

## Accepted Manuscript

Home conservation strategies for tomato (*Solanum lycopersicum*): storage temperature vs. duration – is there a compromise for better aroma preservation?

Catherine M.G.C. Renard, Christian Ginies, Barbara Gouble, Sylvie Bureau, Mathilde Causse

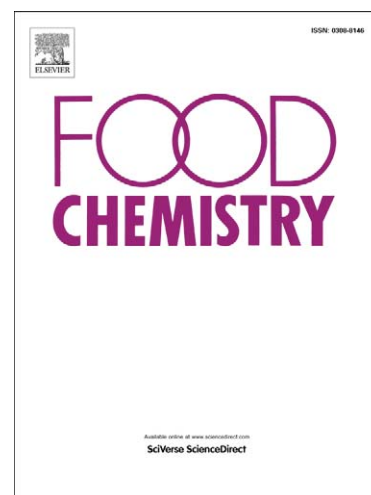
PII: S0308-8146(13)00062-9  
DOI: <http://dx.doi.org/10.1016/j.foodchem.2013.01.038>  
Reference: FOCH 13585

To appear in: *Food Chemistry*

Received Date: 17 August 2012  
Revised Date: 19 November 2012  
Accepted Date: 14 January 2013

Please cite this article as: Renard, C.M.G., Ginies, C., Gouble, B., Bureau, S., Causse, M., Home conservation strategies for tomato (*Solanum lycopersicum*): storage temperature vs. duration – is there a compromise for better aroma preservation?, *Food Chemistry* (2013), doi: <http://dx.doi.org/10.1016/j.foodchem.2013.01.038>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



1 **Home conservation strategies for tomato (*Solanum***  
2 ***lycopersicum*): storage temperature vs. duration – is there a**  
3 **compromise for better aroma preservation?**

4

5 Catherine M.G.C. Renard<sup>1\*</sup>, Christian Ginies<sup>1</sup>, Barbara Gouble<sup>1</sup>, Sylvie Bureau<sup>1</sup> & Mathilde  
6 Causse<sup>2</sup>

7

8 1 INRA, UMR408 Sécurité et Qualité des Produits d'Origine Végétale, F-84000 Avignon,  
9 France

10 1 Université d'Avignon et des Pays de Vaucluse, UMR408 Sécurité et Qualité des Produits  
11 d'Origine Végétale, F-84000 Avignon, France

12 2 INRA, Unité de Génétique et d'Amélioration des Fruits et Légumes, F-84143 Montfavet,  
13 France

14 **ABSTRACT**

15

16 Expression of dissatisfaction with tomato aroma prompted us to lead this study on the impact  
17 of domestic storage conditions on volatile compounds.

18 Two storage modalities (20 °C and 4 °C) and two cultivars (Levovil and LCx) were used.

19 Volatile compounds were analyzed by gas chromatography–mass spectrometry detection after  
20 accelerated solvent extraction. Physical characteristics, lipoxygenase activity, hydroperoxide  
21 lyase activity; linoleic acid and linolenic acid were monitored.

22 Storing tomatoes at 4 °C induced a drastic loss in volatiles, whatever their biosynthetic origin.

23 After 30 days at 4 °C, the concentration of volatiles had decreased by 66 %. Reconditioning

24 for 24 h at 20 °C was able to recover some aroma production after up to 6 days storage at 4

25 °C. Volatile degradation products arising from carotenoids and amino acids increased when

26 tomatoes were kept at 20 °C, while lipid degradation products did not vary.

27 Storing tomatoes at fridge temperature, even for short durations, was detrimental for their

28 aroma. This should be taken into account to formulate practical advice for consumers.

29

30 **Keywords**

31 Storage, volatiles, lipoxygenase, GC-MS, accelerated solvent extraction

32

## 33 1. Introduction

34 In recent years, consumption of fresh tomatoes by French consumers has stagnated, and  
35 market research points to dissatisfaction with the sensory quality of fresh tomatoes on sale.  
36 This motivated the Agence Nationale de la Recherche [French national research agency]  
37 project “QualitomFil”, which aims to find ways to maintain a high quality supply chain from  
38 farm to fork. This study was led under the QualitomFil program. Our hypothesis is that  
39 inadequate storage conditions at the consumer’s home contributes to the perceived flavour  
40 loss. Little is known about the fate of tomatoes at the last link of this chain — the consumer.  
41 The consumer has two main possibilities for tomato storage: at room temperature or in the  
42 refrigerator at around 4 °C. The general physico-chemical characteristics of ripe (light red or  
43 red) tomato, such as soluble solids and acidity, are little modified during home storage  
44 (Auerswald, Peters, Brückner, Krumbein, & Kuchenbuch, 1999; de Leon-Sanchez, Pelayo-  
45 Zaldivar, Rivera-Cabrera, Ponce-Valadez, Avila-Alejandre, Fernandez, et al., 2009; Maul,  
46 Sargent, Sims, Baldwin, Balaban, & Huber, 2000). However, this is not the case with volatile  
47 compounds, and one of the key sensory parameters for tomato frequently mentioned in  
48 consumer complaints is loss of its characteristic aroma. This motivated our investigation of  
49 the fate of tomato volatiles during storage in domestic conditions.  
50  
51 Data, mostly on unripe tomatoes, indicates that low temperature affects volatiles production.  
52 As chilling injury is a well-known risk for this fruit, temperatures > 10 °C are recommended  
53 and relatively few studies can be found dealing with the impact of low temperatures on taste  
54 and flavour volatiles. Stern , Buttery, Teranishi, Ling, Scott & Cantwell (1994) stored  
55 tomatoes harvested mature green, breaker (defined as the maturity stage when the red colour  
56 starts becoming visible at the tip of the tomato) to red-ripe at temperatures from 5 °C to 20

57 °C. Red-ripe tomato held at 5 °C had significantly less volatiles than when stored at 20, 15 or  
58 10 °C for the same duration (6 days). Stern et al. (1994) further verified lack of volatile  
59 development when unripe tomatoes were stored at chilling conditions, and highlighted that  
60 final ripening temperature was a paramount determinant of ultimate odour intensity. Maul et  
61 al. (2000) reported that light-red tomatoes stored at 5 °C are rated by a trained panel as  
62 significantly lower for ripe aroma, sweetness and tomato flavour and significantly higher in  
63 sourness than when stored at 20 °C for the same duration. The concentrations of sugars were  
64 actually higher at 5 °C than at 20 °C while pH and titratable acidity were not statistically  
65 different. The authors also reported significant losses in hexanal, 2+3-methylbutanol, (E)-2-  
66 heptenal, and 2-isobutylthiazole for all fruits stored below 20 °C.

67 De Leon-Sanchez et al. (2009) recently compared sensory analysis, volatiles and alcohol  
68 dehydrogenase activity in tomatoes harvested at the light red stage and stored either at 10 °C  
69 or 20 °C. The first modifications detected at low temperature were a decrease in hexanol and  
70 an increase in 3-methylbutanal, linked to a modification of the aldehyde/alcohol balance,  
71 which the authors explained by a decrease in alcohol dehydrogenase activity during low-  
72 temperature storage. In the sensory evaluation, little difference was reported for storage at 20  
73 °C (up to 14 days), while at 10 °C the assessors detected an increase in “solvent-humidity”  
74 and a decrease in “lemon tea” descriptors.

75

76 Tomato aroma during eating has two main sources: the pre-existing volatiles, and a series of  
77 major volatile compounds known as lipoxygenase-derived products (or LOX products)  
78 (Baysal & Demirdoven, 2007; Riley & Thompson, 1998; Robinson, Wu, Domoney, & Casey,  
79 1995). These volatile compounds result from the oxidative degradation of linoleic and  
80 linolenic acids that occurs when the internal structure of the cells is broken down. This loss of  
81 cellular structure can be provoked by mastication or grinding (Riley & Thompson, 1998; Xu

82 & Barringer, 2010). As a result, the chloroplastic enzymes (lipoxygenase (LOX) and  
83 hydroperoxidelyase (HPL)) come into contact with their cytosolic substrate. LOX products  
84 are therefore produced during mastication (Linforth, Savary, Pattenden, & Taylor, 1994).

85

86 The aim was to investigate the impact of storage in domestic conditions (20 °C and 4 °C) on  
87 tomato volatiles, and to assess means to obviate aroma loss. Volatiles from two varieties of  
88 tomatoes known to differ in intensity of flavour were analyzed at the two storage  
89 temperatures. This study also determined the time-course evolution of potential drivers of  
90 generation “in-mouth” of LOX products, i.e. LOX and hydroperoxide lyase activities and  
91 concentration of linoleic and linolenic fatty acids substrates.

92

## 93 2. Material and methods

94

### 95 2.1 Plant material

96 Plants were grown in a heated glasshouse in Avignon (southeast France) during spring 2008.

97 Fruits were harvested at the red-ripe stage on Levovil, characterized by its large fruits and

98 pharmaceutical sensory attributes, and LCx, an introgressed line carrying five chromosome

99 regions from the Cervil cherry tomato identified as bearing the quantitative trait loci for

100 quality traits, notably tomato aroma intensity (Chaib, Lecomte, Buret, & Causse, 2006) in the

101 Levovil background. In order to eliminate bias linked to maturation patterns, both tomatoes

102 were harvested at the red-ripe stage. To ensure homogeneous maturity and avoid the presence

103 of overripe tomatoes, plants were first unloaded of all their red fruits and 3 days later the

104 fruits which had ripened from orange to red were harvested. Each line was represented by 18

105 plants.

106

### 107 2.2 Chemicals

108 All standard volatile compounds, linolenic acid, yeast alcohol dehydrogenase, soybean type I-

109 B lipoxidase and NADH were purchased from Sigma Aldrich (St Louis, MO). Solvents were

110 from Carlo Erba Reagents (Val de Reuil, France). Hydromatrix<sup>TM</sup> (diatomaceous earth) was

111 from Agilent Technologies (Les Ulis, France). BF<sub>3</sub>/Methanol was purchased from VWR

112 international (Fontenay-sous-Bois, France).

113

### 114 2.3 Tomato storage

115 At harvest, tomatoes were divided into 12 modalities: fresh; storage at 20 °C for 1, 3 or 6

116 days; storage at 4 °C for 1, 6, 15 and 30 days; same, followed by 24 h reconditioning at 20 °C.

117 Each modality comprised 3 replicates of 5 fruits. All storage was carried out in temperature-  
118 controlled, air-circulated chambers. To eliminate effects of temperature on enzyme activity  
119 during the aroma extraction procedure, tomatoes stored at 4 °C were brought back to 20 °C  
120 prior to analysis by incubation (30 min) in water at 20 °C.

121 Tomatoes were characterized at harvest and after storage for weight, firmness and colour.  
122 Fruit colour was measured in the CIE L\*C\*h\* (lightness, chroma and hue) colour space using  
123 a CM-1000R-series Minolta chromameter (Minolta, Ramsey, NJ).

