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

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ABSTRACT

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

Two storage modalities (20 °C and 4 °C) and two cultivars (Levovil and LCx) were used. Volatile compounds were analyzed by gas chromatography–mass spectrometry detection after accelerated solvent extraction. Physical characteristics, lipoxygenase activity, hydroperoxide lyase activity; linoleic acid and linolenic acid were monitored.

Storing tomatoes at 4 °C induced a drastic loss in volatiles, whatever their biosynthetic origin. After 30 days at 4 °C, the concentration of volatiles had decreased by 66 %. Reconditioning for 24 h at 20 °C was able to recover some aroma production after up to 6 days storage at 4 °C. Volatile degradation products arising from carotenoids and amino acids increased when tomatoes were kept at 20 °C, while lipid degradation products did not vary.

Storing tomatoes at fridge temperature, even for short durations, was detrimental for their aroma. This should be taken into account to formulate practical advice for consumers.

Keywords

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

1. Introduction

In recent years, consumption of fresh tomatoes by French consumers has stagnated, and market research points to dissatisfaction with the sensory quality of fresh tomatoes on sale. This motivated the Agence Nationale de la Recherche [French national research agency] project “QualitomFil”, which aims to find ways to maintain a high quality supply chain from farm to fork. This study was led under the QualitomFil program. Our hypothesis is that inadequate storage conditions at the consumer’s home contributes to the perceived flavour loss. Little is known about the fate of tomatoes at the last link of this chain — the consumer. The consumer has two main possibilities for tomato storage: at room temperature or in the refrigerator at around 4 °C. The general physico-chemical characteristics of ripe (light red or red) tomato, such as soluble solids and acidity, are little modified during home storage (Auerswald, Peters, Brückner, Krumbein, & Kuchenbuch, 1999; de Leon-Sanchez, Pelayo-Zaldivar, Rivera-Cabrera, Ponce-Valadez, Avila-Alejandre, Fernandez, et al., 2009; Maul, Sargent, Sims, Baldwin, Balaban, & Huber, 2000). However, this is not the case with volatile compounds, and one of the key sensory parameters for tomato frequently mentioned in consumer complaints is loss of its characteristic aroma. This motivated our investigation of the fate of tomato volatiles during storage in domestic conditions.

Data, mostly on unripe tomatoes, indicates that low temperature affects volatiles production. As chilling injury is a well-known risk for this fruit, temperatures > 10 °C are recommended and relatively few studies can be found dealing with the impact of low temperatures on taste and flavour volatiles. Stern , Buttery, Teranishi, Ling, Scott & Cantwell (1994) stored tomatoes harvested mature green, breaker (defined as the maturity stage when the red colour starts becoming visible at the tip of the tomato) to red-ripe at temperatures from 5 °C to 20

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

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

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

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

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

2. Material and methods

2.1 Plant material

Plants were grown in a heated glasshouse in Avignon (southeast France) during spring 2008. Fruits were harvested at the red-ripe stage on Levovil, characterized by its large fruits and pharmaceutical sensory attributes, and LCx, an introgressed line carrying five chromosome regions from the Cervil cherry tomato identified as bearing the quantitative trait loci for quality traits, notably tomato aroma intensity (Chaib, Lecomte, Buret, & Causse, 2006) in the Levovil background. In order to eliminate bias linked to maturation patterns, both tomatoes were harvested at the red-ripe stage. To ensure homogeneous maturity and avoid the presence of overripe tomatoes, plants were first unloaded of all their red fruits and 3 days later the fruits which had ripened from orange to red were harvested. Each line was represented by 18 plants.

2.2 Chemicals

All standard volatile compounds, linolenic acid, yeast alcohol dehydrogenase, soybean type I-B lipoxidase and NADH were purchased from Sigma Aldrich (St Louis, MO). Solvents were from Carlo Erba Reagents (Val de Reuil, France). Hydromatrix™ (diatomaceous earth) was from Agilent Technologies (Les Ulys, France). BF_3/Methanol was purchased from VWR international (Fontenay-sous-Bois, France).

2.3 Tomato storage

At harvest, tomatoes were divided into 12 modalities: fresh; storage at 20 °C for 1, 3 or 6 days; storage at 4 °C for 1, 6, 15 and 30 days; same, followed by 24 h reconditioning at 20 °C.

