 into a GC (7890A; Agilent Technologies, Inc).

232 VOCs were separated over 60 m, 0.32 mm I.D., 0.5 µm Rx5ms (Restek) with 2 mL min<sup>-1</sup>  
233 <sup>1</sup> helium carrier gas using constant flow and the following programme: initial temperature 35  
234 °C for 5 min, 5 °C min<sup>-1</sup> to 100 °C, then, 15 °C min<sup>-1</sup> to 250 °C, final hold 5 min. Mass spectra  
235 were recorded from m/z 35 – 500 on a time-of-flight mass spectrometer (BenchTOF-dx,  
236 Markes International Ltd).

### 237 *2.9 Analysis of GC-MS data*

238 Initial processing of data from GC-MS measurements was carried out using MSD  
239 ChemStation software (E.02.01.1177; Agilent Technologies, Inc) and was then deconvoluted  
240 and integrated using AMDIS (NIST11) and a custom retention-indexed mass spectral library.  
241 VOCs that were not present in at least two out of the three replicates of one data point, and  
242 compounds abundant in controls, were excluded from statistical analyses. MS spectra from the  
243 deconvolution were searched against the NIST 2011 library (Software by Stein et al., version  
244 2.0 g, 2011). Only compounds scoring > 80 % (in forward and backward fit) were included  
245 into the custom mass spectral library. Putative identifications of VOCs were based on a match  
246 of mass spectra (> 80 %) and a retention index of RI +/- 15.

### 247 *2.10 Statistical analysis*

248 Physiological and nutritional properties, were evaluated per sampling day using  
249 GraphPad Prism 5 software (GraphPad Software,USA) and SPSS statistics 21 (IBM  
250 Corporation, USA), using one-way analysis of variance (ANOVA). All data are presented as  
251 the mean of three biological replicates ± standard deviation (SD). Where data was missing this  
252 was taken into account in the statistical analysis.

253 For VOCs, data were analysed essentially as described in Spadafora et al. (2016) using  
254 R software (version 3.1.3; R core development team 2015) following area normalisation (peak

255 areas were normalised to the total area of the chromatogram) and square root transformation to  
256 reduce the weight of larger components. PerMANOVA (Permutational Multivariate Analysis  
257 of Variance) and CAP analysis (Canonical Analysis of Principal coordinates) statistical tests  
258 (Anderson and Willis, 2003) were performed using the ‘vegan’ package (Oksanen, et al. 2013)  
259 and the ‘BiodiversityR’ package (Kindt and Coe, 2005). This analysis uses the whole VOC  
260 profile as a single variable without making the assumption that each VOC can be treated as an  
261 independent variable. Ordination plots were generated for the storage days and temperature  
262 and a 95 % confidence interval was fitted. Weighted Correlation (Gene) Network Analysis  
263 (WC(G)NA), to display interactions between VOCs, physiological and biochemical parameters  
264 used the WCNA package in R (Langfelder & Horvath 2012) with a soft threshold power of 6,  
265 a deep-split of 3 and module size of 5. WGCNA identifies groups of characters whose change  
266 against the parameter chosen (in this case time or temperature) most closely correlate with each  
267 other and with that parameter (e.g. a negative correlation with increasing temperature). From  
268 within modules that were significantly correlated to that parameter in the WGCNA output,  
269 heatmaps were derived using R from the mean abundance of compounds of the same chemical  
270 class that showed significant correlation with the parameter (time or temperature),

### 271 **3. Results**

#### 272 *3.1 Respiration rate and gaseous atmosphere within the packaging are affected by temperature* 273 *of storage*

274 Respiration rate was significantly affected by all temperature treatments. Throughout  
275 storage, the production of CO<sub>2</sub> by samples stored at 0 °C was significantly lower and relatively  
276 constant when compared to samples stored at 5 °C and 10 °C which by day 9 reached 67.3 and  
277 193.3 mmol CO<sub>2</sub> kg<sup>-1</sup>h<sup>-1</sup>, respectively (Fig. 1a).