124 Fruit texture was measured with a multi-purpose texturometer (Texture analyzer TAplus:  
125 Ametek, Lloyd Instruments Ltd., Fareham, UK). This apparatus registered force/deformation  
126 curves by measuring the reaction force in response to an increasing mechanical constraint  
127 applied to the fruit by a 5 cm flat disc and a 250 N load cell. Probe speed was 20 mm.min<sup>-1</sup>.  
128 Fruit firmness (F) was the force necessary to obtain a deformation corresponding to 3 % of  
129 the fruit diameter.

130

#### 131 *2.4 Accelerated solvent extraction (ASE)*

132 Five tomatoes were ground for 1 min in a blender (Waring-Nova, Grosseron, St Herblain,  
133 France), and approximately 15 g of juice was transferred to a beaker. After 2 minutes, 15 g of  
134 Hydromatrix™ and 16 µg of 4-nonanol (internal standard) were mixed with the tomato juice  
135 to obtain a homogeneous powder while inactivating enzymes. The powder was rapidly  
136 transferred to a 33 ml pressurized extraction cell for immediate extraction. The extractor was  
137 an ASE 200 system (Dionex, Sunnyvale, CA). Extraction conditions were: solvent  
138 dichloromethane, 100 bar, 40 °C, 5 min preheating then 5 min static incubation. The extract  
139 was concentrated to 1 ml by distillation under vacuum (300 Pa, using a Multivapor R12,  
140 Buchi, Rungis, France) prior to gas chromatography. This step showed no significant loss in



141 volatile compounds compared to distillation at ambient pressure (Trad, Ginies, Gaaliche,  
142 Renard, & Mars, 2012).

143

#### 144 2.5 GC-MS

145 Volatile samples (2  $\mu$ l) were injected into a GC-MS system (QP2010; Shimadzu, Kyoto,  
146 Japan) equipped with a UB Wax capillary column [30 m, 0.32 mm i.d., 0.5  $\mu$ m film thickness]  
147 (Interchim, Montluçon, France) as described in Birtic, Ginies, Causse, Page & Renard (2009).

148 A total of 44 volatile compounds were detected (Table 1).

149

150 Detection of LOX volatile oxidation products used a CPSIL 5 CB capillary column [30 m,  
151 0.25 mm i.d., 0.5  $\mu$ m film thickness] (Interchim, Montluçon, France). The injection port was  
152 used in split mode (1/15), and carrier gas (He) velocity was kept at a constant 35  $\text{cm}\cdot\text{s}^{-1}$ . The  
153 initial oven temperature of 40  $^{\circ}\text{C}$  was held for 2 min then ramped up at 3  $^{\circ}\text{C}$  per min to 60  $^{\circ}\text{C}$   
154 then at 10  $^{\circ}\text{C}$  per min to 230  $^{\circ}\text{C}$ . This final temperature was held for 10 min. The mass  
155 spectrometer was operated in electron ionization mode at 70 eV. Single-ion monitoring (SIM)  
156 was used for quantification: ions 55, 44, 41 and 83 were used to monitor 1-penten-3-one,  
157 hexanal, (E)-2-hexenal and (Z)-3-hexenal, respectively.

158

#### 159 2.6 Fatty acids contents

160 Fatty acids were analyzed by GC-MS after transesterification to fatty acid methyl esters  
161 (FAME). Total lipid extract was based on Schäfer (1998), with modifications. 500 mg of  
162 freeze-dried tomato (without seed) was homogenized with 10 g of Fontainebleau sand and  
163 transferred into a 11 ml pressurized extraction cell, and then 0.25 mg of nonadecanoic acid  
164 (internal standard) was introduced. The extractor was an ASE 200 system (Dionex,

165 Sunnyvale, CA), with  $\text{CHCl}_3$ /Methanol (2/1, v/v) as solvent, 100 bars, 120 °C, 5 min  
166 preheating then 5 min static incubation, flushed 50 % and one cycle.  
167 A 1 ml aliquot of extract was transesterified by adding 1 ml of  $\text{BF}_3$ /methanol (10 %) then  
168 incubated at 85 °C for 1 h. After cooling, 2 ml hexane and 2 ml of  $\text{HCO}_3^-$  0.2 mol/L were  
169 added. The tube was vortexed and the organic phase was dried.  
170 1  $\mu\text{L}$  of FAME was injected on the UBWAX capillary column [30 m, 0.25 mm i.d., 0.5  $\mu\text{m}$   
171 film thickness] (Interchim, Montluçon, France). The injection port was operated in splitless  
172 mode for the first 30 s, after which the carrier gas (He) velocity was kept a constant 35  $\text{cm}\cdot\text{s}^{-1}$ .  
173 The initial oven temperature of 50 °C was held for 1 min and then ramped up at 20 °C per min  
174 to 200 °C then at 3 °C per min to 230 °C. This final temperature was held for 15 min. The  
175 mass spectrometer was operated in electron ionization mode at 70 eV with continuous scans  
176 (every 0.5 s) from mass-to-charge ratio (m/z) 50 to 360.  
177 Fatty acid levels were expressed in milligrams of methyl nonadecanoate equivalent.

178

179 *2.7 Enzyme activities*

180 Enzyme extraction was based on Riley, Willemot, & Thompson (1996) and Rodrigo, Jolie,  
181 Van Loey, & Hendrickx (2007). Proteins were extracted by grinding 50 g of tomato pericarp  
182 with 50 g of cold 0.2 mol/L sodium phosphate buffer pH 6.5 containing 2 ml/L of Triton X-  
183 100, followed by centrifugation at 10000 g for 10 min at 4 °C. Protein concentration was  
184 measured by the Bradford method (Bradford, 1976).

185

186 LOX activity was measured by increase in absorbance at 234 nm corresponding to the  
187 generation of conjugated double bonds in linoleic acid (Surrey, 1964). The supernatant (40  
188  $\mu\text{L}$ ) was added to 2 ml of phosphate buffer pH 6 and 40  $\mu\text{l}$  of a linoleic acid solution  
189 (dissolved at 6  $\mu\text{g}/\text{ml}$  in NaOH 24 mmol/L plus Tween 20 10 mg/l). Absorbance at 234 nm

190 was monitored for 5 min. Activity was expressed as absorbance per min per ml of tomato  
191 extract.

192 HPL activity was measured using the NADH-coupled enzyme assay of Vick (1991) as the  
193 decrease in absorbance at 340 nm on oxidation of NaDH, a co-factor for alcohol  
194 dehydrogenase. The hydroperoxylinolenic acid substrate for HPL was prepared according to  
195 Vick (1991). The assay mixture contained 200  $\mu$ l of supernatant, 500  $\mu$ L of substrate, 150  $\mu$ L  
196 of 150 units yeast alcohol dehydrogenase solution, and 50  $\mu$ l of 10 mmol/L NADH. A blank  
197 was performed with the assay mixture to check NADH stability during analysis.

198

### 199 *2.8 Statistical analysis*

200 Results are presented as mean values, and the reproducibility of the results is expressed as a  
201 pooled standard deviation. Pooled standard deviations were calculated for each series of  
202 replicates using the sum of individual variances weighted by the individual degrees of  
203 freedom (Box, Hunter, & Hunter, 1978). PCA was carried out using ExcelStat (Microsoft,  
204 Redmond, WA).

## 205 **3. Results**

### 206 *3.1 Tomato characteristics*

207 The two tomato lines presented the expected general characteristics. In order to eliminate bias  
208 linked to maturation patterns, both tomatoes were harvested at the red-ripe stage. Levovil  
209 were much bigger than LCx (Table 2) and were also less firm. Firmness decreased during  
210 storage for both varieties. For LCx, the decrease was delayed by storage at 4 °C, while for  
211 Levovil temperature had no influence on loss of texture (Fig 1A and B). Both were picked  
212 fully red-ripe as visible from their high saturation values and hue angles close to 45 °. No  
213 significant variations during storage were found for fruit weight, luminance or saturation  
214 (Table 2). Hue angle decreased significantly during storage at 20 °C (but not at 4 °C) for LCx  
215 (Fig. 1C) whereas it increased significantly for storage at 4 °C (but not 20 °C) for Levovil  
216 (Fig. 1D). The time-course evolution of physical characteristics during storage was conform  
217 to the expected behavior of tomatoes, with loss of texture and an increase in red colour  
218 (lycopene production) at 20 °C.

219

220

### 221 *3.2 Volatiles in the fresh tomatoes*

222 Total volatiles concentration was much higher overall in LCx than in Levovil (Fig. 2, Tables  
223 3-5). Eugenol and 2-methoxyphenol were detected only in Levovil, and methyl salicylate was  
224 >50 times higher in Levovil than in LCx (Table 3). The difference in concentrations was also  
225 particularly clear for volatiles originating from phenylalanine and from sulfur-containing  
226 amino acids (Table 4). Benzylalcohol concentration was 22-fold higher, benzaldehyde 7-fold  
227 higher and 2-phenylethanol about 4-fold higher in LCx than in Levovil. All products  
228 identified as originating from sulfur-containing amino acids were 4-7-fold more concentrated

229 in LCx than in Levovil. For products originating from leucine/isoleucine, 3-methyl-1-butanol  
230 was 4-fold higher in LCx whereas 2-isobutylthiazole was slightly higher in Levovil. Furaneol  
231 (from carbohydrates) was also present in higher concentrations in LCx.

232 Noticeable but lower differences were found for the lipid-derived volatiles (Table 5). The  
233 LOX (lipoxygenase) pathway is the origin of the C6 volatiles in tomatoes. The lipid-derived  
234 volatiles are formed from the breakdown of linoleic (C18:2) and linolenic acids (C18:3) that  
235 are cleaved by lipoxygenase to form C13 intermediates, themselves further modified by other  
236 enzymes in the LOX pathway, notably hydroperoxide lyase (HPL). Only (E)-2-hexenal was  
237 7-fold more concentrated in LCx than in Levovil, while for all others the LCx/Levovil ratios  
238 were close to 2. Amounts of the linoleic and linolenic acid precursors as well as global LOX  
239 and HPL activities were similar between LCx and Levovil (Table 6).

240 There were only limited differences between the two lines in terms of products of carotenoid  
241 catabolism (Table 3), except farnesylacetone which was 3-fold higher in LCx.

242

### 243 *3.3 Time-course evolution of volatiles during storage*

#### 244 *3.3.1. General*

245 During storage (Fig. 2), total volatiles increased at 20 °C and decreased markedly at 4 °C for  
246 both LCx and Levovil lines. Reconditioning at 20 °C allowed some recovery of volatile  
247 production for LCx (though not at 30 days) but not for Levovil.

