Effects of postharvest hot air treatments on the quality and antioxidant levels in tomato fruit

Gloria Soto-Zamora\textsuperscript{a}, Elhadi M. Yahia\textsuperscript{b,}\textsuperscript{*}, Jeffrey K. Brecht\textsuperscript{c}, Alfonso Gardea\textsuperscript{a}

\textsuperscript{a}Centro de Investigaci\'on en Alimentaci\'on y Desarrollo, Hermosillo, Sonora 83000, M\'exico
\textsuperscript{b}Facultad de Qu\'imica, Universidad Aut\'onoma de Queretaro, Cd Universidad, Queretaro 76010, Qro., M\'exico
\textsuperscript{c}Horticultural Sciences Department, University of Florida, Gainesville, FL 32611-0690, USA

Received 12 February 2004; received in revised form 3 August 2004; accepted 11 August 2004

Abstract

‘Rhapsody’ tomatoes heated for 24 h in air at 34 or 38 °C were compared to fruit heated in 5% O\textsubscript{2} at 38 °C in order to determine if heat treatment applied in reduced O\textsubscript{2} pressure might reduce stress-related oxidative changes that sometimes accompany heat injury. Fruit were subsequently stored at 4 or 10 °C for up to 30 d. Unheated fruit and those heated in air at 34 °C for 24 h developed the best color during storage at 10 °C. Storage at 4 °C inhibited carotenoid development in all treatments. Fruit heated in air or in 5% O\textsubscript{2} lost the most ascorbic acid and isoascorbic acid. Glutathione reductase activity at the end of storage was similar in all fruit, while glutathione S-transferase activity was higher in fruit that had initially been heated in 5% O\textsubscript{2}. Heating of ‘Rhapsody’ tomato fruit in air at 34 °C for 24 h prior to storage at 10 °C for up to 30 d resulted in the least losses in antioxidant content, and fruit color developed adequately. Reduced O\textsubscript{2} neither improved the efficacy of the heat treatment in reducing chilling injury nor protected tomato fruit from the negative effects of heat treatment.

© 2004 Swiss Society of Food Science and Technology. Published by Elsevier Ltd. All rights reserved.

Keywords: Lycopersicon esculentum; Postharvest; Carotenoids; Lycopene; Ascorbic acid; Isoascorbic acid; Cysteine; Glutathione; Glutathione reductase; Glutathione S-transferase

1. Introduction

Tomato is an important agricultural commodity worldwide. Tomatoes and tomato-based products are considered healthy foods for several reasons. They are low in fat and calories, cholesterol-free, and a good source of fiber. In addition, tomatoes are rich in vitamins A and C, \( \beta \)-carotene, lycopene (Mangels, Holden, Beecher, Forman, & Lanza, 1993) and other antioxidants (Davies & Hobson, 1996). According to epidemiological studies, tomato carotenoids may play an important role in the prevention of diseases such as cancer, cataracts, and heart diseases (Siemensma, 1996; Agarwal & Rao, 2000; Rao & Rao, 2003). Antioxidants work singly and synergistically to prevent or delay oxidative reactions that lead over time to degenerative diseases, including cancer, cardiovascular diseases, cataracts, and other diseases (Block, Patterson, & Subar, 1992).

Nevertheless, tomato fruit are sensitive to chilling injury (CI) at temperatures below 11 °C (Cheng & Shewfelt, 1988). Heat treatments have been reported to ameliorate CI, as well as having other positive effects such as control of ripening, decay and insects (Lurie, Laamim, Lapsker, & Fallik, 1997; Yahia & Ortega, 2000). Heat treatments applied in controlled atmospheres have been investigated only for their role in controlling insects (Ortega & Yahia, 2000; Yahia & Ortega, 2000), but not on other effects on the host fruits such as ameliorating CI. Heat treatments may negatively affect the antioxidant content of fruits and vegetables (Jonsson, 1991), however, the application of these treatments in reduced O\textsubscript{2}-controlled atmospheres may

\textsuperscript{*}Corresponding author. Tel./fax: + 52-442-2281416.
\textit{E-mail address:} yahia@uaq.mx (E.M. Yahia).