278 Packages stored at 10 °C showed a significantly faster accumulation of CO<sub>2</sub> compared to  
279 0 °C, over the entire storage time. Storage at 5 °C resulted in higher CO<sub>2</sub> compared to 0 °C after  
280 day 6 (Fig. 1b).

### 281 *3.1 Colour was most affected at low temperature and firmness at high temperature of storage*

282 Changes in surface colour occurred mainly in samples stored at 0 °C, where lightness  
283 (L\*) values increased from 62.6 to 68.9 by day 2 of storage, remained constant until day 6 and  
284 then decreased to 52.6 by day 9. At 5 and 10 °C L\* remained relatively unchanged throughout  
285 storage and lower than samples stored at 0 °C until day 9 (Fig. 1c; Supplementary Fig. 1).

286 Samples stored at 0 °C showed the highest values for firmness (Fig. 1d). At 10 °C a  
287 significant decrease in firmness was observed between days 2 and 6 while at 5 °C, this decrease  
288 was delayed to between days 6 and 9. By day 9, firmness values when stored at 10 °C were  
289 1.60 N, significantly lower than 2.27 and 2.34 N obtained at 5 and 0 °C, respectively.

### 290 *3.2 Microbial and fungal load increase was delayed at lower temperatures*

291 Although low counts of aerobic mesophilic bacteria and fungi were present on day 0  
292 there was an immediate increase in samples stored at 10 °C reaching 10<sup>4</sup> and 10<sup>6</sup> CFU g<sup>-1</sup> FW,  
293 by day 2 of storage, for microbial and fungal counts respectively (Fig. 2a). Significantly  
294 increased bacterial contamination at 5 °C was only observed by day 6 (10<sup>3</sup> CFU g<sup>-1</sup> FW) and at  
295 0 °C it was delayed until day 9 (10<sup>4</sup> CFU g<sup>-1</sup> FW). Fungal counts increased at a higher rate  
296 particularly at 10 °C, although at 0 °C they remained stable until day 9.

### 297 *3.3 Antioxidant activity and content of total phenolic compounds show similar trends over time 298 and temperature, while catechin concentration rose in some temperature storage regimes.*

299 Antioxidant activity and total phenolics in the melon cubes dropped significantly over  
300 the first 6 days of storage thereafter remaining relatively stable (Supplementary Fig. 2). No  
301 differences between temperatures were observed until day 6, when both were significantly  
302 lower in samples stored at 0 °C compared to 10 °C.

303 Catechin concentration rose steadily at all three temperatures between day 2 and day 9  
304 of storage with a continued increase at day 14 in samples stored at 5 °C (Fig 2c).

305 *3.4  $\beta$ -carotene fell between 6 and 9 days of storage and ascorbic acid content fell dramatically*  
306 *in all temperature regimes*

307 Initial total carotenoid content ( $36.6 \mu\text{g g}^{-1}$  FW) remained stable with no differences  
308 amongst treatments until day 2 of storage, and up to day 9 was better retained at 10 °C. (Fig.  
309 2b).

310  $\beta$ -carotene content increased in the first 2 days of storage at 5 and 10 °C, from 2.81 to  
311  $4.65$  and  $5.86 \mu\text{g g}^{-1}$  FW, respectively (Fig. 2d). At 0 °C  $\beta$ -carotene peaked at day 6 but from  
312 day 9 to 14 it fell at all three storage temperatures.

313 Vitamin C contents did not present significant changes amongst treatments until day 9  
314 (Fig. 2e), but fell significantly immediately after processing, and stayed low until day 6.