248

#### 249 *3.3.2. Global characterization*

250 Principal Component Analysis (PCA) was carried out to obtain a general overview of the  
251 specific volatiles during storage. The original PCA was carried out using all individuals and  
252 volatiles; for readability, Fig. 3 shows the PCA carried out using averages of the three  
253 replicates and variables selected by keeping only one of the most highly-correlated variables.

254 Similar patterns were obtained with the full and the simplified data set. As expected, LCx and  
255 Levovil were clearly differentiated on the first plane of PCA (71% of variance) (Fig. 3A).  
256 LCx was in the same quadrant as most volatiles in the correlation circle (Fig. 3B), which can  
257 be related to its generally higher production of volatiles. The Levovil/LCx separation along  
258 principal component 2 also corresponded to the position of the phenylpropanoid derivatives  
259 on the correlation circle. Methylsalicylate, (eugenol and 2-methoxyphenol are correlated with  
260 methylsalicylate but not represented on the figure) present in Levovil but not in LCx, and 4-  
261 vinyl-2-methoxyphenol, almost exclusively present in LCx (Table 3), were opposed.  
262 Evolutions of sample projections during storage were very different depending on  
263 temperature, but followed similar trends in the two lines (Fig 3A). The evolution observed for  
264 storage at 4 °C was negatively correlated with all volatiles while the evolution for storage at  
265 20 °C was correlated with a majority of detected volatiles, which is in agreement with the  
266 global evolution described in Fig. 2. Levovil samples moved mostly along axis 2 (up with  
267 storage at 20 °C, down with storage at 4 °C) while for LCx the coordinates along axes 1 and 2  
268 were affected to the same extent (up and to the right at 20 °C, down and to the left at 4 °C).  
269 The effects of reconditioning at 20 °C for 24 h (samples in italics) were most marked for  
270 samples kept for shorter storage durations. For tomatoes that had only been stored 24 h at 4  
271 °C, the volatile compositions after a further 24 h at 20 °C were very close to those of the  
272 tomatoes that had only been stored for 24 h at 20 °C. Impact of reconditioning was much  
273 lower for long storage at 4 °C.  
274 The PCA map shows that the volatile compositions of the two lines were initially contrasted  
275 and stayed clearly distinct during storage; however the evolution trends were affected  
276 primarily by storage temperature.  
277  
278 *3.3.3. Evolution of LOX products*

279 The main group of volatiles consisted of the so-called LOX products (Table 5), most of which  
280 correlated with and contributed strongly to axis 1 of the PCA (Fig. 3B), with the exception of  
281 (E,E)-2,4-decadienal and 2-pentylfuran, close to axis 2, and (Z)-3-hexen-1-ol, which had an  
282 intermediate behavior. LOX products are major determinants of the aroma of tomato  
283 products. During storage at 20 °C (Table 3), we noted little significant modification in the  
284 LOX products, which mostly only tended to increase, nor in enzyme activities or substrate  
285 concentrations (Table 6).

286 During storage at 4 °C (Table 3), the production of most LOX products decreased markedly;  
287 after 24 h back at 20°C, LOX products tended to recover in LCx but not in Levovil. For  
288 volatile products from lipid degradation further downstream, such as (E,E)-2,4-decadienal, the  
289 situation was much less clear-cut, and we found a trend towards increased concentrations for  
290 prolonged storage at 4 °C. In contrast, LOX activities remained fairly stable during storage  
291 (Table 6), with values at 4 °C tending to decrease for LCx but increase for Levovil. HPL  
292 activities initially decreased at 4 °C but stabilized after 1 (LCx) or 6 (Levovil) days. Linolenic  
293 and linoleic acid concentrations actually increased during storage at 4 °C (Table 6).

294

#### 295 *3.3.4. Time-course evolution of products from amino acid degradation*

296 Volatiles derived from amino acids (Table 5) were mostly strongly correlated with principal  
297 component 1 (Fig. 3) but showed a diverse range of behaviours during storage. In particular,  
298 2-isobutylthiazole was strongly correlated with and contributed to principal component 2.

299 Production of 2-isobutylthiazole increased markedly at 20 °C in both lines (Table 5). The two  
300 other products from leucine/isoleucine degradation, i.e. 3-methylbutanoic acid and 3-methyl-  
301 1-butanol, were on the diagonal of principal components 1 and 2. 3-Methyl-1-butanol  
302 concentration increased at 20°C in Levovil but not in LCx, and reached similar levels in both  
303 lines after 6 days of storage. Products from the phenylalanine pathway followed a similar

304 pattern 3-methyl-1-butanol. Concentrations of leucine/isoleucine and phenylalanine  
305 degradation products were initially high in LCx and stayed high at 20 °C., whereas in Levovil  
306 they were initially low but increased markedly during storage at 20 °C. Volatiles originating  
307 from sulfur-containing amino acids, illustrated in Fig. 3 by 2-methylthioethanol, increased at  
308 20 °C and for short storage at 4 °C, but decreased upon prolonged storage at 4°C in both lines.  
309 As a rule, volatiles originating from amino-acid degradation tended to increase during storage  
310 at 20 °C and decrease at 4 °C, but the intensity of their variations were very different for LCx  
311 and Levovil.

312

### 313 *3.3.5. Time-course evolution of carotenoid derivatives*

314 Apocarotenoid volatiles are produced by oxidative cleavage of the various carotenoids present  
315 in tomato, of which the major compounds are lycopene and  $\beta$ -carotene. Apocarotenoids are  
316 generally described as having fruity or floral attributes, and have been shown (Vogel, Tieman,  
317 Sims, Odabasi, Clark, & Klee, 2010) to contribute strongly to flavour acceptability. Most  
318 carotenoid-derived compounds were highly correlated with principal component 1 (Fig. 3),  
319 although specific groups emerged (Table 5). One of these groups, illustrated by 6-methyl-5-  
320 hepten-2-one, a lycopene degradation product, was characterized by a marked increase during  
321 storage at 20 °C but stable or slightly decreased concentrations at 4 °C, with very little  
322 recovery during reconditioning. Another typical behaviour is that of dihydroactinidiolide,  
323 originating from  $\beta$ -carotene, which increased in all storage conditions. Carotenoid-derived  
324 volatiles thus showed different time-course evolution patterns during storage, with most  
325 increasing in storage at 20 °C but some also increasing in storage at 4 °C.

326

### 327 *3.3.6. Miscellaneous*



328 Phenylpropanoid derivatives eugenol, methylsalicylate and 2-methoxyphenol (Table 3), as  
329 noted above, were absent or present in only trace amounts in LCx. In Levovil, their  
330 concentrations did not vary significantly during storage at 20 °C but decreased markedly at 4  
331 °C. No particular trend could be noted for furaneol (Table 3) due to its low concentrations and  
332 high variability.

333

#### 334 **4. Discussion**

##### 335 *4.1 Volatiles are lost during storage at 4 °C*

336 The observed time-course evolutions corroborated a loss of aroma for ripe tomatoes under  
337 cold storage. The consumer-perceived loss therefore has a biosynthetic origin and not only a  
338 physico-chemical origin due to eating a colder food. Reconditioning at room temperature as a  
339 strategy to mitigate this loss did not seem very efficient.

340 Storage at 20 °C increased the amounts of volatiles produced and changed their patterns. The  
341 LOX products stayed the most abundant class but their concentrations varied only moderately,  
342 with statistical significance depending on target compound. This is confirmed by the  
343 literature. Zhang, Zeng & Li (2008) compared the volatile composition of mature red and  
344 “stale” tomatoes (storage at 25 °C, but no details of storage duration) by headspace solid-  
345 phase microextraction. They found an increase in the relative area of hexanal and a decrease  
346 of (E)-2-hexenal. This is the pattern observed here for hexanal, while hexenal (sum of (E)-2-  
347 and (Z)-3-hexenal) did not vary. Krumbein, Peters & Brückner (2004) noted increases in  
348 hexanal, (E)-2-heptenal and (E,E)-2,4-decadienal in tomatoes stored at 20 °C for up to 7 (cv  
349 Pronto), 10 (cv Mickey) and 21 days (cv Vanessa). Contrasted behaviours were observed for  
350 (E,E)-2,4-decadienal in LCx and Levovil. Ties & Barringer (2012) also reported moderate  
351 increases in hexanal after 14-day storage of “Campari” tomatoes and an increase in (E)-2-

352 hexenal production from the flesh only. There are also reports of very limited evolution in  
353 LOX pathway substrates and enzymes during storage at 20 °C, as observed by Ties &  
354 Barringer (2012). Krumbein et al. (2004) and Maul et al. (2000) had already reported  
355 increased 6-methyl-5-hepten-2-one and geranylacetone during storage of tomatoes at 20 °C.  
356 Few reports are available on the time-course evolution of volatiles originating from amino  
357 acids in ripe tomato. During storage at 20 °C, Krumbein et al. (2004) and Zhang et al. (2009)  
358 reported increased 2-isobutylthiazole and Maul et al. (2000) found increased 2+3-  
359 methylbutanol. Concerning phenylpropanoid derivatives, Krumbein et al. (2004) observed an  
360 increase in methylsalicylate in three varieties of tomatoes stored at 20 °C. The main evolution  
361 in volatile production during storage at 20 °C was a decrease of the relative proportion of  
362 LOX products, notably relative to the carotenoid degradation products. This can be linked to  
363 loss of the “green” or “grassy” notes as the tomatoes mature, whereas “fruity” aromas  
364 become more marked (Baldwin, Scott, Shewmaker, & Schuch, 2000).

365

366 Tomato volatiles were strongly affected by storage at 4 °C, though red-ripe fruits are less  
367 sensitive to cold than earlier maturation stages. The decrease in production of LOX  
368 compounds under cold conditions has been studied because they are major contributors to  
369 tomato aroma. Maul et al. (2000) had already noted a marked decrease in the production of  
370 LOX compounds (lipid-derived volatiles) during storage at low temperatures, but this loss  
371 cannot be explained in a straightforward manner, and in particular is not explained by a loss  
372 of substrate or global enzyme activity, as exemplified in Ties & Barringer (2012) or Bai,  
373 Baldwin, Imahori, Kostenyuk, Burns, & Brecht (2011). Bai et al. (2011) observed an increase  
374 in total LOX activity after 5-day storage at 5 °C and a decrease in HPL activity with some  
375 recovery after 4-day storage at 20 °C, while Ties & Barringer (2012) found a slight increase  
376 in total LOX activity, confirming our results. Bai et al. (2011) also indicated that the

377 divergence between enzyme activities (and transcript expression) and LOX product  
378 production might be linked to substrate availability. However, as reported by Ties &  
379 Barringer (2012), the concentrations of the LOX substrates linoleic and linolenic acid actually  
380 increased during storage (Table 2). This divergence is therefore most likely linked to the  
381 existence of different isoforms of LOX expressed in ripening tomato. Griffiths, Prestage,  
382 Linforth, Zhang, Taylor, & Grierson (1999) reported no impact on generation of C6 aldehydes  
383 and alcohols in antisense-transgenic tomatoes in which *TomloxA* and *TomloxB* were  
384 inactivated, while the inactivation of *TomloxC* (Chen, Hackett, Walker, Taylor, Lin, &  
385 Grierson, 2004) led to a marked reduction in the production of hexanal, hexenal and hexanol.  
386 Kovács, Fray, Tikunov, Graham, Bradley, Seymour et al. (2009), studying the effect of  
387 ripening mutations on volatile synthesis, also showed that LOX compounds (fatty-acid  
388 derived volatiles) appear intimately linked to *TomloxC* expression. Bai et al. (2011) studied  
389 the expression of *TomloxA*, *B*, *C* and *D* in chilled (5 days at 5 °C) tomatoes and found a  
390 downregulation of all but *TomloxD* upon chilling, together with increased LOX activity. The  
391 existence of a specific isoform downregulated during cold storage might explain the decrease  
392 in LOX volatiles under cold storage without loss of substrates or global LOX activity.  
393 The decrease in phenylpropanoid (eugenol and 2-methylphenol) concentrations in Levovil  
394 during storage at 4 °C could actually be beneficial, leading to a loss (or at least a decrease) of  
395 the “pharmaceutical” aftertaste.