0023-6438/30.00 © 2004 Swiss Society of Food Science and Technology. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.lwt.2004.08.005
prevent this effect. The objective of this work was to study the effect of postharvest hot air treatments in air at 34 or 38 °C or in 5% O₂ at 38 °C for 24 h at 50% RH on the quality and changes in antioxidants in tomato fruit.

2. Materials and methods

Tomatoes (Lycopersicon esculentum, Mill. cv Rhapsody) were grown in a greenhouse (Agros, Colon, Querétaro). Fruit were harvested at the mature-green stage and were brought immediately to the laboratory, selected for uniformity of size, colour, and freedom from defects, and classified into four homogeneous groups of 160 fruit each. Control (unheated) fruit were placed into storage at either 4 °C and 85% RH (vapour pressure deficit of 0.13 kPa) or at 10 °C and 85% RH (vapour pressure deficit of 0.19 kPa) for up to 30 d. A second group was heated in air at 38 °C of 50% RH (vapour pressure deficit of 2.68 kPa), another group was heated in 5% O₂ (balance N₂) at 38 °C and 50% RH (vapour pressure deficit of 3.33 kPa), and the last group was heated in air at 34 °C and 50% RH (vapour pressure deficit of 2.68 kPa). The hot air treatments were repeated using different lots of tomatoes. Immediately after heating, fruits were cooled in water at ambient temperature for 30 min and then placed into storage at 4 or 10 °C as above. Eight, single fruit samples per treatment were evaluated prior to storage and subsequently samples were taken every 3 d during storage.

Heat treatments and controlled atmosphere treatments were conducted inside a gas-tight, temperature-controlled, forced-air chamber (Ortega & Yahia, 2000; Yahia & Ortega, 2000). The chamber (156 cm high, 70 cm wide and 132 cm deep) was constructed from stainless steel (Techni-Systems, Chelan, WA, USA). Chamber temperature was elevated above room temperature for 30 min and then placed into storage at 4 or 10 °C as above. Eight, single fruit samples per treatment were evaluated prior to storage and subsequently samples were taken every 3 d during storage.

Chamber temperature was maintained within ±0.1 °C in the range of 20–60 °C by automatically energizing four 1000 W finned, 230 V electric heater elements as required. Humidity was provided via four atomizing nozzles, each with two ports; one for compressed gas and one for water. When the compressed gas passes through the nozzle it draws distilled water from the humidification water reservoir into the nozzle using the Venturi effect. When more humidity is required, two solenoid valves are turned on simultaneously; each allowing compressed air to flow through the nozzles. Air entrance to the chamber had a velocity of 5 m s⁻¹ and air velocity inside the chamber averaged 3 m s⁻¹. Length of treatment was measured from the time of sealing the chamber and turning-on the heat and gas flow. The O₂ and CO₂ concentrations were controlled by injection of air and N₂. The CO₂ was maintained at less than 1%. An electrochemical O₂ analyser with a range from 0 to 25% and an infrared CO₂ analyser with a range from 0 to 80% (David Bishop Instruments, East Sussex, UK) were used.

Weight loss was evaluated by weighing individual fruit before and after each treatment and at each evaluation. Colour was determined using the CIELAB system with a Minolta CM-2002 colorimeter operated with SpectraQC version 7.2 software (Minolta, Tokyo).

Chlorophyll content was measured in fresh tomato (pericarp plus epidermis) samples. Tomato samples (3 g) were incubated in 15 ml of ethanol for 16 h, homogenized, filtered through two layers of nonwoven sponges, and the absorbance determined with a DU-65 Spectrophotometer (Beckman Coulter, Inc., Fullerton, CA, USA) at 660 and 642.5 nm. Total chlorophyll (mg l⁻¹) was calculated according to the following equation (AOAC, 1997): (7.12 × A660) + (16.8 × A642.5).

The extraction of carotenoids was done according to AOAC (1997) with some modifications. Fresh tomato (pericarp plus epidermis) tissue samples (5 g) were homogenized for 1 min with 15 ml of hexane:acetone:ethanol:toluene (10:7:6:7 v:v), after which 1 ml of methanolic 1 M KOH was added, the mixture was homogenized again, and held for 16 h. The mixture was then filtered, and the absorbance was determined in the spectrophotometer at 470 nm.