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

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

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

2.4 Accelerated solvent extraction (ASE)

Five tomatoes were ground for 1 min in a blender (Waring-Nova, Grosseron, St Herblain, France), and approximately 15 g of juice was transferred to a beaker. After 2 minutes, 15 g of Hydromatrix™ and 16 µg of 4-nonanol (internal standard) were mixed with the tomato juice to obtain a homogeneous powder while inactivating enzymes. The powder was rapidly transferred to a 33 ml pressurized extraction cell for immediate extraction. The extractor was an ASE 200 system (Dionex, Sunnyvale, CA). Extraction conditions were: solvent dichloromethane, 100 bar, 40 °C, 5 min preheating then 5 min static incubation. The extract was concentrated to 1 ml by distillation under vacuum (300 Pa, using a Multivapor R12, Buchi, Rungis, France) prior to gas chromatography. This step showed no significant loss in volatile compounds compared to distillation at ambient pressure (Trad, Ginies, Gaaliche, Renard, & Mars, 2012).

2.5 GC-MS

Volatile samples (2 µl) were injected into a GC-MS system (QP2010; Shimadzu, Kyoto, Japan) equipped with a UB Wax capillary column [30 m, 0.32 mm i.d., 0.5 µm film thickness] (Interchim, Montluçon, France) as described in Birtic, Ginies, Causse, Page & Renard (2009). A total of 44 volatile compounds were detected (Table 1).

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

2.6 Fatty acids contents

Fatty acids were analyzed by GC-MS after transesterification to fatty acid methyl esters (FAME). Total lipid extract was based on Schäfer (1998), with modifications. 500 mg of freeze-dried tomato (without seed) was homogenized with 10 g of Fontainebleau sand and transferred into a 11 ml pressurized extraction cell, and then 0.25 mg of nonadecanoic acid (internal standard) was introduced. The extractor was an ASE 200 system (Dionex, Sunnyvale, CA), with $CHCl_3$/Methanol (2/1, v/v) as solvent, 100 bars, 120 °C, 5 min preheating then 5 min static incubation, flushed 50 % and one cycle.

A 1 ml aliquot of extract was transesterified by adding 1 ml of BF_3/methanol (10 %) then incubated at 85 °C for 1 h. After cooling, 2 ml hexane and 2 ml of HCO_3^- 0.2 mol/L were added. The tube was vortexed and the organic phase was dried.

1 µL of FAME was injected on the UBWAX capillary column [30 m, 0.25 mm i.d., 0.5 µm film thickness] (Interchim, Montluçon, France). The injection port was operated in splitless mode for the first 30 s, after which the carrier gas (He) velocity was kept a constant 35 $cm.s^{-1}$. The initial oven temperature of 50 °C was held for 1 min and then ramped up at 20 °C per min to 200 °C then at 3 °C per min to 230 °C. This final temperature was held for 15 min. The mass spectrometer was operated in electron ionization mode at 70 eV with continuous scans (every 0.5 s) from mass-to-charge ratio (m/z) 50 to 360.

Fatty acid levels were expressed in milligrams of methyl nonadecanoate equivalent.

2.7 Enzyme activities

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

LOX activity was measured by increase in absorbance at 234 nm corresponding to the generation of conjugated double bonds in linoleic acid (Surrey, 1964).The supernatant (40 µL) was added to 2 ml of phosphate buffer pH 6 and 40 µl of a linoleic acid solution (dissolved at 6 µg/ml in NaOH 24 mmol/L plus Tween 20 10 mg/l). Absorbance at 234 nm was monitored for 5 min. Activity was expressed as absorbance per min per ml of tomato extract.

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

2.8 Statistical analysis

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

3. Results

3.1 Tomato characteristics

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

3.2 Volatiles in the fresh tomatoes

Total volatiles concentration was much higher overall in LCx than in Levovil (Fig. 2, Tables 3-5). Eugenol and 2-methoxyphenol were detected only in Levovil, and methyl salicylate was >50 times higher in Levovil than in LCx (Table 3). The difference in concentrations was also particularly clear for volatiles originating from phenylalanine and from sulfur-containing amino acids (Table 4). Benzylalcohol concentration was 22-fold higher, benzaldehyde 7-fold higher and 2-phenylethanol about 4-fold higher in LCx than in Levovil. All products identified as originating from sulfur-containing amino acids were 4-7-fold more concentrated in LCx than in Levovil. For products originating from leucine/isoleucine, 3-methyl-1-butanol was 4-fold higher in LCx whereas 2-isobutylthiazole was slightly higher in Levovil. Furaneol (from carbohydrates) was also present in higher concentrations in LCx.

