Food Chemistry 112 (2009) 146–149

Contents lists available at ScienceDirect

Food Chemistry

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

The influence of storage time on micronutrients in bottled tomato pulp

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ARTICLE INFO

Article history:
Received 11 March 2008
Received in revised form 11 April 2008
Accepted 14 May 2008

Keywords:
Lycopene
β-Carotene
Organic acids
Total phenolics concentration
HMF

ABSTRACT

Micronutrients were determined in bottled tomato pulp stored at room temperature (20.0 ± 1.8 °C) for 0, 60, 120 and 180 days. After 180 days, lycopene content had undergone no significant change ($p > 0.05$); ascorbic, malic and citric acid levels had fallen significantly ($p < 0.001$); and there were significant increases in β-carotene level ($p < 0.001$) and total phenolics concentration ($p < 0.01$). The fall in organic acid levels correlated well with the increase in 5-hydroxymethyl-2-furfural (HMF) ($r^2 > 0.80$).

1. Introduction

Tomato is one of the most extensively marketed vegetable foods, with a worldwide production of 126 million Tm in 2005 (FAOSTAT, 2007). Its processed forms include pulp, purée, sauce, juice, paste and peeled whole tomato (Hayes, Smith, & Morris, 1998; Slimestad & Verheul, 2005). It is an important source of carotenes, organic acids and phenolics (Giovanelli & Paradiso, 2002; Loiudice et al., 1995). Consumption of its components, β-carotene and lycopene, has been related epidemiologically to lower incidence of cardiovascular disease and of prostate, gastrointestinal and epithelial cell cancer (Ishida & Chapman, 2004; Rao & Rao, 2007); its organic acids (which influence its shelf-life and organoleptic properties, such as colour brightness and texture) contribute to acid–base balance for the consumer (Adedeji, Taiwo, Akanbi, & Ajani, 2006); and plant phenolics can have anti-inflammatory, anti-allergic and anti-thrombotic properties and may be beneficial in cardiovascular, neoplastic and neurological pathologies (Kuskoski, Asuero, & Troncoso, 2005).

The bioavailability of the nutrient content of tomato products depends on the processing that they have undergone and on the duration and conditions of storage (Sánchez, Plaza, De Ancos, & Cano, 2006). The effects of processing on lycopene, ascorbic acid and phenolics have been studied by several authors (Abushita, Daood, & Biacs, 2000; Calligaris, Falcone, & Anese, 2002; Dewanto, Wu, Adom, & Liu, 2002; Gahler, Otto, & Bohm, 2003; Noble, 1975; Re, Bramley, & Rice-Evans, 2002; Sahlin, Savage, & Lister, 2004; Shi, Maguer, Kakuda, Liptay, & Niekamp, 1999; Takeoka et al., 2001; Toor & Savage, 2006). However, there has been no comprehensive investigation of the stability of these and other micronutrients in tomato pulp during storage. The findings reported here fill this gap in our knowledge.

2. Materials and methods

2.1. Preparation and storage of tomato pulp

A 50 kg sample of tomatoes (*Lycopersicon esculentum* Mill) for industry (cv. Red Spring) was purchased in Extremadura (Spain) during the summer of 2005, divided into six lots, and processed in a pilot-scale industrial plant in accordance with standard industry practices. The tomatoes of each lot were washed, diced mechanically in 1 cm cubes, heated at 80 °C for 1 min, and pulped at 3000 rpm for 2 min. The pulp was brought to pH 4.3 by addition of citric acid, transferred to 370 ml glass jars at 80 °C, sterilized by autoclaving at 120 °C for 30 min, and rapidly cooled to an average temperature of 24.5 °C by cascading water.

The sterilization process parameters were chosen on the basis of previously determined heat penetration parameters (f_h = 39.84 min, j_h = 1.18) so as to achieve a lethality (*F*-value)

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doi:10.1016/j.foodchem.2008.05.051

equivalent to 11 min at 100 °C, assuming $z = 10$ °C. This F value is commonly used in the tomato processing industry to inactivate spores of the major spoilers of tomato products, *Clostridium pasteurianum* and *Bacillus coagulans* (Anese, Falcone, Fogliano, Nicoliand, & Massini, 2002).