Lycopene was extracted in a manner similar to that for total carotenoids but using freeze-dried tomato tissue samples and, after addition of methanolic 1 M KOH, the samples were heated at 56 °C for 20 min, then immediately placed in a water bath at room temperature. The upper phase was removed and filtered through a 0.2 μm nylon membrane. Lycopene was determined by HPLC (Hewlett Packard 1100 series; Hewlett Packard/Agilent Technologies, Palo Alto, CA) with 4.6 × 150 mm YMC carotenoid C-30 column (YMC, Inc., Wilmington, NC, USA), and a photodiode detector. The mobile phase used was tert-butyl methyl ether:water:methanol in a linear gradient from 15:4:81 to 90:4:6 (v:v) in 90 min, and the flow was 0.75 ml min⁻¹. The absorbance was determined at 471 nm.

Ascorbic and isoascorbic acids were extracted according to Zapata and Dufour (1992). Quantification was done by HPLC equipped with a diode array detector and a 10 μm μBondapak C18 Column (3.9 × 300 mm) with a Sentry, 10 μm μBondapak C18 (3.9 × 20 mm) guard column (Waters, Milford, MA, USA). The mobile phase used was methanol:water (5:95 v:v) containing 5 mM hexadecyltrimethylammonium bromide and 50 mM potassium dihydrogen phosphate at 1.5 ml min⁻¹. Detection was done at 261 nm.
Cysteine, and reduced and oxidized glutathione contents were determined using the method of Farris and Reed (1987) with some modifications. A sample of 0.25 g of freeze-dried tissue was homogenized in 2.5 ml of an aqueous solution containing 10 g kg⁻¹ perchloric acid and 1 mM of bathophenanthroline disulfonic acid. The homogenates were centrifuged at 7800 × g₀, for 20 min at 4 °C, the supernatant collected, the pellets were re-suspended using the same extraction process, and supernatants were combined. A 0.5-ml aliquot of the extract plus 50 μl γ-glutamyl-glutamate (0.5 mM) were carboxymethylated with 50 μl of 100 mM iodoacetic acid dissolved in 0.2 mM m-cresol purple, and the solution was brought to pH 9–10 with a mixture of KOH (2 M) and KHCO₃ (2.4 M), and was incubated in the dark for 15 min. Samples were then derivatized with 1 ml of 1% 2,4-dinitro-1-fluorobenzene and kept in the dark at 4 °C over night. The following day they were centrifuged at 13,000 × g₀, for 15 min and filtered through a 0.2 μm nylon filter before injection in the HPLC. Mobile phase A was 80 g kg⁻¹ methanol. Mobile phase B was prepared by adding 800 ml of a solution containing 1 Kg of sodium acetate in 448 ml of water and 1.39 l of glacial acetic acid added to 3.2 l of 80% methanol. HPLC run was started with 80% A and 20% B for 5 min, followed by a 10 min linear gradient to 1% A and 99% B, maintained for 2 min, and then returned to the initial conditions over a period of 5 min linear gradient, and maintained under that condition for 15 min for re-equilibration of the column. A 50 μl sample of the carboxymethylated and derivatized extract was injected onto a 5 μm Allsphere amino column (250 × 4.6 mm²), the flow rate was 1 ml min⁻¹ and the absorbance was read at 365 nm.

For glutathione reductase (EC 1.6.4.2) activity, 0.15 g freeze-dried tissue samples were homogenized in 2.5 ml of 0.1 M potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 1 mM 2-mercaptoethanol, 0.5% Triton X-100, and 2 g kg⁻¹ PVPP, and centrifuged at 17,400 × g₀, for 20 min. The reaction medium contained 1000 μl of potassium phosphate buffer (0.2 M, pH 7.5), 150 μl of NADPH (2 mM), 150 μl of 20 mM oxidized glutathione (GS-SG), 100 μl of enzyme extract, and 600 μl of water (Iio, Kawaguchi, Sakota, Otonari, & Nitahara, 1993).