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

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

3.3 Time-course evolution of volatiles during storage

3.3.1.General

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

3.3.2. Global characterization

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

Similar patterns were obtained with the full and the simplified data set. As expected, LCx and Levovil were clearly differentiated on the first plane of PCA (71% of variance) (Fig. 3A). LCx was in the same quadrant as most volatiles in the correlation circle (Fig. 3B), which can be related to its generally higher production of volatiles. The Levovil/LCx separation along principal component 2 also corresponded to the position of the phenylpropanoid derivatives on the correlation circle. Methylsalicylate, (eugenol and 2-methoxyphenol are correlated with methylsalicylate but not represented on the figure) present in Levovil but not in LCx, and 4-vinyl-2-methoxyphenol, almost exclusively present in LCx (Table 3), were opposed. Evolutions of sample projections during storage were very different depending on temperature, but followed similar trends in the two lines (Fig 3A). The evolution observed for storage at 4 °C was negatively correlated with all volatiles while the evolution for storage at 20 °C was correlated with a majority of detected volatiles, which is in agreement with the global evolution described in Fig. 2. Levovil samples moved mostly along axis 2 (up with storage at 20 °C, down with storage at 4 °C) while for LCx the coordinates along axes 1 and 2 were affected to the same extent (up and to the right at 20 °C, down and to the left at 4 °C). The effects of reconditioning at 20 °C for 24 h (samples in italics) were most marked for samples kept for shorter storage durations. For tomatoes that had only been stored 24 h at 4 °C, the volatile compositions after a further 24 h at 20 °C were very close to those of the tomatoes that had only been stored for 24 h at 20 °C. Impact of reconditioning was much lower for long storage at 4 °C.

The PCA map shows that the volatile compositions of the two lines were initially contrasted and stayed clearly distinct during storage; however the evolution trends were affected primarily by storage temperature.

3.3.3. Evolution of LOX products

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

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

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

Volatiles derived from amino acids (Table 5) were mostly strongly correlated with principal component 1 (Fig. 3) but showed a diverse range of behaviours during storage. In particular, 2-isobutylthiazole was strongly correlated with and contributed to principal component 2. Production of 2-isobutylthiazole increased markedly at 20 °C in both lines (Table 5). The two other products from leucine/isoleucine degradation, i.e. 3-methylbutanoic acid and 3-methyl-1-butanol, were on the diagonal of principal components 1 and 2. 3-Methyl-1-butanol concentration increased at 20°C in Levovil but not in LCx, and reached similar levels in both lines after 6 days of storage. Products from the phenylalanine pathway followed a similar pattern 3-methyl-1-butanol. Concentrations of leucine/isoleucine and phenylalanine degradation products were initially high in LCx and stayed high at 20 °C., whereas in Levovil they were initially low but increased markedly during storage at 20 °C. Volatiles originating from sulfur-containing amino acids, illustrated in Fig. 3 by 2-methylthioethanol, increased at 20 °C and for short storage at 4 °C, but decreased upon prolonged storage at 4°C in both lines. As a rule, volatiles originating from amino-acid degradation tended to increase during storage at 20 °C and decrease at 4 °C, but the intensity of their variations were very different for LCx and Levovil.

3.3.5. Time-course evolution of carotenoid derivatives

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

3.3.6.Miscellaneous

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

4. Discussion

4.1 Volatiles are lost during storage at 4 °C

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

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

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

Tomato volatiles were strongly affected by storage at 4 °C, though red-ripe fruits are less sensitive to cold than earlier maturation stages. The decrease in production of LOX compounds under cold conditions has been studied because they are major contributors to tomato aroma. Maul et al. (2000) had already noted a marked decrease in the production of LOX compounds (lipid-derived volatiles) during storage at low temperatures, but this loss cannot be explained in a straightforward manner, and in particular is not explained by a loss of substrate or global enzyme activity, as exemplified in Ties & Barringer (2012) or Bai, Baldwin, Imahori, Kostenyuk, Burns, & Brecht (2011). Bai et al. (2011) observed an increase in total LOX activity after 5-day storage at 5 °C and a decrease in HPL activity with some recovery after 4-day storage at 20 °C, while Ties & Barringer (2012) found a slight increase in total LOX activity, confirming our results. Bai et al. (2011) also indicated that the divergence between enzyme activities (and transcript expression) and LOX product production might be linked to substrate availability. However, as reported by Ties & Barringer (2012), the concentrations of the LOX substrates linoleic and linolenic acid actually increased during storage (Table 2). This divergence is therefore most likely linked to the existence of different isoforms of LOX expressed in ripening tomato. Griffiths, Prestage, Linforth, Zhang, Taylor, & Grierson (1999) reported no impact on generation of C6 aldehydes and alcohols in antisense-transgenic tomatoes in which *TomloxA* and *TomloxB* were inactivated, while the inactivation of *TomloxC* (Chen, Hackett, Walker, Taylor, Lin, & Grierson, 2004) led to a marked reduction in the production of hexanal, hexenal and hexanol. Kovács, Fray, Tikunov, Graham, Bradley, Seymour et al. (2009), studying the effect of ripening mutations on volatile synthesis, also showed that LOX compounds (fatty-acid derived volatiles) appear intimately linked to *TomloxC* expression. Bai et al. (2011) studied the expression of *TomloxA, B, C* and *D* in chilled (5 days at 5 °C) tomatoes and found a downregulation of all but *TomloxD* upon chilling, together with increased LOX activity. The existence of a specific isoform downregulated during cold storage might explain the decrease in LOX volatiles under cold storage without loss of substrates or global LOX activity.