396

#### 397 4.2 Comparison of the two cultivars

398 The study was performed on two tomato lines known for their contrasted sensory attributes:  
399 Levovil, characterized by its large fruits and “pharmaceutical” aftertaste, and LCx, an  
400 introgressed line carrying five chromosome regions from the Cervil cherry tomato identified  
401 as bearing the qtls for quality traits, notably for tomato aroma intensity in the Levovil

402 background (Causse, Saliba-Colombani, Lecomte, Duffé, Rousselle, & Buret, 2002; Chaib et  
403 al., 2006). The overall pattern of volatiles in the freshly-harvested red fruits (Tables 3 to 5)  
404 confirms previous research (Birtic et al., 2009; Zanor, Eambla, Chaib, Steppa, Medina,  
405 Granell, et al., 2009). The difference in total volatiles production could be partially explained  
406 by the smaller size and therefore higher peel-to-flesh ratio of LCx, as higher concentrations of  
407 volatiles and their precursors are found in tomato peel (Ties & Barringer, 2012). Eugenol and  
408 2-methoxyphenol are responsible for the presence of “pharmaceutical” aftertaste in Levovil  
409 (Causse et al., 2002; Chaib et al., 2006; Zanor et al., 2009) and were absent in LCx, as in its  
410 Cervil introgression parent (Birtic et al., 2009). The difference between the cultivars could be  
411 due to different glycosidation patterns (Tikunov, de Vos, Paramas, Hall, & Bovy, 2010),  
412 although Birtic et al. (2009) did not find high accumulation of the corresponding glycosides in  
413 Cervil. The two lines also differed in terms of volatiles derived from phenylalanine and  
414 sulphur-containing amino acids, though again this was less marked than in the Cervil/Levovil  
415 comparison (Zanor et al., 2009). Smaller differences could be noted for volatiles derived from  
416 the LOX pathway; concentrations of fatty acid precursors and enzyme activities (LOX and  
417 HPL) were also quite similar. The fatty acid contents were in agreement with the levels  
418 reported by Gray, Prestage, Linforth, & Taylor (1999). Linoleic acid was by far the  
419 predominant fatty acid, with linoleic/linolenic ratio of 7.7 for Levovil and 6.1 for LCx, which  
420 are high values in comparison to those reported by Gray et al. (1999) and Ties & Barringer  
421 (2012). Hexanal originates from linoleic acid while hexenal originates from linolenic acid.  
422 The hexanal/hexenal ratios (0.4 in Levovil, 0.5 in LCx) were about 10-fold lower than the  
423 linoleic/linolenic acid ratio, but still much higher than those found by Gray et al. (1999). This  
424 indicates preferential conversion of linolenic acid, as was also found by Ties & Barringer  
425 (2012). The two lines exhibited the expected characteristics and contrasts, both in terms of  
426 physical characteristics and volatiles production, though the differential was less visible than

427 with Cervil, the origin of the five introgressed chromosome region (Birtic et al., 2009; Chaib  
428 et al., 2006). The main differences concern volatile production, which was much higher in  
429 LCx, and the phenylpropanoid pathway, with only Levovil producing phenylpropanoid  
430 volatiles.

431

## 432 **5. Conclusion**

433

434 The storage conditions of red-ripe tomatoes modified both global volatiles production and  
435 volatiles profile. Cold storage was detrimental for tomato aroma even when starting from red-  
436 ripe fruits. Conservation at 4 °C led to a drop in volatile production and loss of aroma  
437 compounds. Storing tomatoes in a refrigerator thus jeopardizes all the efforts carried out  
438 throughout the supply chain to deliver a high-quality produce. Only in the earliest stages of  
439 fridge storage (< 1 week) could the aroma be restored by reconditioning the tomatoes at room  
440 temperature for 24 h. This conditioning can still have some positive effects after long (> 2  
441 weeks) fridge storage, though with a marked imbalance compared to the fresh tomato. In  
442 particular, there was an increase of the relative importance of carotenoid degradation products  
443 and even some of the down-chain LOX products such as (EE)-2,4-decadienal. Aroma profiles  
444 of red-ripe tomatoes were positively impacted by storage at 20 °C, with an overall increase in  
445 volatile production. This was accompanied by a possible decrease of the “pharmaceutical”  
446 aftertaste in Levovil and an increase in the fruity notes brought by carotenoid degradation  
447 products in both Levovil and LCx. Clearly, conservation at room temperature should be  
448 advocated for consumers. Therefore, further consumer information efforts as well as specific  
449 storage guidelines could be highly beneficial for consumer perceptions of tomato quality.

450

451

452 **Acknowledgements**

453 This work was funded by project ANR-06-PNRA-009 QualitomFil “Elaboration et  
454 valorisation de la qualité organoleptique et nutritionnelle de la tomate tout au long de la  
455 filière” program. The authors thank Antony Rech, Patrice Reling, and Marielle Bogé for their  
456 excellent technical help.

457

458 **References**

- 459 Auerswald, H., Peters, P., Brückner, B., Krumbein, A., & Kuchenbuch, R. (1999). Sensory  
460 analysis and instrumental measurements of short-term stored tomatoes (*Lycopersicon*  
461 *esculentum* Mill.). *Postharvest Biology and Technology*, 15(3), 323-334.
- 462 Bai, J.H., Baldwin, E.A., Imahori, Y., Kostenyuk, I., Burns, J., & Brecht, J.K. (2011).  
463 Chilling and heating may regulate C6 volatile aroma production by different mechanisms in  
464 tomato (*Solanum lycopersicum*) fruit. *Postharvest Biology and Technology*, 60(2), 111-120.
- 465 Baldwin, E.A., Scott, J.W., Shewmaker, C.K., & Schuch, W. (2000). Flavour trivia and  
466 tomato aroma: Biochemistry and possible mechanisms for control of important aroma  
467 components. *HortScience*, 35(6), 1013-1022.
- 468 Baysal, T., & Demirdoven, A. (2007). Lipoxygenase in fruits and vegetables: A review.  
469 *Enzyme and Microbial Technology*, 40(4), 491-496.

- 470 Birtic, S., Ginies, C., Causse, M., Renard, C. M. G. C., & Page, D. (2009). Changes in  
471 volatiles and glycosides during fruit maturation of two contrasted tomato (*Solanum*  
472 *lycopersicum*) lines. *Journal of Agricultural and Food Chemistry*, 57(2), 591-598.
- 473 Box, G.E.P., Hunter, W.G., & Hunter, J. S. (1978). *Statistics for Experimenters*: John Wiley  
474 and Sons, New York.
- 475 Bradford, M. M. (1976). Rapid and sensitive method for quantitation of microgram quantities  
476 of protein utilizing principle of protein-dye binding. *Analytical Biochemistry*, 72(1-2), 248-  
477 254.
- 478 Causse, M., Saliba-Colombani, V., Lecomte, L., Duffé, P., Rousselle, P., & Buret, M. (2002).  
479 QTL analysis of fruit quality in fresh market tomato: a few chromosome regions control the  
480 variation of sensory and instrumental traits. *Journal of Experimental Botany*, 53(377), 2089-  
481 2098.
- 482 Chaib, J., Lecomte, L., Buret, M., & Causse, M. (2006). Stability over genetic backgrounds,  
483 generations and years of quantitative trait locus (QTLs) for organoleptic quality in tomato.  
484 *Theoretical and Applied Genetics*, 112(5), 934-944.
- 485 Chen, G.P., Hackett, R., Walker, D., Taylor, A., Lin, Z.F., & Grierson, D. (2004).  
486 Identification of a specific isoform of tomato lipoxygenase (TomloxC) involved in the  
487 generation of fatty acid-derived flavour compounds. *Plant Physiology*, 136(1), 2641-2651.
- 488 de Leon-Sanchez, F.D., Pelayo-Zaldivar, C., Rivera-Cabrera, F., Ponce-Valadez, M., Avila-  
489 Alejandre, X., Fernandez, F.J., Escalona-Buendia, H.B., & Perez-Flores, L.J. (2009). Effect of  
490 refrigerated storage on aroma and alcohol dehydrogenase activity in tomato fruit. *Postharvest*  
491 *Biology and Technology*, 54(2), 93-100.

- 492 Gray, D.A., Prestage, S., Linforth, R.S.T., & Taylor, A.J. (1999). Fresh tomato specific  
493 fluctuations in the composition of lipoxygenase-generated C6 aldehydes. *Food Chemistry*,  
494 64(2), 149-155.
- 495 Griffiths, A., Prestage, S., Linforth, R., Zhang, J., Taylor, A., & Grierson, D. (1999). Fruit-  
496 specific lipoxygenase suppression in antisense-transgenic tomatoes. *Postharvest Biology and*  
497 *Technology*, 17(3), 163-173.
- 498 Hauck, T., Hübner, Y., Brühlmann, F., & Schwab, W. (2003). Alternative pathway for the  
499 formation of 4,5-dihydroxy-2,3-pentanedione, the proposed precursor of 4-hydroxy-5-methyl-  
500 3(2H)-furanone as well as autoinducer-2, and its detection as natural constituent of tomato  
501 fruit. *Biochimica et Biophysica Acta (BBA) - General Subjects*, 1623(2-3), 109-119.
- 502 Kovács, K., Fray, R.G., Tikunov, Y., Graham, N., Bradley, G., Seymour, G.B., Bovy, A.G., &  
503 Grierson, D. (2009). Effect of tomato pleiotropic ripening mutations on flavour volatile  
504 biosynthesis. *Phytochemistry*, 70(8), 1003-1008.
- 505 Krumbein, A., Peters, P., & Brückner, B. (2004). Flavour compounds and a quantitative  
506 descriptive analysis of tomatoes (*Lycopersicon esculentum* Mill.) of different cultivars in  
507 short-term storage. *Postharvest Biology and Technology*, 32(1), 15-28.
- 508 Lewinsohn, E., Sitrit, Y., Bar, E., Azulay, Y., Ibdah, M., Meir, A., Yosef, E., Zamir, D., &  
509 Tadmor, Y. (2005). Not just colours-carotenoid degradation as a link between pigmentation  
510 and aroma in tomato and watermelon fruit. *Trends in Food Science & Technology*, 16(9), 407-  
511 415.