The jars of sterilized pulp were stored at room temperature (20.0 ± 1.8 °C) for up to 180 days. On days 0, 60, 120 and 180, six jars were randomly selected and removed for analysis.

2.2. Reagents

Lycopene, β-carotene, 2 N Folin-Ciocalteau reagent, 5-hydroxymethyl-2-furfural (HMF) and 2-thiobarbituric acid (TBA) were purchased from Sigma; 0.1 N hydrochloric acid, acetone, 96% ethanol, tetrahydrofuran (THF), 98% 2,6-di-*tert*-butyl-4-methylphenol (BHT), potassium hexacyanoferrate(II) trihydrate, zinc sulphate heptahydrate, trichloroacetic acid (TCA) and anhydrous citric acid from Panreac Química S.A.; 98% gallic acid, L-malic acid, 99% L-ascorbic acid and citric acid were from Acros and sodium bicarbonate from Probus S.A.

2.3. Quantitation of micronutrients

2.3.1. Lycopene and β-carotene

Extraction was performed, following standard procedures (Lugasi et al., 2003; Pedro & Ferreira, 2005; Sadler, Davis & Dezman,1990). Briefly, 2 g of tomato pulp were weighed in a 250 ml Erlenmeyer flask; 50 ml of solvent (50:25:25 hexane/acetone/ethanol containing 2.5% BHT) were added; the flask was covered with aluminium foil, and N_2 was injected for approximately 20 s. The flask was then placed in crushed ice and shaken for 10 min; 10 ml of distilled water were added, and shaking was continued for a further 5 min. A 4 ml sample of the organic (hexane) phase was then obtained using a Pasteur pipette and filtered twice through a 0.2 μm pore-size nylon filter, and 20 μl of the filtrate were injected into an HPLC apparatus equipped with a UV–VIS diode array detector, a degasser, a temperature stabilizer, a SunFire RP_{18} column (5 μm, 25 × 0.46 cm) and a SunFire C_{18} precolumn (5 μm, 1 × 0.46 cm). As mobile phase, 67:27:6 methanol/THF/water was delivered at 2 ml/min, and the columns were thermostatted at 30 °C.

Lycopene and β-carotene were detected at 475 and 450 nm, respectively, and quantified using calibration curves ($r = 0.999$ and $r = 0.994$, respectively) that had previously been constructed with the corresponding standards, which were also employed for identification. For lycopene, the limit of detection was 1×10^{-8} μg/ml, the recovery was 104%, and the measurement and method coefficients of variation 0.88% and 2.64%, respectively. For β-carotene, the limit of detection was 5.98×10^{-5} μg/ml, the recovery 93.75%, and the measurement and method coefficients of variation 1.49% and 4.24%, respectively.

2.3.2. Organic acids

Organic acids were determined as by Vázquez-Odériz, Vázquez-Blanco, López-Hernández, Simal-Lozano, and Romero-Rodríguez (1994). Approximately 20 g of sample were weighed into a 200 ml beaker; 60 ml of 4.5% (*w*/*v*) metaphosphoric acid were added; the beaker was covered with aluminium foil, and the mixture was stirred magnetically for 15 min and then filtered through Whattmann 135 paper. A 5 ml sample of the filtrate was made up to 10 ml with 4.5% metaphosphoric acid and passed twice through a (0.22 μm)-mesh nylon filter, and 20 μl of this ultra filtrate were subjected to HPLC at 25 °C using a Spherisorb ODS2 RP_{18} column (5 μm, 25 × 0.46 cm), a Spherisorb ODS2 C_{18} precolumn (5 μm, 1 × 0.46 cm) and, as mobile phase, a 0.4 ml/min flow of Milli-Q water brought to pH 2.2 with sulphuric acid. Ascorbic acid was detected at 245 nm, and malic and citric acids at 215 nm. Concentrations (mg/(100 g fresh mass)) were read from calibration curves constructed using the corresponding standards ($r = 0.999$ in all cases). For method validation parameters see Vázquez-Odériz et al. (1994).

