

Effects of Cutting and Maturity on Lycopene Concentration of Fresh-Cut Tomatoes during Storage at Different Temperatures

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Abstract

To investigate the changes in lycopene concentration of fresh-cut tomato during storage, tomato fruits at different stages of maturity were cut into 7 mm slices and stored at temperatures varying from 2°C to 16°C. To assess the effect of cutting, intact fruit were stored in an additional experiment at 5°C. Cutting did not change the accumulation of lycopene in fruit stored at 5°C, compared to intact fruit. The lycopene concentration of the tomato slices stored at different temperatures showed net increases of lycopene concentration for all maturity stages tested at temperatures of 8°C and higher. At lower temperatures no increases or small decreases were observed. The lycopene concentration was considered to be the net result of production and degradation of lycopene. Kinetic models for different pathways were tested. The reaction rate constant for lycopene formation at 8°C was estimated at $2.16 \pm 0.24 \cdot 10^{-2}$ µg/mg.day. The estimated activation energy for the lycopene formation (92.4 ± 7.3 kJ/mol) indicates that this process is highly dependent on temperature. The degradation rate constant was estimated at $2.99 \pm 0.61 \cdot 10^{-2}$ (1/day) and, over the temperature range studied, the degradation rate was not dependent on temperature.

INTRODUCTION

Data on the concentration and retention of bioactive compounds in minimally processed fruit and vegetables are sparse (Lindley, 1998). Fresh cut tissues are exposed to oxidative stress that can cause membrane damage and alter the composition and concentration of antioxidant compounds, including carotenoids (Chen and Djuric, 2001). Carotenoids are known to be susceptible to oxidation in the presence of light, oxygen and low pH, (Shi and Maguer, 2000) all conditions likely to occur when the fruit or vegetable tissue is cut. Cutting has been shown to induce an increase in the activity of lipoxygenase (Karakurt and Huber, 2003) that, in turn, can promote the co-oxidation of carotenoids (Biacs and Daood, 2000). Because the enzymatic systems are functional in fresh-cut fruit, the biosynthesis of pigments associated with ripening continues to occur during storage (Mencarelli and Saltveit, 1988; Campbell et al., 1990). Thus, it is expected that both synthesis of lycopene due to ripening and degradation of lycopene due to oxidation occurs during storage of fresh-cut tomato.

The objective of this research was to investigate the changes in lycopene concentration of fresh-cut tomato during storage. The processes of lycopene production and degradation are expected to depend on the stage of maturity of the fruit at processing and on the storage temperature.

MATERIALS AND METHODS

Harvesting, Processing and Storage

1. Experiment 1. Tomatoes ('Durinta') grown in the same greenhouse in Wageningen (The Netherlands) were harvested in a single day in October 2002 according to five colour stages, corresponding to the following grades of the tomato colour scale (ref. The Greenery): I = grade 3-4; II = grade 5; III= grade 7; IV = grade 9; V= grade 11. After harvest, the fruit were sanitised with sodium hypochlorite solution (100 ppm) and sliced in 7-mm thick transversal slices. The third and fourth slice from the stem end were taken and placed in a covered plastic petri dish and stored at 2°C, 5°C, 8°C, 12°C, and 16°C. For each maturity x temperature x storage time combination, 3 petri dishes with 2 slices from a same fruit were analysed separately.

2. Experiment 2. Tomatoes ('Belissimo') grown in the same greenhouse in Made (The Netherlands) were harvested in a single day in April 2003 in the following colour stages: I, II and IV. After harvest, the fruit were sanitised and cut as described for Experiment 1 and stored at 2°C, 5°C, 8°C, 12°C, and 16°C. The first and the last slice were discarded while the others were grouped in order to obtain one batch for each colour. For each maturity x temperature x storage time combination, 5 petri dishes with 3 slices taken at random were analysed separately.

3. Experiment 3. Tomatoes ('Belissimo') were harvested at Made (The Netherlands) in May 2003 in the same colour stages described for Experiment 2. Groups of 6 fruit similar in colour, shape and size were grouped. Three were stored intact and three were sliced in 7-mm thick transversal slices. The first and last slices from the stem end were discarded while the following four were stacked in the same position they had in the fruit. For each maturity x temperature x storage time combination, 5 replicates corresponding to a tray with 3 fruit were analysed separately. Both sliced and intact tomatoes were stored at 5°C.

