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Effect of refrigerated storage (12.5 °C) on tomato (*Solanum lycopersicum*) fruit flavor: A biochemical and sensory analysis

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ABSTRACT

Refrigeration is the main postharvest technology for increasing shelf life of horticultural products; however, it has a detrimental effect on tomato (*Solanum lycopersicum*) flavor. The objective of the present study was to evaluate the effect of refrigerated storage at 12.5 °C on tomato aroma profile, sensory quality, and consumer's flavor perception. C6 and 3-methyl butanol volatile levels were determined by GC. Enzyme activity and gene expression of alcohol dehydrogenase (ADH) and lipoxygenase (LOX), involved in the oxylipin biosynthetic pathway were also determined. A quantitative descriptive analysis (QDA) and a consumer test were carried out to compare the effect of refrigerated storage on flavor perception at 10 °C and 12.5 °C to non-refrigerated tomatoes. Refrigerated storage at 12.5 °C caused a general decrease on total aroma volatiles that were detected from 9 d onward. A lack of accumulation of hexanal, hexanol and *cis*-3-hexenol, a transitory increase of *trans*-2-hexenol and the accumulation of 3-methyl butanol were observed. Trained judges perceived these changes as an increase of the musty/damp descriptor which was higher in fruit stored at 10 °C than at 12.5 °C. Tomatoes stored at 10 °C were the less preferred by consumers perceived as less fresh and with the presence of off odors. Consumers did not find differences between tomato stored at 12.5 °C and 20 °C. Results showed that tomato fruit stored at 12.5 °C maintain a better sensory quality than those stored at 10 °C.

1. Introduction

The main objective of research on postharvest physiology and technology is to maintain quality and minimize loses of horticultural products. Quality for fresh consumption has been traditionally based on external physical characteristics such as color, size, and absence of superficial damage and defects. However, hidden quality attributes such as flavor, aroma and nutritional value have not been part of this selection. Currently, the number of consumers asking for horticultural products with higher quality in aroma and flavor is increasing (Baldwin et al., 2000; Bruhn et al., 1991).

The main postharvest technology for increasing horticultural products shelf life is refrigeration. However, it has been reported that this technology affects tomato fruit aroma and flavor and therefore its sensory quality (Baldwin et al., 2011; Díaz de León-Sánchez et al., 2009; Maul et al., 2000; Stern et al., 1994). Baldwin et al. (2011) showed that an increase on the temperature of refrigerated storage combined with the use of 1-MCP maintained a better sensory quality of tomato fruit with an adequate shelf life. This study represents an attempt to find the better storage conditions for maintaining tomato fruit sensory quality without compromising shelf life, taking into account both producer and consumer needs.

Tomato characteristic flavor is the result of complex interactions among organic acids, sugars and more than 400 volatile compounds (Baldwin et al., 2000; Sanz et al., 1997). These volatiles are derived from different metabolic pathways such as lipids, amino acids and carotenoids catabolism (Sanz et al., 1997). Traditionally flavor research has used ortho-nasally measured odor threshold and odor units to establish which aroma volatiles are important in contributing to aroma. With this approach only

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about 16–30 aroma volatiles were predicted to contribute to tomato flavor (Baldwin et al., 2000; Buttery et al., 1987). However, this approach has revealed to be too simplistic to explain the high complexity of our perception of flavor and aroma. Tieman et al. (2012), using a metabolomics approach and looking at variation in flavor associated with sugars, acids and aroma volatiles, conclude that some of the most abundant volatiles do not contribute to consumer liking, whereas other less abundant do. Also, the authors showed that some aroma volatiles contribute to sweetness perception independent of sugar concentration.

In previous studies from this research group, it was shown that the use of the lower limit of the recommended range of storage temperature for light-red tomato (10 °C) changes the aroma profile of the fruit after 6 d of storage and these modifications were detected by a trained sensory panel (Díaz de León-Sánchez et al., 2009). In the present study, the effect of refrigeration at 12.5 °C (a higher temperature) on tomato flavor and aroma perception was determined by a trained panel and complemented by a consumer test to determine if using a higher temperature decreased the effect on the aroma profile perception.

Some of the considered impact aroma volatiles of tomato come from the oxidative degradation of fatty acids by the oxylipins/lipoxygenase pathway (Buttery et al., 1971; Yilmaz, 2001). The key enzymes of this pathway are lipoxygenases (LOX), hydroperoxide lyases (HPL) together with 3Z, 2N-enal isomerases and alcohol dehydrogenase (ADH). Lipoxygenases catalyze the dioxygenation of polyunsaturated fatty acids, such as linoleic and linolenic acids, converting them into their corresponding hydroperoxides (HOPs). HPLs cut the HPOs producing oxiacids and short-chain aldehydes. ADH acts transforming aldehydes in their corresponding alcohols.