The decrease in phenylpropanoid (eugenol and 2-methylphenol) concentrations in Levovil during storage at 4 °C could actually be beneficial, leading to a loss (or at least a decrease)of the “pharmaceutical” aftertaste.

4.2 Comparison of the two cultivars

The study was performed on two tomato lines known for their contrasted sensory attributes: Levovil, characterized by its large fruits and “pharmaceutical” aftertaste, and LCx, an introgressed line carrying five chromosome regions from the Cervil cherry tomato identified as bearing the qtls for quality traits, notably for tomato aroma intensity in the Levovil background (Causse, Saliba-Colombani, Lecomte, Duffé, Rousselle, & Buret, 2002; Chaib et al., 2006). The overall pattern of volatiles in the freshly-harvested red fruits (Tables 3 to 5) confirms previous research (Birtic et al., 2009; Zanor, Eambla, Chaib, Steppa, Medina, Granell, et al., 2009). The difference in total volatiles production could be partially explained by the smaller size and therefore higher peel-to-flesh ratio of LCx, as higher concentrations of volatiles and their precursors are found in tomato peel (Ties & Barringer, 2012). Eugenol and 2-methoxyphenol are responsible for the presence of "pharmaceutical" aftertaste in Levovil (Causse et al., 2002; Chaib et al., 2006; Zanor al., 2009) and were absent in LCx, as in its Cervil introgression parent (Birtic et al., 2009). The difference between the cultivars could be due to different glycosidation patterns (Tikunov, de Vos, Paramas, Hall, & Bovy, 2010), although Birtic et al. (2009) did not find high accumulation of the corresponding glycosides in Cervil. The two lines also differed in terms of volatiles derived from phenylalanine and sulphur-containing amino acids, though again this was less marked than in the Cervil/Levovil comparison (Zanor et al., 2009). Smaller differences could be noted for volatiles derived from the LOX pathway; concentrations of fatty acid precursors and enzyme activities (LOX and HPL) were also quite similar. The fatty acid contents were in agreement with the levels reported by Gray, Prestage, Linforth, & Taylor (1999). Linoleic acid was by far the predominant fatty acid, with linoleic/linolenic ratio of 7.7 for Levovil and 6.1 for LCx, which are high values in comparison to those reported by Gray et al. (1999) and Ties & Barringer (2012). Hexanal originates from linoleic acid while hexenal originates from linolenic acid. The hexanal/hexenal ratios (0.4 in Levovil, 0.5 in LCx) were about 10-fold lower than the linoleic/linolenic acid ratio, but still much higher than those found by Gray et al. (1999). This indicates preferential conversion of linolenic acid, as was also found by Ties & Barringer (2012). The two lines exhibited the expected characteristics and contrasts, both in terms of physical characteristics and volatiles production, though the differential was less visible than with Cervil, the origin of the five introgressed chromosome region (Birtic et al., 2009; Chaib et al., 2006). The main differences concern volatile production, which was much higher in LCx, and the phenylpropanoid pathway, with only Levovil producing phenylpropanoid volatiles.

5. Conclusion

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

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Caption to figures

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

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

□ : Levovil, stored at 20 °C; ◊ : Levovil, stored at 4 °C; ♦ : Levovil, stored at 4 °C then reconditioned for 24 h at 20 °C.

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

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

□: Levovil, stored at 20 °C; ◊: Levovil, stored at 4 °C; ♦: Levovil, stored at 4 °C then reconditioned for 24 h at 20 °C.

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

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

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

Coding of the sample points: L, levovil; X, LCx; the first number gives storage temperature, followed by duration of storage. The “+1” indicates that the sample was left for 1 day at 20 °C after storage at 4 °C. Thus L_4_15+1 designated tomatoes of the Levovil variety, stored at 4 °C for 15 days then left for 1 day at 20 °C to recover volatile production. Blue denotes storage at 4 °C, and red denotes storage at 20 °C.