2.3.3. Total phenolics concentration

Following Velioglu, Mazza, Gao, and Oomah (1998) and Valverde, Periago, Provan, and Chesson (2002), phenolics were extracted by treating 200 mg of freeze-dried sample with 2 ml of 80:20 (*v*/*v*) methanol–water containing 1% (*v*/*v*) of HCl, covering with ice-flakes, mixing for 2 h in an orbital shaker at 200 rpm, and centrifuging the mixture for 5 min at 5000 rpm. Total phenolics concentration in the supernatant was quantified using Folin–Ciocalteau reagent as by Spanos and Wrolstad (1990) and Toor, Savage, and Lister (2006), as follows; a mixture of 100 μl of supernatant, 0.9 ml of distilled water and 5 ml of 0.2 N Folin–Ciocalteau reagent was neutralized with 4 ml of 7.5% (*w*/*v*) sodium bicarbonate, vortexed for 20 s, and incubated for 90 min at 23 °C (Kim et al., 2005). Absorbance at 765 nm was measured in a UV–Vis spectrophotometer, and total phenolics concentration (expressed in terms of gallic acid equivalents, mg GAE/100 g fresh mass) was read off from a calibration curve ($r = 0.994$) that had previously been constructed using appropriate standards. The method and measurement coefficients of variation were 2.56% and 0.76%, respectively.

2.3.4. 5-Hydroxymethyl-2-furfural (HMF)

HMF concentration (mg/kg fresh mass) was determined by clarifying 5 g samples with Carrez solutions (Porreta & Sandei, 1991) and measuring absorbance at 443 nm (Min & Zhang, 2003).

2.4. Statistical analysis

Results presented are means ± standard deviations of six replicates. The effects of storage time on individual dependent variables were evaluated by ANOVA using a fixed-effects model and taking $p < 0.05$ as the minimum criterion for statistical significance. Differences detected by ANOVA were identified using Tukey's test. All statistical calculations were performed using SPSS 15 for Windows.

3. Results and discussion

Table 1 lists the micronutrient concentrations measured after 0, 60, 120 and 180 days' storage, together with the corresponding ANOVA results.

Lycopene content showed no significant change during storage. Tamburini, Sandei, Aldini, De, and Leoni (1999) similarly found no change in the lycopene content of tomato purée during 1 year's storage. By contrast, Lin and Chen (2005) and Markovic, Hruskar, and Basic (2007) observed a significant fall in lycopene content during similar storage periods at 25 °C. The stability of lycopene in the present study may have been favoured both by the thermal inactivation of enzymes that expose it to oxidants by destroying the cell wall (Tamburini et al., 1999), and by the non-removal of tomato skin, which may protect lycopene from oxidation (Akanbi & Oludemi, 2004). It is also possible that lycopene may have been stabilized by the presence of other antioxidants (ascorbic acid, tocopherol, β-carotene, and phenolics) (Takeoka et al., 2001).

β-Carotene levels increased significantly during storage, by some 46% after 180 days. This finding contrasts with the 40% loss recorded after storage of tomato juice for 210 days at 22 °C (Dietz & Gould, 1986), the loss of over 70% from tomato juice stored for 90 days at 25 °C (Lin & Chen, 2005), and the loss of 12% from tomato soup stored for 60 days at 30 °C (Vashista, Kawatra, &

Table 1
Concentrations of micronutrients in bottled tomato pulp stored for various times and results of the one-factor analyses of variance (storage time)

