Scientia Horticulturae 213 (2016) 42–48

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Nutritional qualities and aroma volatiles of harvested red pepper fruits stored at suboptimal temperatures

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ARTICLE INFO

Article history:
Received 7 June 2016
Received in revised form 9 October 2016
Accepted 13 October 2016

Keywords:
Capsicum annuum
Postharvest
Quality
Shelf life

ABSTRACT

Red pepper fruits cv. Cannon stored at suboptimal temperatures of 1.5 or 4 °C for 3 weeks followed by 3 days of shelf-life simulation were evaluated by analyzing selected quality traits and quantifying candidate aroma-related volatiles with a Gas Chromatograph-Mass Spectrometer to understand the quality associated with nutritional level and aroma. Analysis of variance showed that storage temperature and containment in microperforated Xtend® bags significantly influenced the nutritional qualities, with significant interaction between temperature and packaging material. Vitamin C, total phenolic and hydrophilic antioxidant contents were not significantly reduced ($\alpha = 0.05$) at suboptimal storage temperatures of 4 or 1.5 °C in Xtend packaging, compared with those stored at the optimum storage temperature of 7 °C in Xtend® packaging. Aromatic volatiles, which create fruity, spicy, pungent, floral sweet, floral green and bell pepper-like odor notes, were observed. Most of the volatiles decreased in quantity at reduced storage temperatures when Xtend packaging was used. Particularly, 2-isobutyl-3-methoxypyrazine – the source of bell pepper-like aroma notes was found to decrease at lower storage temperatures but the fruits did not lack aroma. Thus quality and aroma of red pepper fruits can be maintained at suboptimal temperatures of 4 or 1.5 °C, which can serve as a quarantine treatment that would simultaneously eliminate Mediterranean fruit fly (*Ceratitis capitata*).

1. Introduction

Bell pepper (*Capsicum annuum* L.) is an important fruit crop, widely cultivated worldwide, and is available in numerous colors. The fruit is a good source of polyphenols, antioxidants, and vitamin C, which are important contributors to human nutrition (Howard et al., 1994), therefore, it is considered one of the most important export fruits worldwide.

Bell pepper fruits can be exported from Mediterranean countries to countries that are free of the Mediterranean fruit fly (*Ceratitis capitata*), but must be subjected to mandatory cold-based – 18 days at 1.1–2.2 °C (US-FDA, 2002) – or heat-based (Neven, 2003) quarantine treatment. Cold-based quarantine treatments of 16–18 days at 1.1–2.2 °C are not feasible for bell peppers because of their susceptibility to chilling injury (CI) at temperatures below 7 °C (Lim et al., 2007). Postharvest heat treatments have been reported to induce cold-temperature tolerance in fruits, and to reduce development of CI symptoms during cold storage (Ghasemnezhad et al., 2008; Sapitnitskaya et al., 2006). An additional way to reduce CI is by using plastic packaging materials: polyethylene bags significantly reduced CI in bell peppers stored at 5 °C (Kosson, 2003).

Recently, cold-based quarantine packaging of bell pepper fruits in plastic film following hot water rinsing over brushes (HWRB) at 55 °C for 15 s enabled pepper storage for 3 weeks at 1.5 or 4 °C, with external fruit quality maintained (Bar-Yosef et al., 2009; Fallik et al., 2012). To date, there are few data regarding changes in aroma and nutritional status during storage, particularly in relation to suboptimal-temperature storage at 1.5 °C – the current practice with respect to postharvest cold quarantine.

Polyphenols in general are well known for their antioxidant qualities and their contributions to the sensory and nutritive qualities of fruit, particularly with regard to color, taste, aroma and flavor (Tomás-Barberán and Espín, 2001). Epidemiological studies have shown that increased consumption of polyphenols may be inversely associated with the occurrence of numerous chronic diseases, including stroke, cardiovascular disease, and cancers. Fruits and vegetables, which are rich in antioxidant molecules, are known for their health-promoting effects against degenerative diseases

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http://dx.doi.org/10.1016/j.scienta.2016.10.015
0304-4238/

(Ames et al., 1993). Vitamin C is required for prevention of scurvy and maintenance of healthy skin, gums and blood vessels; and vitamin C concentration in bell pepper tissue ranged from about 76 to 243 mg per 100 g, and increased as fruits matured (Howard et al., 1994).

During the current decade, consumers have become increasingly demanding with regard to new aromas and flavors, therefore flavor and pungency are now considered important quality parameters (Eggink et al., 2012). The aim of the present study was to investigate the quantitative changes in aroma-related volatiles and nutritional levels of commercial red bell pepper cultivars, as related to their external qualities, and as affected by storage under suboptimal temperatures selected as quarantine treatments. To the best of our knowledge, this is the first such study regarding bell pepper fruits.

2. Materials and methods

2.1. Plant materials

Red pepper fruits cv. Cannon (indeterminate type) of uniform size – about 190 ± 10 g each – without defects or diseases, obtained from Zeraim, Gedera, Israel, were harvested at nearly 90% coloration, once about 6 weeks (based on fruits quality) from December 2013 to March 2014, i.e., three harvests, in the Arava Valley in southern Israel. The fruits underwent HWRB treatment at 55 °C for 15 s, according to commercial practice (Fallik et al., 1999). Then packed in 20 μm thick with high microperforation of 0.0016% (holes of 0.8 mm each) microperforated bags, which are manufactured from proprietary blends of polymeric materials having O_2 permeances 24–48 × 10^{-14} mol s^{-1} m^{-2} Pa^{-1} (according to ATM D1434 at 5 °C) and water vapor transmission rate of ~2 × 10^{-10} mol s^{-1} m^{-2} Pa^{-1} (according to ASTM E96 test at 5 °C) (Xf-100–Xtend®; Stepac, Tefen, Israel) and stored at 1.5, 4, or 7 °C for 21 days followed by 3 days of shelf-life simulation at 20 °C. Unpackaged HWRB-treated fruits served as controls.