- 512 Linforth, R.S.T., Savary, I., Pattenden, B., & Taylor, A.J. (1994). Volatile compounds found  
513 in expired air during eating of fresh tomatoes and in the headspace above tomatoes. *Journal of*  
514 *the Science of Food and Agriculture*, 65(2) 241-247.
- 515 Mathieu, S., Cin, V.D., Fei, Z.J., Li, H., Bliss, P., Taylor, M.G., Klee, H.J., & Tieman, D.M.  
516 (2009). Flavour compounds in tomato fruits: identification of loci and potential pathways  
517 affecting volatile composition. *Journal of Experimental Botany*, 60(1), 325-337.
- 518 Maul, F., Sargent, S. A., Sims, C.A., Baldwin, E.A., Balaban, M.O., & Huber, D.J. (2000).  
519 Tomato flavour and aroma quality as affected by storage temperature. *Journal of Food*  
520 *Science*, 65(7), 1228-1237.
- 521 Mayer, F., Takeoka, G.K., Buttery, R.G., Whitehand, L.C., Naim, M.N., & Rabinowitch, H.D.  
522 (2008). Studies on the aroma of five fresh tomato cultivars and the precursors of cis- and  
523 trans-4,5-epoxy-(E)-2-decenals and methional. *Journal of Agricultural and Food Chemistry*,  
524 56(10), 3749-3757.
- 525 Riley, J.C.M., & Thompson, J.E. (1998). Ripening-induced acceleration of volatile aldehyde  
526 generation following tissue disruption in tomato fruit. *Physiologia Plantarum*, 104, 571-576.
- 527 Riley, J.C.M., Willemot, C., & Thompson, J.E. (1996). Lipoxygenase and hydroperoxide  
528 lyase activities in ripening tomato fruit. *Postharvest Biology and Technology*, 7(1/2), 97-107.
- 529 Robinson, D.S., Wu, Z.C., Domoney, C., & Casey, R. (1995). Lipoxygenases and the quality  
530 of foods. *Food Chemistry*, 54(1), 33-43.
- 531 Rodrigo, D., Jolie, R., Van Loey, A., & Hendrickx, M. (2007). Thermal and high pressure  
532 stability of tomato lipoxygenase and hydroperoxide lyase. *Journal of Food Engineering*,  
533 79(2), 423-429.

- 534 Schäfer, K. (1998). Accelerated solvent extraction of lipids for determining the fatty acid  
535 composition of biological material. *Analytica Chimica Acta*, 358(1), 69-77.
- 536 Schwab, W., Davidovich-Rikatani, R., & Lewinsohn, E. (2008). Biosynthesis of plant-derived  
537 flavour compounds. *The Plant Journal*, 54(4), 712-732.
- 538 Stern, D.J., Buttery, R.G., Teranishi, R., Ling, L., Scott, K., & Cantwell, M. (1994). Effect of  
539 storage and ripening on fresh tomato quality, Part 1. *Food Chemistry*, 49(3), 225-231.
- 540 Surrey, K. (1964). Spectrophotometric method for determination of lipoxidase activity. *Plant*  
541 *Physiology*, 39(1), 65-70.
- 542 Ties, P., & Barringer, S. (2012). Influence of lipid content and lipoxygenase on flavour  
543 volatiles in the tomato peel and flesh. *Journal of Food Science*, 77(7), C830-C837.
- 544 Tikunov, Y., Lommen, A., de Vos, C.H.R., Verhoeven, H.A., Bino, R.J., Hall, R.D., & Bovy,  
545 A.G. (2005). A novel approach for nontargeted data analysis for metabolomics. Large-scale  
546 profiling of tomato fruit volatiles. *Plant Physiology*, 139(3), 1125-1137.
- 547 Tikunov, Y.M., de Vos, R.C.H., Paramas, A.M.G., Hall, R.D., & Bovy, A.G. (2010). A role  
548 for differential glycoconjugation in the emission of phenylpropanoid volatiles from tomato  
549 fruit discovered using a metabolic data fusion approach. *Plant Physiology*, 152(1), 55-70.
- 550 Trad, M., Ginies, C., Gaaliche, B., Renard, C.M.G.C., & Mars, M. (2012). Does pollination  
551 affect aroma development in ripened fig [*Ficus carica* L.] fruit? *Scientia Horticulturae*, 134,  
552 93-99.

- 553 Ullrich, F., & Grosch, W. (1987). Identification of the most intense volatile flavour  
554 compounds formed during autoxidation of linoleic acid. *Zeitschrift für*  
555 *Lebensmitteluntersuchung und -forschung A*, 184(4), 277-282.
- 556 Vick, B.A. (1991). A spectrophotometric assay for hydroperoxide lyase. *Lipids*, 26(4), 315-  
557 320.
- 558 Vogel, J.T., Tieman, D.M., Sims, C.A., Odabasi, A.Z., Clark, D.G., & Klee, H.J. (2010).  
559 Carotenoid content impacts flavour acceptability in tomato (*Solanum lycopersicum*). *Journal*  
560 *of the Science of Food and Agriculture*, 90(13), 2233-2240.
- 561 Xu, Y.C., & Barringer, S. (2010). Comparison of volatile release in tomatillo and different  
562 varieties of tomato during chewing. *Journal of Food Science*, 75(4), C352-C358.
- 563 Zanor, M.I., Rambla, J.L., Chaib, J., Steppa, A., Medina, A., Granell, A., Fernie, A.R., &  
564 Causse, M. (2009). Metabolic characterization of loci affecting sensory attributes in tomato  
565 allows an assessment of the influence of the levels of primary metabolites and volatile organic  
566 contents. *Journal of Experimental Botany*, 60(7), 2139-2154.
- 567 Zhang, Z.M., Zeng, D.D., & Li, G.K. (2008). Study of the volatile composition of tomato  
568 during storage by a combination sampling method coupled with gas chromatography/mass  
569 spectrometry. *Journal of the Science of Food and Agriculture*, 88(1), 116-124.

570 **Caption to figures**

571

572 Figure 1: Time-course evolution of firmness and hue angle for LCx and Levovil tomatoes  
573 stored at 20 °C and 4 °C. Each point is the average (and standard deviation) of three replicates  
574 of 5 tomatoes each.

575 ○: LCx, stored at 20 °C; △: LCx, stored at 4 °C; ▲: LCx; stored at 4 °C then reconditioned  
576 for 24 h at 20 °C.

577 □: Levovil, stored at 20 °C; ◇: Levovil, stored at 4 °C; ◆: Levovil, stored at 4 °C then  
578 reconditioned for 24 h at 20 °C.

579

580 Figure 2: Time-course evolution of the sum of volatiles of LCx and Levovil tomatoes stored  
581 at 20 °C and 4 °C. Each point is the average (and standard deviation) of three replicates of 5  
582 tomatoes each.

583 ○: LCx, stored at 20 °C; △: LCx, stored at 4 °C; ▲: LCx, stored at 4 °C then reconditioned  
584 for 24 h at 20 °C.

585 □: Levovil, stored at 20 °C; ◇: Levovil, stored at 4 °C; ◆: Levovil, stored at 4 °C then  
586 reconditioned for 24 h at 20 °C.

587

588 Figure 3: Principal component analysis of the volatiles composition of tomatoes as a function  
589 of their storage conditions.

590 Variables were selected based on a PCA carried out with all variables and all samples,  
591 followed by eliminating all but one of each highly correlated variables group. Each point is  
592 the average of three replicates of 5 tomatoes each.

593 Panel A: map of samples (tomatoes) projected onto the principal components 1 x 2 plane.

594 Coding of the sample points: L, levovil; X, LCx; the first number gives storage temperature,  
595 followed by duration of storage. The “+1” indicates that the sample was left for 1 day at 20 °C  
596 after storage at 4 °C. Thus L\_4\_15+1 designated tomatoes of the Levovil variety, stored at 4  
597 °C for 15 days then left for 1 day at 20 °C to recover volatile production. Blue denotes storage  
598 at 4 °C, and red denotes storage at 20 °C.

599 Panel B: variables (volatile compounds) projected on the correlation circle defined by  
600 principal components 1 x 2. Each arrow corresponds to one of the variables. The colours  
601 denote biochemical origins: blue for volatiles derived from fatty acid degradation by LOX,  
602 purple from carotenoids, green for amino acids, orange for phenylpropanoids, and red from  
603 carbohydrates.

604

605 **Tables**

606 Table 1: Volatile compounds quantified: retention index, odourant potential and biosynthetic origin

Volatile compound	RI	Contribution to tomato aroma <sup>b</sup>	Biosynthetic origin
Hexanal	a	++	Lipid <sup>c,e</sup>
(E)-2-hexenal	a	+	Lipid <sup>c,e</sup>
(Z)-3-hexenal	a	+++	Lipid <sup>c,e</sup>
1-penten-3-one	a	+	Lipid <sup>c,e</sup>
(E)-2-pentenal	1115		Lipid <sup>c,e</sup>
1-penten-3-ol	1168		Lipid <sup>c,e</sup>
3-methyl-1-butanol	1219	+	Leucine/isoleucine <sup>c,e</sup>
2-pentylfuran	1245		Lipid <sup>c</sup>
1-pentanol	1262		Lipid <sup>c,e</sup>
2-methylthioacetaldehyde	1282		Sulfur-containing amino acid <sup>b,f</sup>
1-octen-3-one	1313	+	Lipid <sup>b,h</sup>
2-hexanol	1326		Lipid <sup>c</sup>
(Z)-2-heptenal	1341		Lipid <sup>c</sup>
6-methyl-5-hepten-2-one	1354		Open chain carotenoid <sup>c</sup> , lycopene <sup>e</sup>
1-hexanol	1366		Lipid <sup>c</sup>
(Z)-3-hexen-1-ol	1399		Lipid <sup>c</sup>
2-isobutylthiazole	1422	+	Leucine/isoleucine <sup>c,e</sup>
(E)-2-octenal	1449	+	
Methional	1470	+	Sulfur-containing amino acid <sup>b,f</sup>
Benzaldehyde	1550		Phenylalanine <sup>e</sup>
2-methylthioethanol	1554		Sulfur-containing amino acid <sup>f</sup>
$\beta$ -cyclocitral	1642		Cyclic carotenoid <sup>c</sup> , $\beta$ -carotene <sup>d</sup>
Phenylacetaldehyde	1671	++	Phenylalanine <sup>e</sup>
3-methylbutanoic acid	1701	+	Leucine/isoleucine <sup>c,e</sup>
(E,E)-2,4-nonadienal	1723		Lipid <sup>b</sup>
3-methylthiopropanol	1741		Sulfur-containing amino acid <sup>f</sup>
Geranial	1754		Open chain carotenoid <sup>c</sup> , lycopene <sup>d</sup>
(E,Z)-2,4-decadienal	1786	+++	Lipid <sup>c</sup>
Methyl salicylate	1804		Phenylpropanoid <sup>c</sup>
(E,E)-2,4-decadienal	1820	+++	Lipid <sup>c</sup>
Geranylacetone	1874		Open chain carotenoid <sup>c</sup> , phytoene phytofluene <sup>d</sup>
2-methoxyphenol	1889		Phenylpropanoid <sup>c</sup>
Benzyl alcohol	1905		Phenylalanine <sup>e</sup>
2-phenylethanol	1939		Phenylalanine <sup>e</sup>
$\beta$ -ionone	1962	+	Cyclic carotenoid <sup>c</sup> , $\beta$ -carotene <sup>d</sup>
<i>Cis</i> -4,5-epoxy-(E)—2-decenal	2005		Lipid <sup>b</sup>
5,6-Epoxy $\beta$ -ionone	2016		Cyclic carotenoid <sup>c</sup> , $\beta$ -carotene <sup>d</sup>
<i>Trans</i> -4,5-epoxy-(E)—2-decenal	2025	++	Lipid <sup>b</sup>
Furaneol	2057	+++	Carbohydrates <sup>f,g</sup>
Pseudo-ionone (unknown configuration)	2154		Cyclic carotenoid <sup>c</sup> , $\beta$ -carotene <sup>d,f</sup>