Sample Preparation

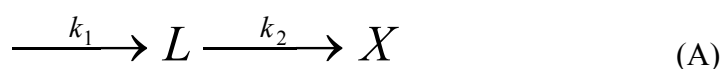
In all experiments slices from fruit at stages IV and V were analysed daily irrespective of temperature. Slices stored at 12°C and 16°C were analysed daily irrespective of stage of maturity. All the other combinations were analysed every other day. After removal from the storage chambers, the slices were frozen immediately with liquid nitrogen and kept at -20°C until freeze-drying. Until analysis, the freeze-dried and powdered samples were stored in the dark, under nitrogen atmosphere at 4°C (Experiment 1) or at -80°C (Experiments 2 and 3). In Experiments 1 and 3 the whole slice was analysed, while in Experiment 2 only the pericarp was analysed.

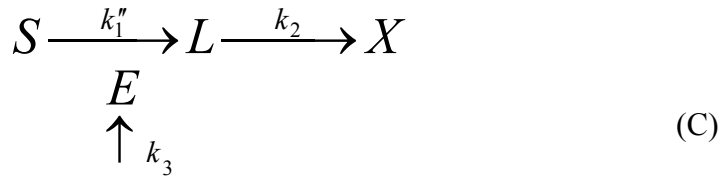
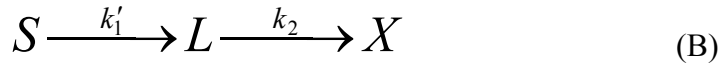
Lycopene Extraction and Analysis

In all the three experiments, the lycopene extraction was based on the methods described by Epler et al. (1993), Konings and Roomans (1997) and García-Plazaola and Becerril (1999). The lycopene concentration was measured by High Performance Liquid Chromatography (HPLC) with an Alltima C18, 3 µm, 100 mm x 4.6 mm (Alltech) column at 25°C. The mobile phase used was 75% methanol + 0.05 M ammonium acetate + 0.05% TEA and 25% ethyl acetate + 0.05% TEA. The flow rate 1 ml/min and detection of lycopene was at 470 nm.

Modelling

The lycopene concentration of the tomato slices was considered to be the net result of both production and degradation of lycopene according to the following three investigated reaction pathways:





where: k_1 reaction rate constant of lycopene formation
 k_2 reaction rate constant of lycopene degradation
 k_3 reaction rate constant of enzyme system formation
 L lycopene
 X lycopene degradation products
 S substrate for lycopene
 E enzyme system for lycopene formation

From the reaction pathways, sets of differential equations can be derived that describe the concentration of lycopene in time:

For pathway A:

$$\frac{dL}{dt} = k_1 - k_2 L \quad (1)$$

For pathway B:

$$\frac{dS}{dt} = -k'_1 \cdot S \quad (2)$$

$$\frac{dL}{dt} = k'_1 S - k_2 L \quad (3)$$

For pathway C:

$$\frac{dE}{dt} = k_3 \quad (4)$$

$$\frac{dS}{dt} = -k''_1 E \cdot S \quad (5)$$

$$\frac{dL}{dt} = k''_1 E \cdot S - k_2 L \quad (6)$$

k_1 rate constant formation of lycopene
 $[k_1: \mu\text{g}/\text{mg}\cdot\text{day}; k'_1: 1/\text{day}; k''_1: \text{mg}/(\mu\text{g}\cdot\text{day})]$
 k_2 rate constant degradation of lycopene $[1/\text{day}]$
 k_3 rate constant formation of enzyme system $[\mu\text{g}/\text{mg}\cdot\text{day}]$
 L lycopene concentration $[\mu\text{g}/\text{mg}]$
 S substrate concentration $[\mu\text{g}/\text{mg}]$
 E enzyme system concentration $[\mu\text{g}/\text{mg}]$
 t time $[\text{day}]$

The conversion of S into L is on an equimolar basis. Since the actual substrate(s) for lycopene have not been taken into consideration, the units are expressed in μg and not in moles.

Reactions are assumed to be temperature dependent as described by a rearranged Arrhenius equation:

$$k = k_{ref} \exp\left(\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right) \quad (2)$$

k_{ref} rate constant at reference temperature ($k_{1,ref}$: [$\mu\text{g}/\text{mg}\cdot\text{day}$], $k_{2,ref}$: [1/day])
 T_{ref} reference temperature [K]
 E_a activation energy [J/mole]
 R gas constant [J/(mole K)]

The reference temperature was set to 281 K (8°C), the median storage temperature that was applied.

Fitting of the data to the model was done using the software Athena Visual Workbench (www.athenavisual.com). The lycopene concentrations measured for the different experimental settings (stage of maturity at harvest, cultivars and tissue - pericarp or whole slice) were treated as different responses to allow for their different initial concentrations of lycopene. The data from all temperatures were fitted simultaneously to the model using the determinant criterion (Stewart et al., 1992) to estimate the parameters ($L_{t=0}$, $S_{t=0}$, $E_{t=0}$, $k_{1,ref}$, $E_{a,1}$, $k_{2,ref}$, $E_{a,2}$, $k_{3,ref}$ and $E_{a,3}$) together with their confidence intervals.