Glutathione S-transferase (EC 2.5.1.18) was assayed in the same manner as for glutathione reductase. The reaction medium contained 1585 μl of 0.1 M potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 15 μl of 150 mM CDNB (1-chloro-2,4-dinitrobenzene), 100 μl of 75 mM reduced glutathione (GSH), and 300 μl of enzyme extract (Iio et al., 1993).

Protein content was determined according to Bradford (1976) using bovine serum albumin as the standard. Statistical significance was determined by analysis of variance (P < 0.05), and means comparisons (Student’s t, P < 0.05), using a computer program (JMP 4.0.4, SAS Institute Inc.). Treatments were repeated twice with different lots of tomatoes.

3. Results and discussion

Temperature in the heat treatment chamber increased during the first 3 h and then stabilized at the set temperature, and the desired O₂ concentration was obtained within the first 0.5 h of the treatment (Fig. 1).

Weight loss increased during storage and was exacerbated by heat treatment and higher storage temperature (Fig. 2). Control (not heated) fruit had the least weight loss, with a final value of 5.8% at both 4 and 10 °C storage. Fruit heated in air at 34 °C for 24 h and stored at 4 °C had a final weight loss of 8.8 g kg⁻¹, while those stored at 10 °C had a weight loss of 9.8 g kg⁻¹. Fruit heated in air at 38 °C for 24 h and stored at 4 °C had a final weight loss of 10.5 g kg⁻¹, while those stored at 10 °C had a final weight loss of 11.2 g kg⁻¹. Exposure to 5% O₂ while heating fruit at 38 °C for 24 h reduced weight loss compared to heating in air. Fruit from the reduced O₂ heat treatment stored at 4 and 10 °C had final weight losses of 8.0 g kg⁻¹ and 9.8 g kg⁻¹, respectively. Storage duration, storage temperature, and treatment had significant effects on weight loss. Kumar, Ghuman, and Gupta (1999) and Lurie and Sabehat (1997) reported similar results.

Fruit heated in air at 38 °C for 24 h then stored at 4 °C or 10 °C showed only a very slight increase in a* value and a slight decrease in hue angle value, and they did not

![Fig. 1. Temperature and oxygen behaviour during the application of the hot air treatments.](image-url)
develop adequate red colour (Fig. 2). Fruit heated in air at 34 °C for 24 h then stored at 10 °C and the nonheated (control) fruit stored at 10 °C developed the highest a* value and smallest hue angle value, followed by fruit heated in air at 38 °C for 24 h then stored at 10 °C, nonheated fruit stored at 4 °C, and fruit heated in air at 34 °C for 24 h and stored at 4 °C. Similar results were obtained by Arias, Lee, Logendra, and Janes (2000) who reported an increase in a* value (red colour) due to fruit ripening. Storage duration, storage temperature, and treatment as well as their interactions had significant effects on a* and hue angle values (P < 0.001, \( r^2 = 0.84 \)). The correlation (r) of some colour components with total carotenoids and lycopene content were as follows: a*: 0.84 and 0.85, hue value: 0.83 and 0.83, a*/b*: 0.83 and 0.82, and (a*/b*)²: 0.65 and 0.64, respectively.

Chlorophyll decreased during storage (Fig. 3) and the decrease was greater in fruit that were not heated and in those heated in air at 34 °C for 24 h. Chlorophyll slightly decreased in 10 °C-stored fruit that were previously heated in air or in 5% \( \text{O}_2 \) at 38 °C for 24 h. Storage duration, treatment and temperature interactions had a significant effect (P < 0.05, \( r^2 = 0.73 \)). Sozzi, Cascone, and Fraschina (1996) showed a decrease in tomato chlorophyll content during storage. The increase in temperature from 21 to 40 °C in 48 h decreased the degradation of chlorophyll (Sozzi et al., 1996). Chlorophyll content in the pericarp of tomato fruit was also reported to decrease during hot air treatments at 35 to 40 °C (Lurie & Klein, 1991).

Total carotenoids were highest in fruit that were not heated and those heated in air at 34 °C for 24 h then stored at 10 °C (Fig. 3). They were lower in fruit heated in air at 38 °C for 24 h, and stored for up to 30 d at 4 °C and 10 °C (Fig. 3). Data are the average of 6 observations. Vertical bars indicate standard error of the mean.