---

Eugenol	2197	+	Phenylpropanoid <sup>c</sup>
4-vinyl-2-methoxyphenol	2225		Phenylpropanoid <sup>c</sup>
Dihydroactiniodiolide	2387		$\beta$ -carotene <sup>d</sup>
Farnesylacetone (unknown configuration)	2389		Phytoene, phytofluene <sup>d</sup>

---

- 607 a: quantification on DB5 column; b: from Mayer, Takeoka, Buttery, Whitehand, Naim, & Rabinowitch (2008);
- 608 c: from Tikunov, Lommen, de Vos, Verhoeven, Bino, Hall et al. (2005); d: from Lewinsohn, Sitrit, Bar, Azulay,
- 609 Ibdah, Meir et al. (2005); e: from Mathieu, Cin, Fei, Li, Bliss, Taylor, et al. (2009); f: from Schwab,
- 610 Davidovitch-Rikatani, & Lewinsohn (2008); g: from Hauck, Hübner, Brühlmann & Schwab (2003); h: from
- 611 Ullrich & Grosch (1987).

Table 2: Time-course evolution of the physical characteristics of tomatoes during storage at different temperatures. For all data, n = 3 repetitions of 5 tomatoes each.

	Storage		Weight (g)	Firmness (N)	Colour		
	Temp	Duration (days)			L*	C*	h* (degrees)
Levovil	20 °C	0	128	24.5	37.9	31.9	43.2
		1	129	23.6	37.4	30.4	44.9
		3	123	21.4	37.2	30.2	43.5
		6	129	17.1	37.3	31.9	41.9
	4 °C	1	125	26.2	37.4	32.8	43.5
		6	129	18.3	38.4	32.1	46.5
		15	127	14.0	38.3	31.9	49.7
		30	125	15.5	37.0	28.3	49.6
	4 °C + 1 day 20 °C	1	119	26.7	37.6	32.2	42.2
		6	130	16.9	37.2	31.8	44.1
		15	127	17.6	37.6	29.4	47.9
		30	125	15.8	37.0	27.5	48.8
LCx	20 °C	0	31	40.3	37.8	30.6	42.7
		1	27	40.0	37.9	30.7	39.5
		3	27	36.0	36.3	30.2	37.9
		6	29	26.1	36.8	30.1	38.4
	4 °C	1	29	37.6	38.1	30.0	41.0
		6	31	37.7	36.7	29.6	39.7
		15	31	20.6	35.4	28.9	40.0
		30	33	31.6	35.3	30.5	41.1
	4 °C + 1 day 20 °C	1	31	41.1	36.4	30.0	39.3
		6	30	30.8	36.1	28.8	39.6
		15	32	22.3	36.0	28.4	40.0
		30	30	16.6	37.1	28.6	42.8
<i>Pooled standard deviation</i>			<i>42 / 6</i>	<i>5.4</i>	<i>1.4</i>	<i>2.9</i>	<i>2.5</i>



Table 3: Time-course evolution of volatiles of carotenoid origin, phenylpropanoid origin and carbohydrate origin in stored tomatoes ( $\mu\text{g}/\text{kg}$  fresh weight, in 4-nonanol equivalents). For all data,  $n = 3$  repetitions of 5 tomatoes each.

Storage	Carotenoid origin										Phenylpropanoid origin						Carbohydrate origin
	Open-chain carotenoids					cyclic carotenoids											
	Temp	Duration (days)	6-methyl-5-hepten-2-one	Geranial	Geranyl acetone	Farnesyl acetone	$\beta$ -ionone	Epoxy $\beta$ -ionone	Dihydro actinidiolide	Pseudo ionone	$\beta$ cyclo citral	methyl salicylate	2-methoxy phenol	Eugenol	4-vinyl-2-methoxy phenol	Furaneol	
Levovil	20°C	0	81	23	36	71	8	10	21	11	3	76	35	74	8	7	
		1	86	24	36	57	7	7	17	10	3	83	19	55	5	5	
		3	152	27	54	83	8	8	21	12	3	85	27	63	4	11	
		6	358	37	75	122	9	10	28	17	3	77	23	55	3	3	
		1	89	22	28	43	7	8	17	9	3	95	31	62	5	4	
	4°C	6	96	25	26	33	4	6	21	7	2	53	24	35	3	26	
		15	96	34	39	62	5	5	25	15	3	29	24	15	1	40	
		30	45	15	16	27	4	3	28	6	1	5	10	4	1	21	
		1	100	30	51	75	8	8	18	12	3	108	60	31	6	6	
		6	52	15	20	27	5	5	20	6	2	69	27	58	5	13	
4°C + 1 day 20°C	15	54	18	30	50	6	6	5	28	5	40	17	36	2	26		
	30	43	14	20	40	6	6	4	33	7	3	3	6	1	29		
	LCx	0	156	22	82	214	16	18	37	10	5	2	0	0	35	34	
		1	190	29	103	275	18	17	38	14	5	2	0	0	14	23	
		3	236	44	143	367	21	20	47	23	6	2	0	0	11	17	
		6	381	66	207	508	29	26	61	35	8	3	0	0	6	12	
		1	133	22	71	186	14	13	33	9	4	2	1	0	15	29	
	4°C	6	156	37	85	172	14	15	40	12	5	2	2	0	9	20	
		15	90	28	52	89	10	11	48	9	4	2	0	0	15	3	
		30	72	23	81	152	12	10	51	10	4	1	0	0	13	16	
1		163	31	109	274	18	18	40	14	5	2	0	0	13	28		
6		145	36	109	240	18	18	49	15	5	1	0	0	9	8		
4°C + 1 day 20°C	15	81	23	59	96	11	13	48	12	4	2	0	0	18	6		

30	68	20	65	121	12	11	50	9	3	1	0	0	16	14
<i>Pooled standard deviation</i>	26.6	5.9	16.1	39.5	2.9	2.1	4.1	2.9	0.7	18.6	12.2	6.6	4.4	10.6

Table 4: Time-course evolution of volatiles of amino acid origin in stored tomatoes ( $\mu\text{g}/\text{kg}$  fresh weight, in 4-nonanol equivalents). For all data,  $n = 3$  repetitions of 5 tomatoes each.

Storage	Leucine or isoleucine				Phenylalanine			Sulfur-containing amino acids				
	duration (days)	3-methyl-1-butanol,	2-isobutyl-thiazole	3-methylbutanoic acid,	Benzaldehyde	Phenyl acetaldehyde	2-phenyl ethanol	Benzyl Alcohol	2-methylthio acetaldehyde	2-methylthio ethanol	3-methylthio propanol	methional
Levovil	20 °C	0	69	12	51	16	9	52	50	1	28	5
		1	123	18	78	14	12	56	48	2	37	12
		3	306	34	338	16	17	134	66	4	44	16
	4 °C	6	523	66	545	14	34	243	43	5	60	27
		1	93	25	42	14	10	58	50	2	39	5
		6	28	14	10	8	4	24	27	2	61	3
4 °C + 1 day 20 °C	20 °C	15	10	14	3	5	3	1	4	2	40	2
		30	5	9	3	3	2	1	3	0	3	0
		1	100	17	61	12	13	27	28	2	43	10
	4 °C	6	51	12	34	6	5	4	5	2	35	6
		15	9	12	4	4	5	1	3	1	8	1
		30	6	8	6	4	3	1	4	0	0	0
LCx	20 °C	0	308	7	291	109	17	258	1100	5	93	37
		1	327	8	288	69	16	218	734	7	109	46
		3	297	10	255	44	14	208	308	9	136	42
	4 °C	6	349	23	226	35	17	189	158	12	200	66
		1	254	5	79	55	9	234	769	3	91	46
		6	47	4	19	29	9	84	189	5	107	9
4 °C + 1 day 20 °C	20 °C	15	25	2	14	17	4	1	11	2	36	3
		30	13	1	10	14	5	1	4	0	1	1
		1	318	6	216	51	11	177	584	6	125	67
	4 °C	6	295	5	120	28	6	22	66	7	189	86
		15	38	1	30	14	9	2	7	1	14	9
		30	15	0	16	12	4	1	4	1	2	3

<i>Pooled standard deviations</i>	49.0	4.8	59.7	5.6	3.6	20.8	49.8	1.1	24.6	9.8	1.2
-----------------------------------	------	-----	------	-----	-----	------	------	-----	------	-----	-----

ACCEPTED MANUSCRIPT

Table 5: Lipid-derived volatiles in stored tomatoes ( $\mu\text{g}/\text{kg}$  fresh weight, in 4-nonanol equivalents, except for 1-penten-3-one, hexanal, (E)-2-hexenal and (Z)-3-hexenal, in  $\mu\text{g}/\text{kg}$  fresh weight). For all data,  $n = 3$  repetitions of 5 tomatoes each.