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

Tables

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

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

Eugenol	2197	+		Phenylpropanoid[c]
4-vinyl-2-methoxyphenol	2225			Phenylpropanoid[c]
Dihydroactiniodiolide	2387			β-carotene[d]
Farnesylacetone (unknown configuration)	2389			Phytoene, phytofluene[d]

a: quantification on DB5 column; b: from Mayer, Takeoka, Buttery, Whitehand, Naim, & Rabinowitch (2008); c: from Tikunov, Lommen, de Vos, Verhoeven, Bino, Hall et al. (2005); d: from Lewinsohn, Sitrit, Bar, Azulay, Ibdah, Meir et al. (2005); e: from Mathieu, Cin, Fei, Li, Bliss, Taylor, et al. (2009); f: from Schwab, Davidovitch-Rikatani, & Lewinsohn (2008); g: from Hauck, Hübner, Brühlmann & Schwab (2003); h: from 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 Temp	Duration (days)	Weight (g)	Firmness (N)	Colour L*	C*	h* (degrees)
Levovil		0	128	24.5	37.9	31.9	43.2
	20 °C	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		0	31	40.3	37.8	30.6	42.7
	20 °C	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
Pooled standard deviation			*42 / 6*	*5.4*	*1.4*	*2.9*	*2.5*

Table 3: Time-course evolution of volatiles of carotenoid origin, phenylpropanoid origin and carbohydrate origin in stored tomatoes (µg/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	β-ionone	Epoxy β-ionone	Dihydroactinidiolide	Pseudo ionone	β cyclocitral	methyl salicylate	2-methoxy phenol	Eugenol	4-vinyl-2-methoxy phenol	Furaneol
Levovil		0	81	23	36	71	8	10	21	11	3	76	74	35	8	7
	20°C	1	86	24	36	57	7	7	17	10	3	83	55	19	5	5
		3	152	27	54	83	8	8	21	12	3	85	63	27	4	11
		6	358	37	75	122	9	10	28	17	3	77	55	23	3	3
	4°C	1	89	22	28	43	7	8	17	9	3	95	62	31	5	4
		6	96	25	26	33	4	6	21	7	2	53	35	24	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
	4°C + 1 day 20°C	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	58	27	5	13
		15	54	18	30	50	6	5	28	5	2	40	36	17	2	26
		30	43	14	20	40	6	4	33	7	2	3	6	3	1	29
LCx		0	156	22	82	214	16	18	37	10	5	2	0	0	35	34
	20°C	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
	4°C	1	133	22	71	186	14	13	33	9	4	2	1	0	15	29
		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
	4°C + 1 day 20°C	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
		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
Pooled standard deviation	*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 (µg/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			
	Temp	duration (days)	3-methyl-1-butanol,	2-isobutyl thiazole	3-methyl butanoic acid,	Benz aldehyde	Phenyl acetaldehyde	2-phenyl ethanol	Benzyl Alcohol	2-methylthio acetaldehyde	2-methylthio ethanol	3-methylthio propanol	methional
Levovil		0	69	12	51	16	9	52	50	1	28	5	1
	20 °C	1	123	18	78	14	12	56	48	2	37	12	4
		3	306	34	338	16	17	134	66	4	44	16	7
		6	523	66	545	14	34	243	43	5	60	27	10
	4 °C	1	93	25	42	14	10	58	50	2	39	5	3
		6	28	14	10	8	4	24	27	2	61	3	1
		15	10	14	3	5	3	1	4	2	40	2	3
		30	5	9	3	3	2	1	3	0	3	0	1
	4 °C + 1 day 20 °C	1	100	17	61	12	13	27	28	2	43	10	3
		6	51	12	34	6	5	4	5	2	35	6	1
		15	9	12	4	4	5	1	3	1	8	1	2
		30	6	8	6	4	3	1	4	0	0	0	2
LCx		0	308	7	291	109	17	258	1100	5	93	37	10
	20 °C	1	327	8	288	69	16	218	734	7	109	46	10
		3	297	10	255	44	14	208	308	9	136	42	9
		6	349	23	226	35	17	189	158	12	200	66	10
	4 °C	1	254	5	79	55	9	234	769	3	91	46	6
		6	47	4	19	29	9	84	189	5	107	9	2
		15	25	2	14	17	4	1	11	2	36	3	1
		30	13	1	10	14	5	1	4	0	1	1	2
4 °C + 1 day 20 °C		1	318	6	216	51	11	177	584	6	125	67	9
		6	295	5	120	28	6	22	66	7	189	86	7
		15	38	1	30	14	9	2	7	1	14	9	2
		30	15	0	16	12	4	1	4	1	2	3	3

Pooled standard deviations	*49.0*	*4.8*	*59.7*	*5.6*	*3.6*	*20.8*	*49.8*	*1.1*	*24.6*	*9.8*	*1 .2*