315 *3.5 Both time and temperature of storage are discriminated by the volatile organic compound*  
316 *profile*

317 Based on comparison to custom libraries derived from NIST11, a total of 82 compounds  
318 were identified in the aroma profile of the fresh-cut melon cubes throughout the storage period  
319 and across all three temperatures (Supplementary Table 1). Esters were the largest group and  
320 the most abundant in the VOC profile (55 VOCs). They were split into acetate esters (27), and  
321 non-acetate esters (28). Other VOCs included sulphur compounds (5), alcohols (3), aldehydes  
322 (3), terpenes (2), alkanes (2), organic acids (2), ketones (2), nitrile compounds (1), anhydrides  
323 (1), aromatic compounds (1), and trienes (1). Four compounds could not be identified.

324 The relatively most abundant three VOCs across all samples were an acetate ester: ethyl  
325 acetate and two non-acetate esters: ethyl butanoate and ethyl 2-methyl-butanoate  
326 (Supplementary Table 2; Supplementary Fig. 3). Ethyl acetate was by far the most abundant  
327 VOC with a mean abundance over all the samples that was 1.76 times the abundance of the

328 next most abundant VOC (ethyl butanoate). In no sample was any one VOC >28.6 % of the  
329 total VOC signal.

330 The number of VOCs fell significantly over the time of storage ( $P < 0.001$ ) from a mean  
331 of 69 in fresh-cut to 44 after 14 days, and the ratio of all non-acetate to all acetate esters rose  
332 significantly ( $P < 0.05$ ) over the storage period at 0 °C (from 0.54 to 1.0). However, at 5 °C  
333 and 10 °C there was no significant change in ester ratio over time (Fig. 2f).

334 The overall pattern of abundance of VOC profiles (abundance of each VOC as a  
335 proportion of the total profile abundance) differed significantly amongst days of storage  
336 (PerMANOVA,  $P < 0.001$ ,  $R^2 = 0.492$ ), temperature of storage (PerMANOVA,  $P < 0.001$ ,  $R^2$   
337 = 0.136) and showed a significant interaction between days and temperature of storage  
338 (PerMANOVA,  $P < 0.005$ ,  $R^2 = 0.143$ ) (Fig. 3). Overall the PerMANOVA analysis accounted  
339 for 77.1 % of the variation of the data set. Linear discrimination plots produced from CAP  
340 separated days of storage with a percentage of correct classification of 100 % ( $P < 0.001$ ) (Fig.  
341 3a). Fresh-cut was clearly separated from all the storage days, and each day was clearly  
342 separated from all other time-points on the basis of its VOC profile using a 95% confidence  
343 interval. The CAP also separated temperature of storage with 83.3 % ( $P < 0.001$ ) correct  
344 classification separating fresh-cut from all the stored samples, and clearly separating melon  
345 cubes stored at 10 °C from those stored at lower temperatures, but the 0 °C and 5 °C stored  
346 melon were not discriminated. (Fig. 3b).

347 CAP on storage time and temperature combined into a single category (10 samples)  
348 resulted in correct classification of 86.7 % ( $P < 0.001$ ) (Fig. 3c). Fresh-cut was clearly separated  
349 from all other samples; at day 2 VOCs from samples stored at 5 °C were discriminated from  
350 the other two temperatures. At day 6 samples stored at 10 °C were well-separated from all the  
351 other later time points, although the samples stored at the two lower temperatures were  
352 discriminated from each other and from samples stored for 14 days. By day 14, samples stored

353 at 5 and 10 °C were not discriminated from each other by the VOC profiles, however the  
354 sample held at 0 °C was clearly discriminated. (Fig. 3c).