Fig. 2. Weight loss (g kg⁻¹), a* value and hue angle value in tomato fruit that were not heated or heated in air at 38 °C, in 5% \( \text{O}_2 \) at 38 °C, or in air at 34 °C, and 50% RH for 24 h, and stored for up to 30 d at 4 °C or 10 °C. Data on weight loss are the average of nine observations and a* and hue data are the average of 18 observations. Vertical bars indicate standard error of the mean.

Fig. 3. Total chlorophyll, carotenoids, and lycopene contents in tomato fruit that were not heated or heated in air at 38 °C, in 5% \( \text{O}_2 \) at 38 °C, or in air at 34 °C, and 50% RH for 24 h, and stored for up to 30 d at 4 °C or 10 °C. Data are the average of six observations. Vertical bars indicate standard error of the mean.
that α-carotene exists only in small quantities in tomatoes.

The main symptom of abnormal ripening in tomatoes exposed to high temperatures was found to be the deficiency in the synthesis of lycopene (Hamauza & Chachin, 1995). Short periods of exposure to high temperatures had a significant negative effect on the ripening of tomato fruit at 20 °C after 4–8 d of storage at 2.5 °C (Saltveit & Cabrera, 1987). On the other hand, Sozzi et al. (1996) reported an increase in lycopene content in heat-treated tomatoes; however, lycopene decreased as the temperature increased from 21 to 40 °C for 48 h. Cheng, Flores, Shewfelt, and Chang (1988) observed that hot air at ≥38 °C inhibited lycopene synthesis in tomato fruit. Lurie, Handros, Felix, and Shapiro (1996) reported that low temperature inhibited ripening as well as lycopene synthesis in tomato fruit.

The ascorbic acid content in fruit that were not heated initially increased but then decreased to a value of 1.45 g kg⁻¹. There was a general decreasing tendency in heated fruit that the lowest ascorbic acid values were obtained in fruit heated in air or 5% O₂ at 38 °C for 24 h (Fig. 4). Storage duration, storage temperature, treatments, and their interactions had significant effects (P < 0.05, r² = 0.98). The isoascorbic acid content in nonheated fruit had values between 0.7 and 1.35 g kg⁻¹, and was higher in fruit stored at 10 than those stored at 4 °C (Fig. 4). Heat, especially 38 °C in air or 5% O₂, resulted in lower isoascorbic acid levels. All factors and their interactions had significant effects (P < 0.05, r² = 0.91). Dehydroascorbic acid content did not change in fruit that were not heated, but decreased in heated fruit, reaching the lowest concentration in fruit heated in air or 5% O₂ at 38 °C for 24 h (data not shown). The highest content of total ascorbic acid was attained in nonheated fruit (15.75 g kg⁻¹ fresh weight), which was similar to the 16–22 g kg⁻¹ reported by Abushita et al. (2000).

Nonheated fruit held at 4 °C presented variations in the concentration of cysteine between 2.6 and 4.6 g kg⁻¹ of freeze-dried tomato (Fig. 5). Nonheated fruit held at 10 °C had an increased concentration from 2.57 to 7.73 g kg⁻¹. Cysteine initially increased in fruit heated in air at 38 °C and held at 4 °C. Cysteine also increased in fruit heated in air at 38 °C and held at 10 °C, reaching a maximum concentration of about 4.6 g kg⁻¹. Fruit heated in 5% O₂ at 38 °C and held at 4 °C had a concentration ranging between 1.8 and 3.4 g kg⁻¹. Fruit heated in 5% O₂ at 38 °C and held at 10 °C had a concentration of 2.2–3.3 g kg⁻¹. In fruit heated in air at 34 °C and held at 4 °C, the concentration varied between 1.7 and 4.3 g kg⁻¹. In fruit heated in air at 34 °C and held at 10 °C there was an increase in cysteine concentration from 2.5 to 7.4 g kg⁻¹. Storage duration, storage temperature, and treatment, as well as their interactions had a statistically significant effect on the concentration of cysteine (P < 0.001, r² = 0.95).