Storage time (days)	Lycopene[a]	β-Carotene[a]	Total phenolics concentration[b]	Malic acid[a]	Ascorbic acid[a]	Citric acid[a]	HMF[c]
0	14.0 ± 0.68	0.72 ± 0.08	9.42 ± 2.07	20.1 ± 0.52	8.64 ± 0.50	450 ± 17.06	3.95 ± 0.87
60	14.5 ± 1.88	0.89 ± 0.12	9.77 ± 1.82	18.7 ± 0.91	6.38 ± 0.87	433 ± 20.51	4.48 ± 0.34
120	12.8 ± 0.55	0.85 ± 0.12	11.4 ± 1.42	12.3 ± 1.00	4.46 ± 0.45	321 ± 22.40	9.06 ± 0.83
180	14.5 ± 0.60	1.05 ± 0.08	12.5 ± 1.66	9.91 ± 0.90	4.10 ± 0.47	131 ± 2.63	9.94 ± 0.15
ANOVA	NS	***	**	***	***	***	***

Statistical significance levels: NS, not significant; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.
[a] mg/(100 g fresh mass).
[b] (mg GAE)/(100 g).
[c] mg/(kg fresh mass).

Sehgal, 2003). As in the case of lycopene, the discrepancy may be due to the inclusion of tomato skins in the stored pulp. Reboul et al. (2005) found that tomato paste that was 6% skin had 99% more β-carotene than skin-free paste so, in the present study, measured β-carotene levels may have increased due to the hydrolysis, during storage, of β-carotene–protein complexes present in skin.

Total phenolics concentration was almost 33% higher after 180 days' storage than at the beginning of the study. Lavelli and Giovanelli (2003) suggested that the increased total phenolics concentrations of stored tomato products may be due to hydrolysis processes. Lavelli, Hippeli, Peri, and Elstner (1999) point to two specific causes: the release of free hydroxyl groups through hydrolysis of flavonoid glycosides, and the release of phenolics by cell walls.

The concentrations of all three organic acids fell significantly during storage: malic acid by 51%, ascorbic acid by 53%, and citric acid by 71%. However, there were differences in the dynamics of this process: loss of malic acid was greatest between days 60 and 120, and only became statistically significant at this latter time; loss of ascorbic acid was already statistically significant after 60 days and continued at approximately the same rate for a further 60 days, but levelled off thereafter; while loss of citric acid increased at an accelerating rate, becoming statistically significant after 120 days. In previous studies, ascorbic acid losses of 44% and 53% have been observed in tomato products stored, respectively at 20 °C (Saccani et al. (2001) and 30 °C (Vashista et al., 2003), and a 60% loss from tomato juice was reported following 180 days' storage by Wiese and Dalmasso (1994). The losses of malic and ascorbic acids are probably attributable to oxidation; in particular, oxidation of ascorbic acid to dehydroascorbic acid is followed by hydrolysis of the latter to 2,3-diketogulonic acid, which then undergoes polymerization to other nutritionally inactive products (Dewanto et al., 2002).

HMF concentration rose by 152% during the study. This rise correlated well with losses of malic acid ($r^2 = 0.978$; see Table 1), ascorbic acid ($r^2 = 0.815$; see Table 1) and citric acid ($r^2 = 0.815$), suggesting a relationship between organic acid loss and HMF formation. A similar pattern was observed in stored tomato juice by Min and Zhang (2003), who conclude that carbonyl groups released by the degradation of ascorbic acid react with lysine, glutamic acid and other amino acids to form non-enzymatic browning products. Kus, Gogus, and Eren (2005) likewise reported that heating tomato products caused non-enzymatic browning due to their sugar and organic acid contents, while Resnik and Chirife (1979) rated malic acid as a good catalyst of the degradation of fructose and glucose.

In conclusion, in this study, the lycopene content of bottled tomato pulp remained stable during 180 days' storage; β-carotene and total phenolics concentrations rose significantly, while the concentrations of malic, ascorbic and citric acids all underwent significant reductions that correlated well with an increase in HMF concentration.

Acknowledgement

This work was financially supported by the Ministerio de Ciencia y Tecnología (Referencia AGL 2002-03018 ALI).

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