### 355 *3.6 Correlation analysis of VOC profiles with physiological and biochemical parameters*

356 WCNA was used to correlate changes in the patterns of VOC profiles with changes in  
357 the physiology and nutritional content of the melon cubes over the first six days of storage at  
358 the different storage temperatures. The analysis clustered the parameters analysed into nine  
359 modules (Fig. 4a; Supplementary Table 3). Four modules (blue, brown, pink and turquoise)  
360 showed statistically significant negative correlation with both temperature and day of storage  
361 while three modules (black, green and red) showed negative correlation only with day of  
362 storage and one module was positively correlated only with temperature (yellow). The highest  
363 negative correlation was with day of storage (brown module,  $R^2 = -0.85$ ) and positive (yellow  
364 module,  $R^2 = 0.64$ ) with temperature. All of the 45 VOCs whose change correlated significantly  
365 with time were negatively correlated with this parameter. Furthermore, of the 28 VOCs that  
366 correlated with change in temperature, only four (ethyl (2Z)-2-butenate, (3E)-3-hexen-1-yl  
367 acetate, butyl acetate and isobutyl acetate) were positively correlated with an increase in  
368 temperature, as was respiration rate. Thus overall, the WGCNA revealed a predominantly  
369 negative correlation between VOCs and both increasing days and temperature.

370 Two heat maps were created using individual VOCs (grouped into chemical families)  
371 physiological and phytochemical characters from these modules that correlated significantly  
372 with day of storage (Fig. 4b; Supplementary Table 4) or temperature (Fig. 4c; Supplementary  
373 Table 4). VOCs that were significantly negatively correlated with time of storage, were  
374 dominated by non-acetate esters (19) followed by acetate esters (11). Vitamin C, total phenolics  
375 and total carotenoids were also negatively correlated with days of storage. There was a close  
376 correlation between the fall in abundance vitamin C and a nitrile compound: 3-  
377 methylbutanenitrile (Fig 4b). The decrease of non-acetate esters and two terpenes (limonene



378 and eucalyptol) correlated with the fall in total antioxidants over time. The fall in total phenolics  
379 and carotenoids over time correlated (though less tightly) with the reduction in terpenes, non-  
380 acetate esters and 3-methylbutanenitrile.

381 VOCs that were negatively correlated with temperature of storage were also highly  
382 dominated by non-acetate esters (16) with only two acetate esters in this category. Change in  
383 temperature of storage correlated with changes in VOC profiles, physiological parameters and  
384 phytochemical content (Fig. 4c). Vitamin C content correlated negatively with rising  
385 temperature of storage and showed a close correlation with the change in terpenes, represented  
386 by limonene. The fall in  $\beta$ -carotene was also closely correlated with the fall in limonene. In  
387 contrast the rise in respiration rate with temperature correlated most closely with the rise in  
388 three acetate esters: (3E)-3-hexen-1-yl acetate, butyl acetate and isobutyl acetate.

389

#### 390 **4. Discussion**

391 Effects of changing the temperature by 5 °C increments supports previous studies  
392 indicating that respiration rate, firmness, and colour are all adversely affected by an increase  
393 in storage temperature between 0 and 5 °C (Aguayo et al., 2004). The big change in respiration  
394 rate from 0 - 10 °C is also in agreement with previous studies (Watada et al., 1999). Colour  
395 changes have been reported during storage of fresh-cut melon (Amaro et al., 2012). In this  
396 study, L\* values were highest between days 2 and 6, when storage was performed at 0 °C.  
397 Changes in L\* value can be an indicator of water soaking (Bai et al., 2001) a disorder often  
398 observed in fresh-cut cantaloupe melon, however, no evidence of water soaking was visually  
399 detected in this study. At the higher storage temperature (10 °C), the firmness decrease  
400 occurred earlier and was more noticeable as also previously noted (Aguayo et al., 2004).