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

Storage			From linoleic acid							From linolenic acid												
	Temp	Duration (days)	Hexanal	2-hexanol	Hexanol	(E,E)-2,4-Deca dienal	2,4-Deca dienal	*Cis*-4,5-Epoxy-2-decenal	*Trans*-4,5-Epoxy-2-decenal	(E)-2-Hexenal	(Z)-3-Hexenal	1-penten-3-one	(E)- 2-Pentenal,	1-penten-3-ol	2-pentyl furan,	1-Pentanol	(Z)-2-Heptenal,	(Z)-3-Hexen-1-ol	(E)- 2-Octenal,	1octen-3-one	(E,E)-2,4-nona dienal
Levovil		0	741	81	23	27	9	103	310	66	1 669	115	25	55	71	171	94	101	58	10	7
	20 °C	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
		6	1 024	68	20	29	12	121	336	168	2 048	112	24	56	65	157	114	62	48	8	10
	4 °C	1	894	68	14	19	7	93	271	86	2120	115	25	51	43	124	90	61	36	8	6
		6	259	67	12	21	4	39	106	60	1262	117	26	43	49	57	39	113	20	6	3
		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
	4 °C + 1 day 20 °C	1	776	75	12	30	12	99	265	112	1762	114	24	54	59	140	98	50	51	9	10
		6	325	92	8	21	4	42	112	86	1292	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
LCx		0	1815	106	53	52	34	144	372	490	3024	203	45	119	98	308	180	257	126	23	15
	20 °C	1	1536	90	37	44	35	149	374	371	3508	221	51	119	82	284	170	160	95	18	11
		3	1530	76	44	36	22	178	485	306	3518	217	53	121	83	290	177	227	87	19	10
		6	1899	79	61	38	65	163	417	267	3455	220	51	112	83	321	206	197	87	19	12
	4 °C	1	1217	99	44	39	27	116	303	275	3436	192	48	108	81	224	133	265	80	16	7
		6	739	69	31	36	52	74	188	245	2675	181	44	96	66	165	98	181	55	14	7
		15	417	75	37	43	5	64	166	177	1715	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
	4 °C + 1 day 20 °C	1	1235	121	39	41	31	134	339	239	3274	196	50	109	75	249	153	195	86	19	9
		6	979	80	37	40	37	95	248	237	3011	214	52	113	86	215	137	229	76	18	8
		15	681	66	27	38	6	90	226	249	2489	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

Pooled standard deviation	*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*

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.

		Storage	Enzymes		Unsaturated fatty acids (mg/kg)	
	Temp	Duration (days)	LOX	HPL	Linoleic acid	Linolenic acid
Levovil		0	0.23	0.108	142	19
	20 °C	1	0.23	0.114	98	13
		3	0.25	0.086	123	16
		6	0.23	0.092	141	17
	4 °C	1	0.21	0.083	147	21
		6	0.34	0.058	172	31
		15	0.28	0.071	173	35
		30	0.34	0.067	165	34
4 °C + 1 day 20 °C		1	0.32	0.085	160	22
		6	0.37	0.069	154	29
		15	0.32	0.084	190	33
		30	0.29	0.081	182	39
LCx		0	0.32	0.106	139	23
	20 °C	1	0.45	0.118	135	23
		3	0.55	0.060	137	22
		6	0.41	0.078	113	18
	4 °C	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 day 20 °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
Pooled standard deviation			*0.095*	*0.0082*	*22.1*	*2.7*