Storage	From linoleic acid				From linolenic acid																	
	Duration (days)	2-Hexanol	(E,E)-2,4-Deca dienal	Cis-4,5-Epoxy-2-decinal	(E)-2-Hexenal	(Z)-3-Hexenal	1-penten-3-one	(E)-2-Penten-3-ol	1-penten-2-furanol	(Z)-2-Pentanol	(Z)-3-Hexenol	(E)-2-Octenal	1-Octen-3-one	(E,E)-2,4-nonadienal								
Levovil	20 °C	0	741	81	23	27	9	103	310	66	1 669	115	25	55	71	171	94	101	58	10	7	
		1	731	70	16	23	8	91	263	106	1 775	116	25	55	59	131	87	76	39	7	7	
		3	832	69	20	27	12	109	291	149	2 027	123	26	62	64	151	104	76	45	8	8	
	4 °C	6	1 024	68	20	29	12	121	336	168	2 048	112	24	56	65	157	114	62	48	8	10	
		1	894	68	14	19	7	93	271	86	2 120	115	25	51	43	124	90	61	36	8	6	
		6	259	67	12	21	4	39	106	60	1 262	117	26	43	49	57	39	113	20	6	3	
	4 °C + 1 day 20 °C	15	202	54	7	44	4	19	45	32	512	78	19	27	114	38	14	36	18	4	2	
		30	111	48	10	40	3	12	27	22	170	49	12	16	99	34	14	41	17	4	1	
		1	776	75	12	30	12	99	265	112	1 762	114	24	54	59	140	98	50	51	9	10	
	LCx	20 °C	6	325	92	8	21	4	42	112	86	1 292	119	23	48	44	72	46	48	24	7	2
			15	202	50	7	42	4	23	58	57	419	82	19	25	119	43	25	41	21	4	1
			30	108	48	7	39	3	11	26	25	109	48	12	18	82	32	14	40	17	3	1
4 °C		0	1 815	106	53	52	34	144	372	490	3 024	203	45	119	98	308	180	257	126	23	15	
		1	1 536	90	37	44	35	149	374	371	3 508	221	51	119	82	284	170	160	95	18	11	
		3	1 530	76	44	36	22	178	485	306	3 518	217	53	121	83	290	177	227	87	19	10	
4 °C		6	1 899	79	61	38	65	163	417	267	3 455	220	51	112	83	321	206	197	87	19	12	
		1	1 217	99	44	39	27	116	303	275	3 436	192	48	108	81	224	133	265	80	16	7	
		6	739	69	31	36	52	74	188	245	2 675	181	44	96	66	165	98	181	55	14	7	
4 °C + 1 day 20 °C		15	417	75	37	43	5	64	166	177	1 715	153	35	75	74	117	65	313	31	10	4	
		30	408	57	24	65	6	36	86	218	744	146	34	54	89	69	45	182	27	7	3	
		1	1 235	121	39	41	31	134	339	239	3 274	196	50	109	75	249	153	195	86	19	9	
4 °C	6	979	80	37	40	37	95	248	237	3 011	214	52	113	86	215	137	229	76	18	8		
	15	681	66	27	38	6	90	226	249	2 489	231	50	88	80	124	98	184	41	14	6		
	30	394	48	24	60	6	31	73	180	966	173	40	62	57	72	49	186	26	8	3		

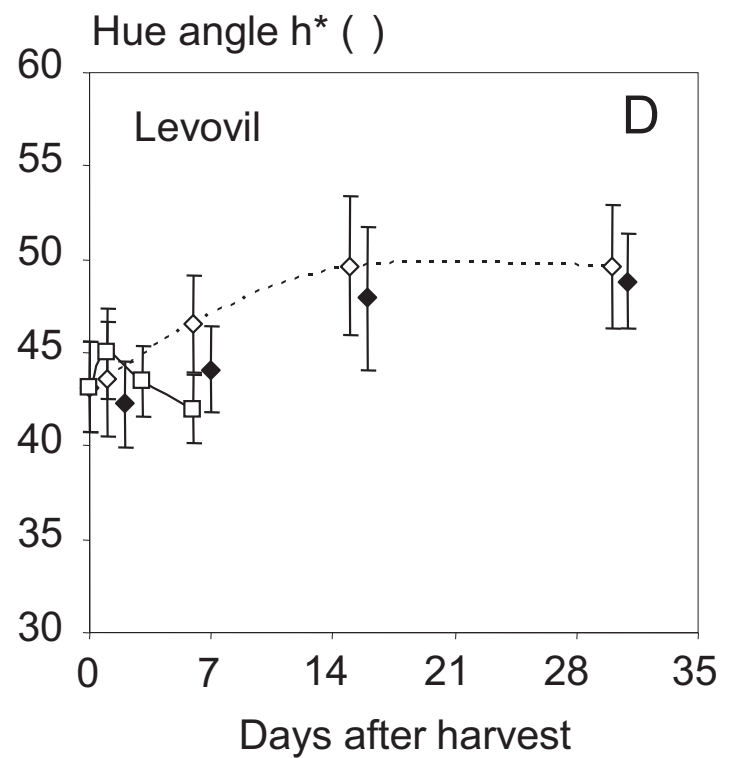
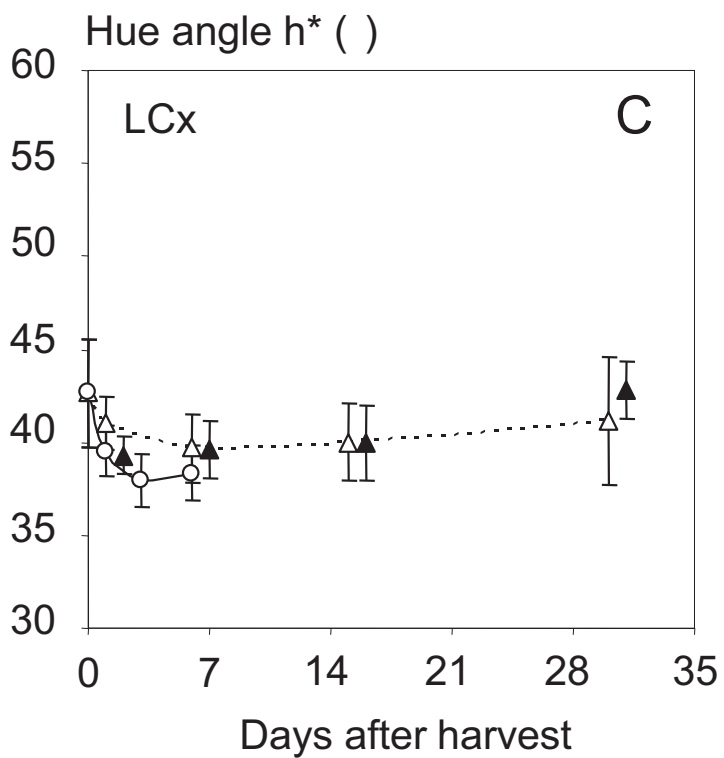
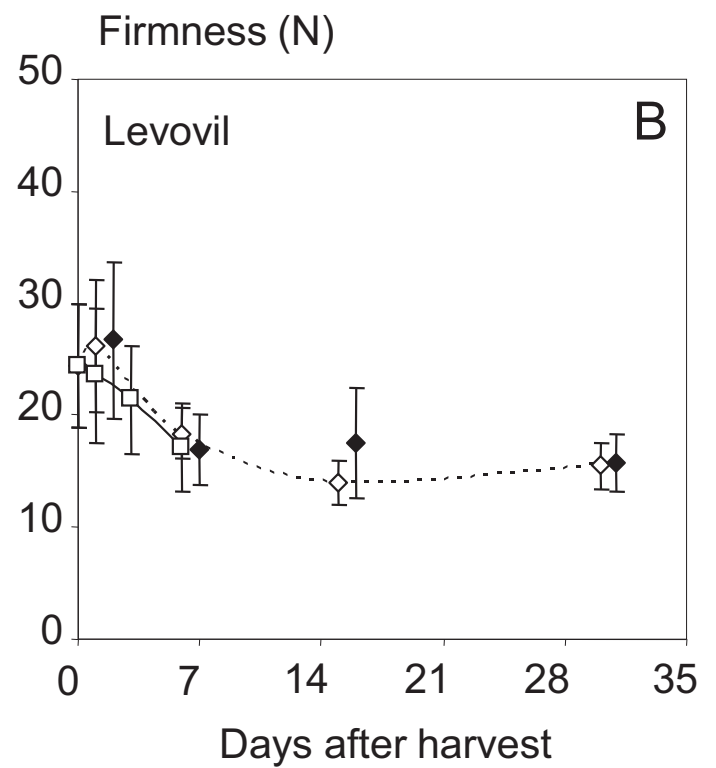
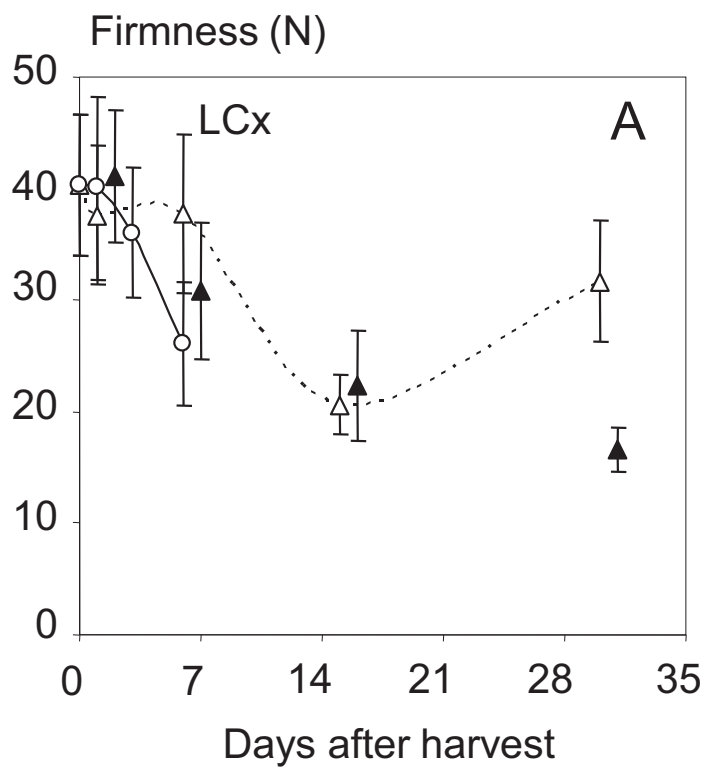
<i>Pooled standard deviation</i>	199.0	10.9	11.1	7.9	12.5	19.8	55.2	51.4	471.6	29.7	5.3	8.0	22.0	32.9	18.5	67.8	11.7	2.3	2.2
----------------------------------	-------	------	------	-----	------	------	------	------	-------	------	-----	-----	------	------	------	------	------	-----	-----

ACCEPTED MANUSCRIPT

Table 6: Time-course evolution of LOX pathway enzymes and substrates in tomatoes during storage at different temperatures. Lipoxygenase (LOX) and hydroperoxylase (HPL) activities are given as variation of absorbance per min per ml of tomato extract in their respective assays. For all data, n = 3 repetitions of 5 tomatoes each.