401 There was a clear temperature-dependent shift in the timing of increase of microbial  
402 populations seen in this study. The very steep rise in microbial load at 10 °C, also probably

403 contributes to the peak in respiration rate seen at 9 days of storage. In agreement with  
404 Lamikanra et al., (2000) microbial growth in melon stored at 10 °C started to increase  
405 exponentially within 2 days of storage, while at 5 °C this was delayed until day 9. The maximal  
406 levels of microbial load reached in this study within the 14 day period (approx.  $10^7$  CFU/g) are  
407 also in line with previous reports (Ayhan and Chism, 1998). Microbial counts under  $10^6$  CFU  
408  $g^{-1}$  are considered acceptable for fresh-cut produce (Gilbert et al., 2000). These levels of  
409 contamination were reached by day 6 in samples stored at 10 °C, and by day 9 in samples stored  
410 at 5 °C, while samples stored at 0 °C never reached this contamination limit, indicating that this  
411 very low storage temperature is optimal for a shelf-life assessment based on only on microbial  
412 load and not considering also quality factors.

413 The content of total antioxidants, phenolics, and carotenoids, all showed a similar pattern  
414 of change with a decrease until day 6-9 and then a rise. This late rise may be due to further  
415 stress responses elicited by the long storage time or a release of more metabolites from the cells  
416 as they degrade increasing extraction efficiency. Total antioxidant level was not greatly  
417 affected by temperature, whereas total phenolics and total carotenoids were reduced at lower  
418 temperatures in the first 6-9 days compared to the melon stored at 10 °C. Thus, lower storage  
419 temperatures (0-5 °C) may in fact be detrimental to phenolic compound retention. Moreover,  
420 the pattern for individual phenolics and carotenoids, such as catechin and  $\beta$ -carotene, is  
421 complex. The rise in catechin content at 5 and 10 °C, between day 2 and 14, may be a response  
422 to wounding damage that stimulates secondary metabolite production (Brecht, 1995). The fall  
423 in  $\beta$ -carotene after day 6 may be attributed to carotenoid degradation resulting from exposure  
424 to oxygen, and cellular disruption caused by wounding that exposes the carotenoids to  
425 lipoxygenase action (Britton and Khachik, 2009). The rapid reduction in ascorbic acid within  
426 2 days of storage is likely due to the relatively low acidity of melon flesh, since an acid  
427 environment is required for maintaining ascorbic acid stability (Kalt, 2005). Its rise at day 9

428 may be due to an increased softening of the tissue making its extraction more efficient rather  
429 than an actual increase in concentration within the tissue.

430 A similar number of VOCs was detected compared to other studies (e.g. Allwood et al.,  
431 2014) but unlike some studies where the most abundant three VOCs made up over 60% of the  
432 total signal (Bauchot et al., 1998) in this study the maximum content of the three most abundant  
433 VOCs was only 46% of the total signal. This may be due to the properties of the method of  
434 collection used which reduces saturation by single compounds. As found in other studies (e.g.  
435 Beaulieu, 2006b) the majority of the VOCs were represented by esters (68%), in this study  
436 equally divided between acetate and non-acetate esters when considered across all samples  
437 together. Of the 24 VOCs identified as characteristic impact flavor or aroma compounds  
438 (CIFACs; Beaulieu, 2006b) twelve were also found in this study, and many of the other 70  
439 VOCs identified in this study were also found in previous studies (e.g. Wang et al., 1996, from  
440 *C. melo* var. *reticulatus* cv. Makdimon; Aubert and Bourger, 2004, from a mixture of cultivars  
441 of *C. melo* var. *cantalupensis*). More recently Allwood et al. (2014) also found the most  
442 abundant esters in a range of cultivars of *C. melo* to include ethyl butanoate, propyl acetate,  
443 butyl acetate, and 2-methylbutyl acetate which are amongst the top 10 most abundant VOCs in  
444 this study. Two of the three most abundant VOCs, ethyl butanoate and ethyl 2-methyl-  
445 butanoate are also noted as amongst the most potent odorants (Bauchot et al., 1998).