2.2. Chilling injury (CI) and chilling index (CINX)

A fruit with a sunken pitting deeper than 2 mm on the skin or calyx was considered damaged, and CI was expressed as the percentage of the total initial number of fruits that were damaged.

The severity of chilling injury was expressed as the CINX, on a 0–3 scale, on which: 0 = no chilling injury; 1 = minor damage – less than 10% of the peel affected; 2 = moderate damage – 10–30% of peel affected; and 3 = severe damage – more than 30% affected. The index was calculated as follows (Bar-Yosef et al., 2009):

$$\text{CINX} = [(N_u \times 0) + (N_{mi} \times 1) + (N_{mo} \times 2) + (N_s \times 3)]/N_t$$

in which: N_u, N_{mi}, N_{mo}, and N_s, respectively, are numbers of fruits with no, minor, moderate, and severe damage.

The CI and CINX were calculated for four 5-kg export cartons of bell pepper fruits.

2.3. Nutritional quality parameters

2.3.1. Vitamin C

Vitamin C content of the bell pepper fruits was measured and calculated in milligrams per 100 g with the HI3850 Ascorbic Acid Test Kit (Hanna Instruments, Smithfield, RI, USA). According to the test kit, 2 g of fresh bell paper fruit was homogenized at high speed for 1 min with 10 mL deionized water in 50 mL vial. The homogenate was filtered by filter paper and kept on the ice. 1 mL of homogenate was mixed with 49 mL deionized water in a beaker. Then 1 mL HI3850A-0 reagent and 4 drops of starch as an indicator were added. HI3850C-0 reagent was added in drops (each drop contains 10 μL), swirled and counted the drops, until a persistent blue color was developed.

2.3.2. Antioxidants content

The antioxidant activity was measured by means of Vinokur and Rodov's (2006) modified version of the 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) (Sigma–Aldrich, Rehovot, Israel) discoloration method. In our study, only hydrophilic fractions were isolated from 100 mg of freeze-dried powder by stepwise extraction with acetate buffer, acetone, and hexane, and repeated partitioning of water-soluble and water-insoluble portions. Antioxidant activity was evaluated by discoloration of the ABTS + radical cation. The radical was generated in acetate buffer medium at pH 4.3, to facilitate the activities of the hydrophilic antioxidants. The final reaction mixture contained 150 μmol of ABTS and 75 μmol of potassium persulfate ($K_2S_2O_8$) in 249 mL of acetate buffer at pH 4.3. Incubation of the reaction mixture at 45 °C for 1 h was sufficient to generate ABTS+. The resulting stock solution of ABTS+ can be stored for up to 3 days at 4 °C without significant loss of properties. The discoloration test was performed in a 96-well microplate by adding 3 μL of test sample to 300 μL of ABTS+ and comparing the optical density at 734 nm after 15 min of incubation at room temperature, with that of a blank sample. Final results were calculated by comparing the absorbance of the samples with that of the standard (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (Sigma–Aldrich). The antioxidant levels in the samples were determined as Trolox equivalents (TE) according to the formula

$$\text{TE} = (A_{\text{sample}} - A_{\text{blank}})/(A_{\text{standard}} - A_{\text{blank}}) \times C_{\text{standard}},$$

where A is the absorbance at 734 nm and C is the concentration of Trolox (mmol). To calculate the TE antioxidant capacity (TEAC) per unit weight of plant tissue, we used the formula, TEAC (mmol TE/mg) = (TE × V)/(1000 × M) in which V is the final extract volume and M is the amount of tissue extracted.

2.3.3. Total phenolics (polyphenols)

Total phenolic contents in bell peppers were determined with the use of Folin-Ciocalteu reagent (Sigma-Aldrich, Rehovot, Israel). Folin's and Ciocalteu's phenol reagent is a combination of phosphotungstic and phosphomolybdic compounds; reduction of the reagent's compounds by oxidation of phosphorus releases blue oxidative byproducts of tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}). One milliliter of double-distilled water (DDW) was added to a vial containing 100 mg of lyophilized pepper tissue. The vial was shaken in an Orbital Shaker Incubator, M.R.C. (Thermo Fisher Scientific, Waltham, MA, USA) at 220 rpm for 1 h at 37 °C and centrifuged for 10 min at 17,968 × *g* and 4 °C (Sorvall RC 6+, Thermo Fisher Scientific, Waltham, MA, USA). The upper layer was removed from the vial and centrifuged again, as before, for another 15 min. The total phenolics test was performed in a 96-well micro plate (Thermo Fisher Scientific, Waltham, MA, USA) by adding 15 μL of the pepper extract, 860 μL of DDW, 25 μL of Fortin-Ciocalteu reagent and 100 μL of 20% (w/v) Na_2CO_3. After 1 h of incubation at room temperature, the intensity of blue coloration in the 96-well plate was measured with an Enspire2300 multi-label reader (Perkin Elmer, Boston, USA) by recording the light absorption at 765-nm wavelength, which was compared with that in a 20 mmol solution of gallic acid that was used as a standard. The results were expressed as mmol gallic acid equivalent (GAE).

The contents of vitamin C, hydrophilic antioxidants, and total phenolics were measured in five different composite samples, each taken from 3 fruits, i.e., a total of 15 fruits in each treatment in every experiment (i.e., harvest).