Reduced glutathione concentration in nonheated fruit ranged between 0.35 and 0.74 g kg⁻¹ (Fig. 5). Fruit heated in air at 38 °C and held at 4 °C initially had a
decreased concentration and later remained constant at about 0.26 g kg\(^{-1}\). In fruit heated in air at 38 °C and held at 10 °C, there was a fluctuation in the concentration of reduced glutathione between 0.33 and 0.48 g kg\(^{-1}\). Fruit heated in 5% O\(_2\) at 38 °C and held at 4 °C had concentrations between 0.11 and 0.28 g kg\(^{-1}\), while those held at 10 °C had a final concentration of 0.24 g kg\(^{-1}\). Heating in air at 34 °C increased the concentration of the reduced glutathione, with a final concentration of 0.7 g kg\(^{-1}\). All the factors and their interactions had a significant effect on the concentration of reduced glutathione (\(P<0.05, r^2=0.83\)).

Oxidized glutathione concentration in nonheated fruit ranged between 0.13 and 0.43 g kg\(^{-1}\) (Fig. 5). In fruit heated in air at 38 °C and held at 4 °C it ranged between 0.14 and 0.33 g kg\(^{-1}\). The concentration initially decreased in fruit heated in air at 38 °C and held at 10 °C for up to 13 days of storage, and then increased, reaching a concentration of 0.31 g kg\(^{-1}\). Heating in 5% O\(_2\) at 38 °C decreased the concentration from 0.23 to 0.12 g kg\(^{-1}\). Fruit heated in air at 34 °C and held at 4 °C initially had an increased concentration from 0.2 to 0.34 g kg\(^{-1}\), which eventually decreased to 0.24 g kg\(^{-1}\). Fruit heated in air at 34 °C and held at 10 °C had a concentration ranging between 0.17 and 0.44 g kg\(^{-1}\). All the factors and their interactions (except the interaction “storage temperature-treatment”) had a significant effect on the concentration of oxidized glutathione (\(P<0.05, r^2=0.72\)).

The lowest levels of the three metabolites (cysteine, reduced glutathione, and oxidized glutathione) were found in fruit heated in 5% O\(_2\), which were the most damaged. Fuchs, Pesis, Zauberman, and Tabachnik (1981) reported that the quantity of free sulphhydryl groups increased in tomato fruit as they ripened either on the vine or in storage. They suggested that sulphhydryl groups were contributed by cysteine and reduced glutathione. In the present work, we observed that these changes are mainly due to the increase in the cysteine concentration, because glutathione concentration did not increase very significantly. On the other hand, in this study we observed higher levels of reduced glutathione than oxidized glutathione, contrary to what was reported by Jiao and Wang (2000) in juice of blackberry. Kosower and Kosower (1978) reported that the majority of glutathione in the cell is maintained in the reduced state.

Heat treatment of ‘Rhapsody’ tomato fruit in air at 34 °C and 50% RH for 24 h did not reduce antioxidant content, as compared to nonheated fruit, and the fruit developed normal colour during storage at 10 °C. However, exposure of fruit to 38 °C for 24 h in air or in 5% O\(_2\) caused significant negative effects, including greater losses in weight and antioxidants, and the fruit did not develop normal colour. Hot air treatments, with or without reduced O\(_2\), did not protect ‘Rhapsody’ tomatoes from the effects of chilling injury during storage at 4 °C (data not shown). Storage at 4 °C inhibited tomato fruit ripening and accelerated antioxidant losses, but resulted in less weight loss. Carotenoid and lycopene contents can be effectively predicted from hue angle values.

4. Conclusion

Heat treatment of ‘Rhapsody’ tomato fruit in air at 34 °C and 50% RH for 24 h did not reduce antioxidant content, as compared to nonheated fruit, and the fruit developed normal colour during storage at 10 °C. However, exposure of fruit to 38 °C for 24 h in air or in 5% O\(_2\) caused significant negative effects, including greater losses in weight and antioxidants, and the fruit did not develop normal colour. Hot air treatments, with or without reduced O\(_2\), did not protect ‘Rhapsody’ tomatoes from the effects of chilling injury during storage at 4 °C (data not shown). Storage at 4 °C inhibited tomato fruit ripening and accelerated antioxidant losses, but resulted in less weight loss. Carotenoid and lycopene contents can be effectively predicted from hue angle values.

References