446 In previous studies a high degree of wounding (thin slicing) was associated with a rapid  
447 loss of esters within the first day of storage at 4 °C (Lamikanra and Richard, 2002) while this  
448 was not found when the melon was cut into thicker slices (Beaulieu, 2006b). In this study the  
449 slicing was most similar to that of Beaulieu (2006b) and again there was no dramatic loss of  
450 esters supporting the hypothesis that the loss is due to excessive wounding. Using melon  
451 wedges from  $\frac{3}{4}$  slip fruit (as used in this study) held at 4 °C for 14 days Beaulieu (2006b) found  
452 the ratio of non-acetate to acetate esters changed over time with a steady increase over the first

453 12 days of storage from around 0.5 to 2.7; thereafter the ratio remained fell back to 2.5. In this  
454 study the change in this ratio at 0 °C and 5 °C shows a similar trend: although the change was  
455 not as great by 14 days, there was a similar pattern of acceleration of change at later time points.  
456 However, at 10 °C there was much less change in the ratio. This suggests that at this  
457 temperature the negative effects of storage on aroma may be less pronounced. Beaulieu (2006b)  
458 hypothesised that the relative increase in non-acetate esters during storage is due to a limitation  
459 in the supply of acetyl-CoA or a preferential hydrolysis of acetate esters by esterases due to  
460 differences in steric hindrance. Differences seen in this study amongst temperatures of storage  
461 might reflect differential activity of esterases at different temperatures or perhaps a greater  
462 availability of acetyl-CoA though the higher metabolic rate seen at 10 °C.

463 The separation of temperatures of storage seen using the whole VOC profile is consistent  
464 with this marked difference between storage at the lower two temperatures compared to 10 °C.  
465 Although at later time-points individual sample separation becomes less clear-cut. This  
466 indicates that the whole VOC profile could be used reliably at earlier stages of storage to detect  
467 breaches in the cold chain. At later stages the rise in microbial load may also be contributing  
468 to the VOC profiles, especially at the higher two temperatures.

469 Of particular interest is the correlation between changes in specific classes of VOCs and  
470 nutritionally-relevant metabolites over the first half of the storage period where visible changes  
471 may be less evident. The nitrile VOC, 3-methylbutanenitrile whose loss correlates with loss of  
472 vitamin C over time was previously identified in a medium shelf-life Charentais cantaloupe  
473 melon cultivar (*C. melo* L. var. *cantalupensis*, cv. Match; Lignou et al., 2014), significantly  
474 associated with stage of fruit maturity. It was also associated with fruit ripening in tomato  
475 (Wang et al., 2016). This VOC, along with other short-branched-chain amino acid-related  
476 VOCs was one of the few VOCs detected in whole tomato fruit (Rambla et al., 2015), and its  
477 level did not increase with fruit homogenization. Detection of 3-methylbutanenitrile was also

478 reported to be better via TD than SPME (Rambla et al. 2015). Hence use of this marker to  
479 detect loss of vitamin C in intact melons may be possible and best assessed using TD. Loss of  
480 total antioxidants over time could also be assessed through the close correlation to loss of non-  
481 acetate esters, and the terpenes limonene and eucalyptol.

482 Vitamin C and  $\beta$ -carotene levels also fell with increasing temperature and were closely  
483 correlated with the fall of a single terpene: limonene. In citrus juice, a fall in limonene also  
484 correlates with a fall in Vitamin C when the juice is subjected to heat treatments (Pérez et al.,  
485 2005), thus indicating that limonene may provide a potential marker for assessing breaches in  
486 the cold chain that may have affected vitamin C content.

487

## 488 **5. Conclusions**

489 Overall the choice of storage temperature needs to strike a compromise: 0 °C is best for  
490 reducing microbial load and preserving vitamin C but 10 °C is better for preservation of  
491 phenolics and flavour-related VOCs. TD may be preferable to SPME for an accurate analysis  
492 of the proportions of VOC components and the whole VOC profile provides a good indicator  
493 for day and temperature of storage. Two useful VOC markers are identified for changes in  
494 vitamin C: 3-methylbutane nitrile in relation to storage time and limonene for cold chain  
495 breaches.