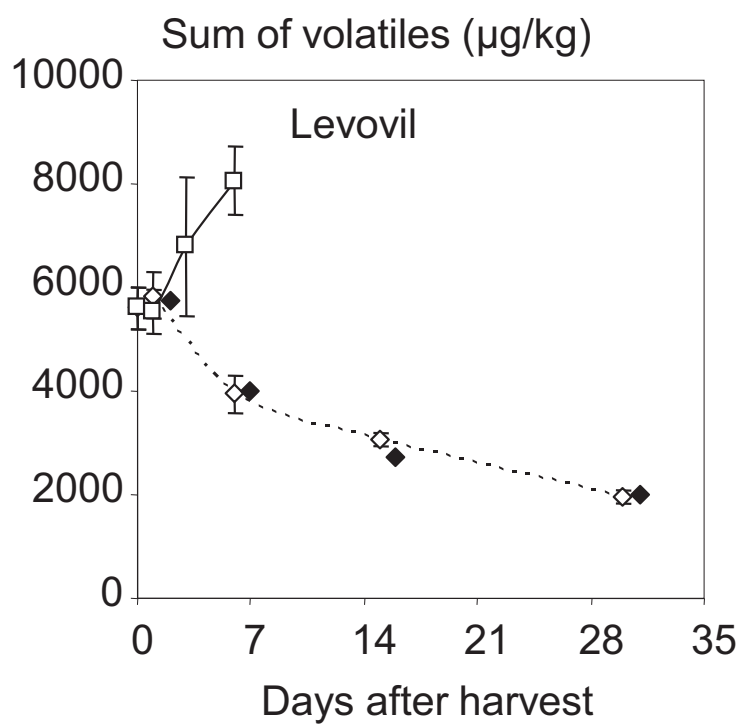
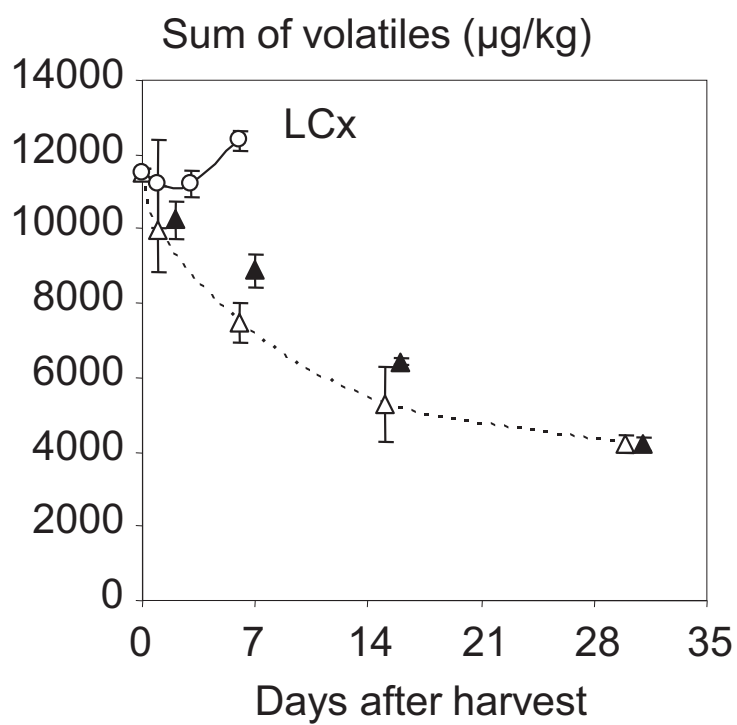
Temp	Storage Duration (days)	Enzymes		Unsaturated fatty acids (mg/kg)			
		LOX	HPL	Linoleic acid	Linolenic acid		
20 °C	0	0.23	0.108	142		19	
	1	0.23	0.114	98		13	
	3	0.25	0.086	123		16	
	6	0.23	0.092	141		17	
	1	0.21	0.083	147		21	
	6	0.34	0.058	172		31	
4 °C	15	0.28	0.071	173		35	
	30	0.34	0.067	165		34	
	1	0.32	0.085	160		22	
4 °C + 1 day 20 °C	6	0.37	0.069	154		29	
	15	0.32	0.084	190		33	
	30	0.29	0.081	182		39	
	0	0.32	0.106	139		23	
	1	0.45	0.118	135		23	
	3	0.55	0.060	137		22	
20 °C	6	0.41	0.078	113		18	
	1	0.35	0.066	94		14	
	6	0.37	0.076	145		24	
	15	0.33	0.070	139		27	
	30	0.29	0.041	201		43	
	4 °C	1	0.45	0.073	109		13
6		0.32	0.087	127		21	
15		0.35	0.094	Not done		35	
30		0.32	0.060	176		41	
<i>Pooled standard deviation</i>		0.095	0.0082	22.1		2.7	

Renard et al.  
Tomato storage  
Fig. 1

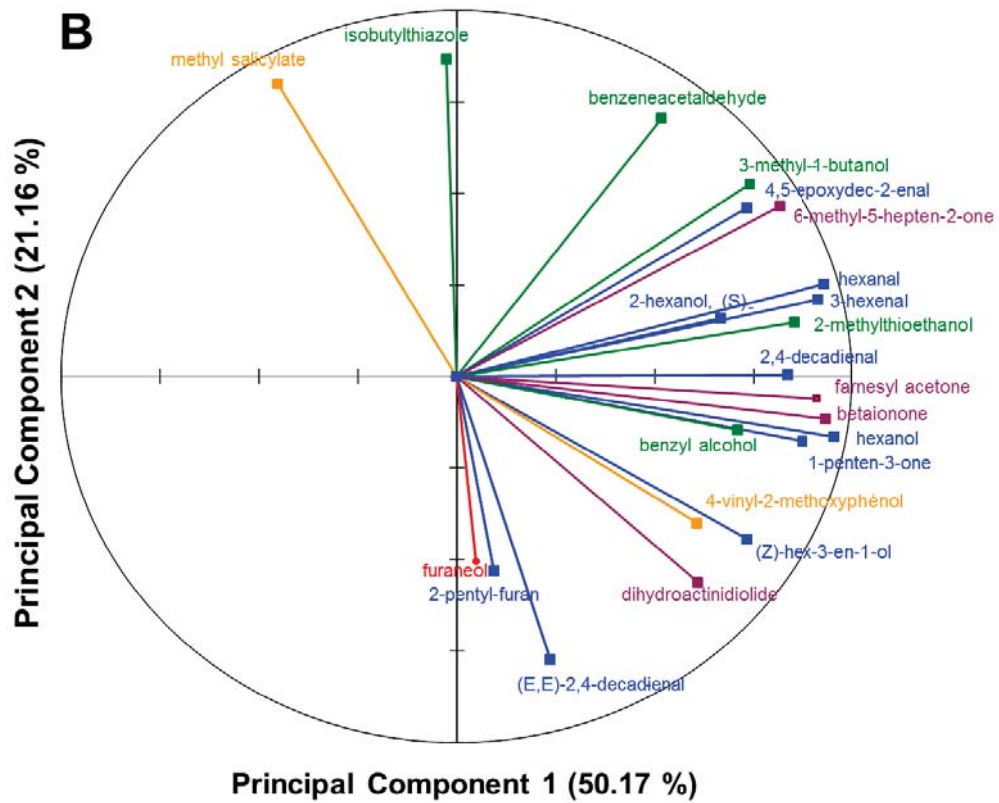
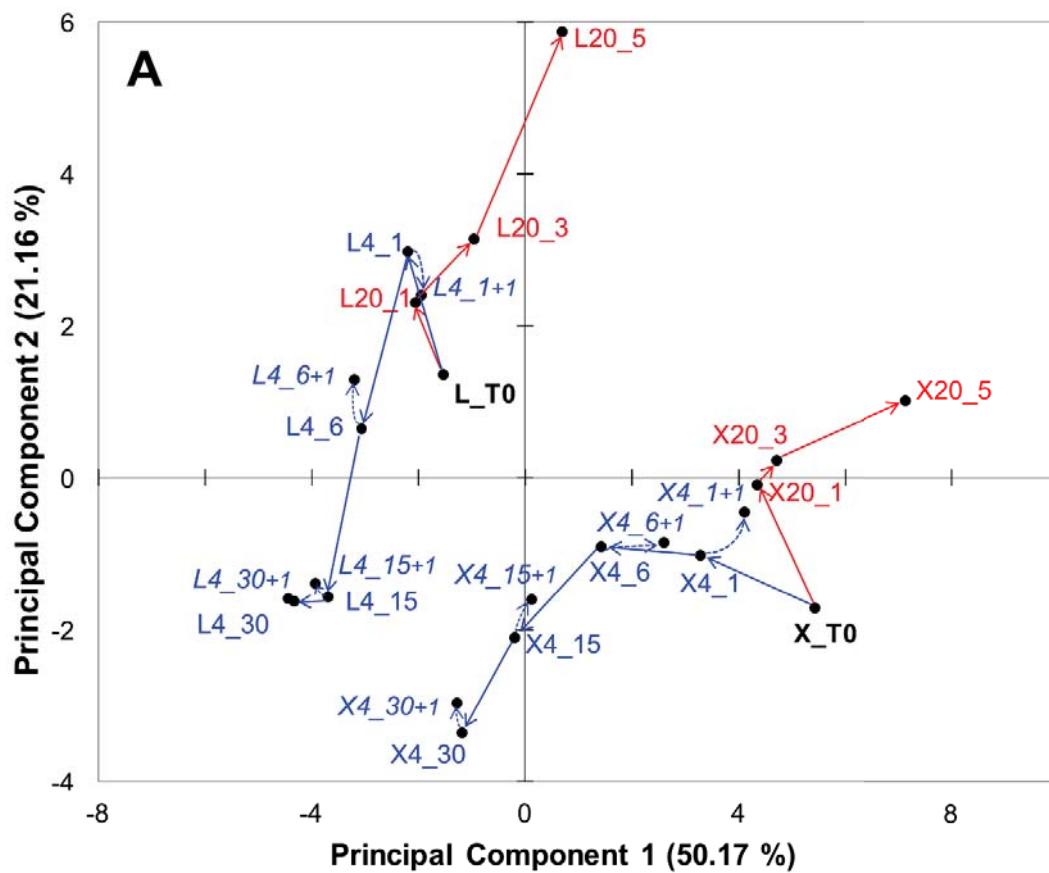




Renard et al.  
Tomato storage  
Fig. 2



Renard et al.  
Tomato storage  
Fig. 3



## Highlights

- We study evolution of volatiles in two contrasted tomato lines at 20°C and 4°C
- Storage at 4°C is detrimental for volatile production and hence aroma
- Volatile levels are not restored by one day at 20°C
- Storage at 20°C up to 1 week leads to increased volatiles, with acceptable texture loss
- This information should be taken up in consumer's information

ACCEPTED MANUSCRIPT