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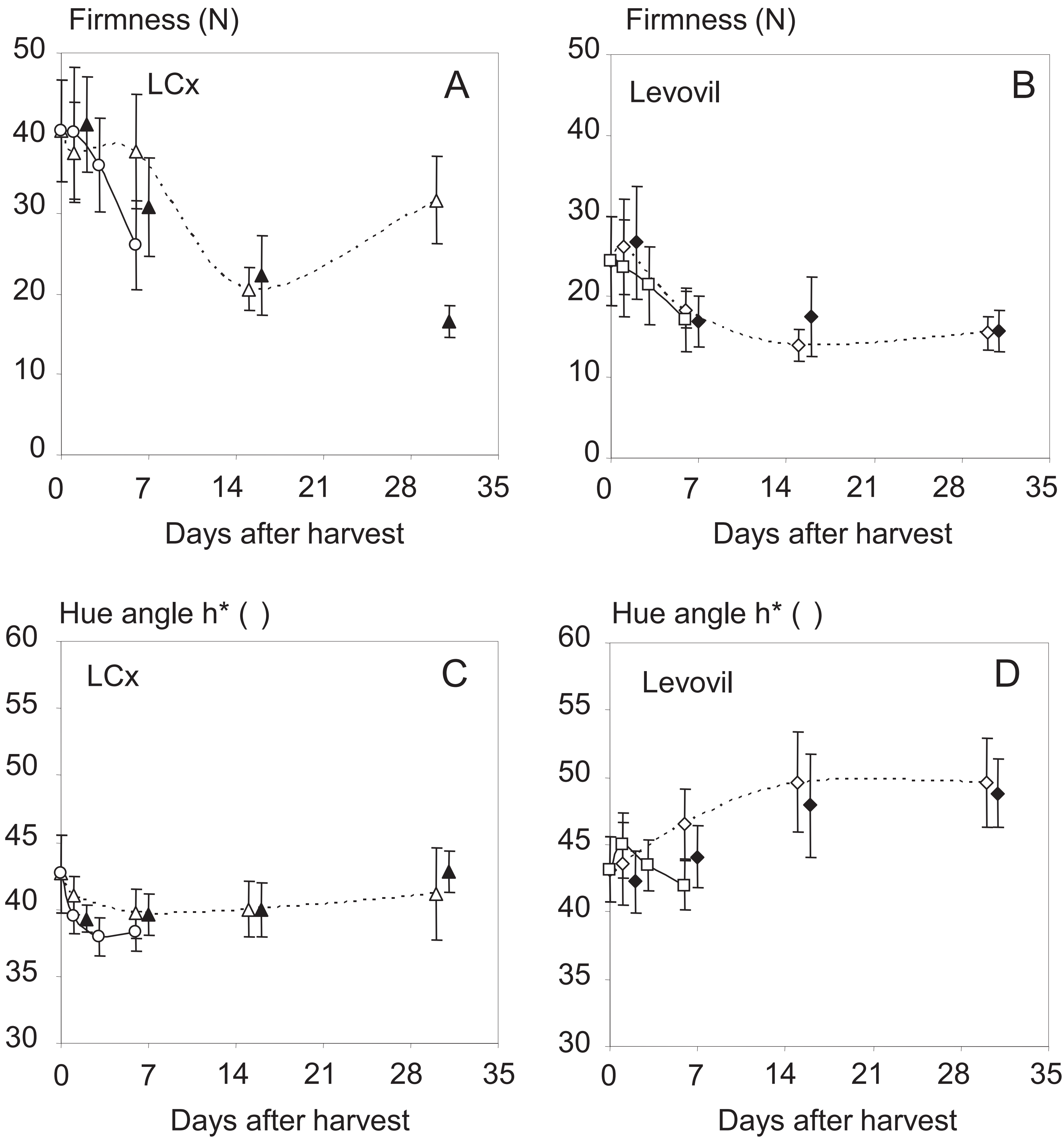
Tomato storage
Fig. 1



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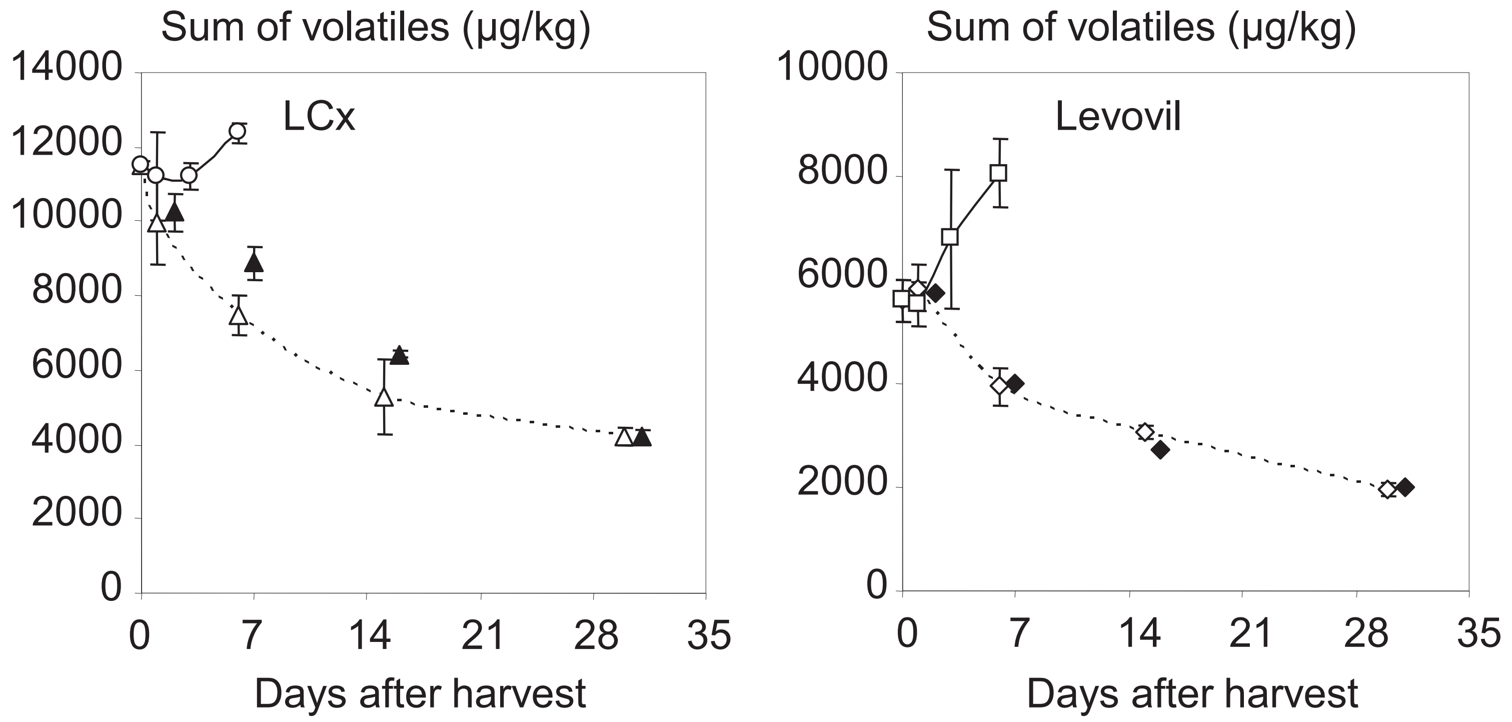
Tomato storage
Fig. 2