496

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## 502 **Conflict of interest**

503 The authors declare no conflict of interest.

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#### 508 **Supplementary Data**

509 **Supplementary Table 1.** List of VOCs detected across all samples and codes for physiological  
510 and biochemical data

511 **Supplementary Table 2.** Abundance of VOCs across all samples

512 **Supplementary Table 3.** Output from WGCNA

513 **Supplementary Table 4.** Characters significantly correlated with day or temperature

514 **Supplementary Fig. 1.** Changes in colour attributes through storage of melon cubes for 14  
515 days at three temperatures: 0, 5 and 10 °C: (a) Hue, (b) C and (c) a\*

516 **Supplementary Figure 2** - Changes in (a) total antioxidants (b) total phenolics,

517 **Supplementary Figure 3** Mean VOC abundance (+/- SD) across all samples (data from Supp.  
518 Table 2)

519

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635

## 636 **Figure Legends**

637 **Figure 1.** Physiological responses: (a) respiration rate, (b) CO<sub>2</sub>, (c) colour (L\*) and (d) firmness  
638 in melon cubes stored at 0 °C, ( —●— ), 5 °C ( —■— ) and 10 °C ( —▲— ) during 14 days of storage  
639 (mean ± S.E.; n= 3). Lowercase letters above symbols indicate statistically different values  
640 between the different temperatures at each time point, upper case letters (H= 10, M = 5 and L  
641 = 0 °C) indicate significant differences between each time point or to the fresh cut control for  
642 each temperature using ANOVA and Tukey’s range test (P < 0.05). Only significant differences  
643 are shown at each time point

644 **Figure 2.** Microbial growth and metabolite content: changes in (a) bacterial and fungal  
645 Growth, (b) total carotenoids, (c) catechin, (d) β-carotene, (e) vitamin C(f) ratio of non-acetate:  
646 acetate esters; n=3, in melon cubes stored at 0 °C  
647 ( —●— ), 5 °C ( —■— ) and 10 °C ( —▲— ) during 14 days of storage (mean ± S.E.). Lowercase letters  
648 indicate statistically different values between the different temperatures at each time point,  
649 upper case letters (H= 10, M = 5 and L = 0 °C) indicate significant differences between each  
650 time point or the fresh cut control, using ANOVA and Tukey’s range test (P < 0.05). Only  
651 significant differences are shown at each time point. For microbial growth, solid line and lower  
652 case letters are used for fungal growth; upper case letters and broken line for bacterial growth.

653 **Figure 3.** Canonical Analysis of Principal coordinates based on all VOCs from melon using  
654 TD-GC-TOF-MS: A CAP model was produced for melon samples stored (a) for 0, 2, 6, and  
655 14 days, (b) at 0, 5 or 10°C and (c) combined time (0, 2, 6, and 14 d) and temperature (0, 5 or  
656 10°C) into a single sample category. The plots use the first two linear discriminants (LD); each  
657 ellipse represents the 95% confidence interval. Percentage of correct classifications was 100%  
658 ( $P < 0.001$ ,  $n = 9$ ) for days of storage (a), 83.3% ( $P < 0.001$ ,  $n = 9$ ) for temperature of storage  
659 (b) and 86.7% ( $P < 0.001$ ,  $n = 3$ ) for combined days of storage and temperature.

660 **Figure 4.** Multi-trait correlation analysis of physiological indicators, nutritionally relevant  
661 phytochemicals and VOCs in melon stored at three different temperatures (0, 5 °C and 10 °C)  
662 over a 14 day storage period. (a) WCNA modules: the score and significance ( $P$  values in  
663 brackets) are according to a Pearson analysis, (b) and (c) heat maps of multi-trait correlation  
664 analysis based on (b) days of storage and (c) temperature nutritionally relevant phytochemicals  
665 ( and VOCs. Blue indicates a low content, green intermediate and red a high content  
666 for each character.