There are six genes of LOX that are expressed in tomato fruit (Chen et al., 2004; Shen et al., 2014). Chen et al. (2004) demonstrated by specifically depleting *TomloxC* expression, that this isoform was responsible for the synthesis of C6 aldehydes and alcohols derived from fatty acids, such as hexanal, hexenal and hexenol, considered impact volatiles for tomato aroma. Furthermore, Kovacs et al. (2009) suggested that the negative effect on flavor associated with the use of tomato mutants in which fruit ripening is inhibited/delayed, might be directly correlated to a decrease in the production of volatiles derived from fatty acids as a result of a decrease on *TomloxC* gene expression. Recent evidence showed that *TomloxC* also participates in the biosynthesis of C5 volatile compounds, such as 1-penten-3-ol, 1-penten-3-one, pentanal, (*Z*)-2-penten-1-ol and 1-pentanol (Shen et al., 2014). Díaz de León-Sánchez et al. (2009) observed an increase in the 3-methylbutanal/3methylbutanol and hexanal/hexanol ratio in tomato stored at 10 °C. These changes correlated with a decrease in ADH enzyme activity. Also, these authors observed an increase in *trans*-2-hexenal and *trans*-3-hexenol levels in refrigerated tomato compared to fruit ripened at room temperature. Therefore, it is of interest to analyze the effect of refrigeration on enzyme activity and gene expression of LOX, an enzyme acting earlier than ADH in the biosynthetic pathway of volatiles derived from fatty acids.

Sensory evaluation is an important tool to determine if the human palate perceives the chemical changes. Díaz de León-Sánchez et al. (2009) performed a quantitative descriptive analysis (QDA) to determine if the effect of refrigerated storage at 10 °C on the tomato aroma profile was detected by a trained panel. The judges detected considerable modifications in some of the descriptors generated for describing tomato aroma when comparing refrigerated tomato with freshly harvested fruit. In the present study, a QDA was carried out to determine if refrigeration of tomato fruit at 12.5 °C (a higher temperature) had a smaller effect on the tomato aroma profile perception by trained judges. Also, a consumer test was performed to determine if potential tomato consumers could perceive any aroma or flavor changes in refrigerated tomato compared to non-refrigerated tomato.

The objective of the present work was to study the effect of refrigerated storage at 12.5 °C on tomato aroma profile and to evaluate consumer's perception. The effect of refrigeration on LOX and ADH activities and gene expression was also analyzed.

2. Materials and methods

2.1. Plant material and treatments

Saladette tomato fruit (*Solanum lycopersicum*) '7705' were harvested in a commercial greenhouse in the state of Hidalgo, Mexico, at a light red stage. Fruit were selected for color and size uniformity, divided randomly into two groups and stored at 12.5 °C or 20 °C (control) with an 85 ± 2% relative humidity. Two independent samples (three replicates of 6 fruit) were obtained from each temperature after 0, 2, 4, 6, 9 and 15 d of storage. One sample was used to evaluate the quality parameters and determine aroma volatile levels. From the second sample, a small part was separated to determine ADH activity in the fresh tissue and the rest was frozen with liquid nitrogen and stored for LOX activity determination and RNA extraction.

For the sensory analysis, three sets of 20 fruit each were stored at 10 °C, 12.5 °C and the whole tomatoes were sampled after 6 and 15 d and compared to fresh tomatoes by quantitative descriptive analysis (QDA). For the consumer tests, only tomatoes stored for 15 d were used.

2.2. Determination of quality parameters

2.2.1. Weight loss

Three replicates of 6 tomatoes were weighed using an analytic balance at the beginning of the experiment and at each sampling time (2, 4, 6, 9 and 15 d). Cumulative weight loss was expressed as percentage loss of the initial total weight.

2.2.2. Color and texture

Tomato color was determined with a colorimeter Chroma Meter CR-400 (Konica Minolta, Osaka, Japan) on three locations of each fruit. Lightness (L^*), chroma (C^*) and hue (h) values were registered. Firmness was measured using a penetrometer FT 327 (Effe-Gi, Milan, Italy) and results were reported in Newtons (N).

2.2.3. Titratable acidity (TA) and soluble solid contents (SSC)

To determine TA and SSC content, tomato juice was obtained with a manual juice extractor. SSC was determined with an Atago N-1 alfa hand-held refractometer (Atago Co., Ltd., Tokyo, Japan). TA was measured by titration with 0.1 mol L^{-1} NaOH and it is reported as percentage of citric acid, the dominant acid in tomato fruit.

2.3. Aroma volatiles

To limit the formation of aroma artifacts by oxidative and enzymatic reactions 2 mL of a 0.5 mol L^{-1} EDTA in 0.625 mol L^{-1}

Table 1
Descriptors generated by the trained panel for the five analyzed volatile compounds used in this study.

Volatile	Descriptor
3-methyllbutanal	Rancid–vinegary
3-methyllbutanol	Medicinal-alcohol
Trans-3-hexenol	Musty/damp
Hexanol	Floral-vegetable
Hexanal	Green apple