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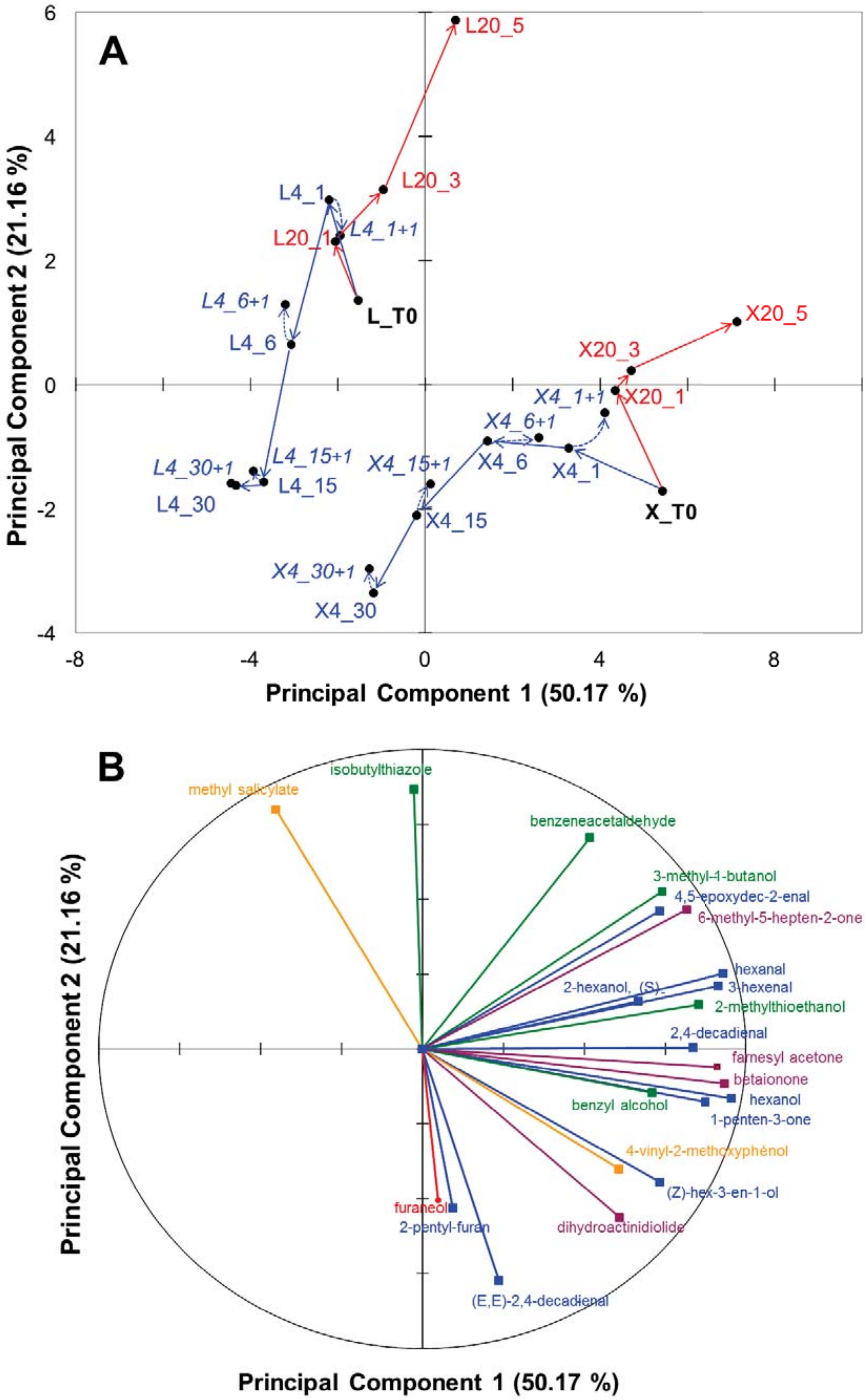
Tomato storage
Fig. 3



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