RESULTS AND DISCUSSION

Raw Data

Cutting did not change the accumulation of lycopene in fruit stored at 5°C, compared to intact fruit (Fig. 1). After 9 days storage, the lycopene concentration of fruit at stage I and II hardly changed compared to the initial concentration of both intact and sliced fruit. The same was observed for stage IV after 5 days storage, although in this case a slight decrease was observed in intact fruit.

Despite the high variability in the lycopene data, there was a clear tendency for the lycopene concentration to increase with time at 8°C and higher (Fig. 2). This trend was more pronounced for the less mature stages (I-II). There was a tendency for the lycopene concentration of red fruit (stages IV and V) to decrease at temperature lower than 8°C. This does not eliminate the possibility that lycopene degradation occurs at other stages of maturity, since the concentration is a result of both synthesis and degradation.

Alternatively, lycopene could be protected by other antioxidants that are preferentially oxidised. Vitamin C and vitamin E have been shown to reduce the degradation of lycopene in the presence of lipoxygenase, suggesting that regeneration of lycopene by ascorbic acid occurs during the course of co-oxidation (Biacs and Daood, 2000). The stability of lycopene in the food matrix in conditions where pure lycopene should be easily oxidised was reviewed by Nguyen and Schwartz (1998) and Shi and Maguer (2000).

Modeling Lycopene Production and Degradation during Storage

Simulations of the three proposed pathways (A, B and C), with hypothetical values for the rate constants, resulted in different patterns of lycopene accumulation during storage (Fig. 3). For pathway A, the simulated lycopene concentration increased with time but the apparent rate decreased at higher concentrations because of the increasing importance of the degradation process at higher concentrations. This effect was even more obvious in pathway B, due to the additional effect of decreasing concentrations of lycopene substrate after long storage time. In pathway C, there was a lag-phase in the formation of lycopene because the activity of the enzyme system is low in the beginning and only increases after some time during storage. In this case, the apparent formation rate also decreased with time due to the reduced concentration of substrate and the increasing importance of the degradation process at higher concentrations of lycopene.

Because of the observed high variability in the lycopene concentration of the tomato slices during storage it was not possible to select the most suitable pathway based

on our data. In the present study, all pathway models were able to fit the data. However, because of the increasing number of parameters required when going from pathway A to B and then to C, the accuracy of the parameter estimation was very low for model B and C. Therefore, it was decided to use the simplest pathway A in order to obtain kinetic parameters for lycopene formation and degradation as a function of temperature. In Fig. 2, the fits of the kinetic model based on pathway A to the data of Experiment 2 are shown. Similar graphs were obtained for Experiment 1.

The reaction rate constant for lycopene formation at 8°C was $2.16 \pm 0.24 \cdot 10^{-2}$ µg/mg.day. The estimated activation energy for the lycopene formation (92.4 ± 7.3 kJ/mol) indicated that this process was highly dependent on temperature. In the temperature range investigated, the formation rate was approximately 4 times faster with 10°C temperature increase. The degradation rate had an estimated rate constant of $2.99 \pm 0.61 \cdot 10^{-2}$ (1/day). The confidence interval around the estimated value was about 20%, which is double the confidence interval for the formation rate constant. For the degradation reaction no temperature dependency could be observed ($E_a = 0$). For a chemical reaction this is unexpected, but it might be explained by the fact that this reaction is oxygen dependent. Because oxygen is less soluble at higher temperatures the overall reaction rate will not increase as much as if it was a constant, and in fact it does not seem to increase at all. For the degradation reaction of lycopene during storage of tomato pulp in air a very low temperature dependency has been reported (Sharma and Le Maguer, 1996). These authors found an increase of degradation rates of only 35% with 10°C temperature rise in the range of 20 to 25°C. To compare the actual degradation and formation rates, the degradation rate constant has to be multiplied by the actual lycopene concentration which starts at about 0.1 µg/mg. This always results in a net synthesis of lycopene at the early maturity stages. Since the degradation rate is not dependent on temperature, at temperatures higher than 8°C the formation rate will become increasingly dominant.

The data for 'Durinta' and 'Belissimo' were analyzed separately to investigate a possible cultivar effect on the rate constants. The degradation rate constants for both cultivars were very similar and well within their confidence intervals. There was a tendency for a 50% higher rate constant for lycopene formation for 'Belissimo', but this difference was within the overlap of both confidence intervals so no definite conclusion about possible cultivar effect on formation could be drawn from our data. Difference in lycopene formation capacity in different cultivars is well known in literature. (Dumas et al., 2003) reported difference in lycopene concentration of one to four folds between different cultivars.

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Figures

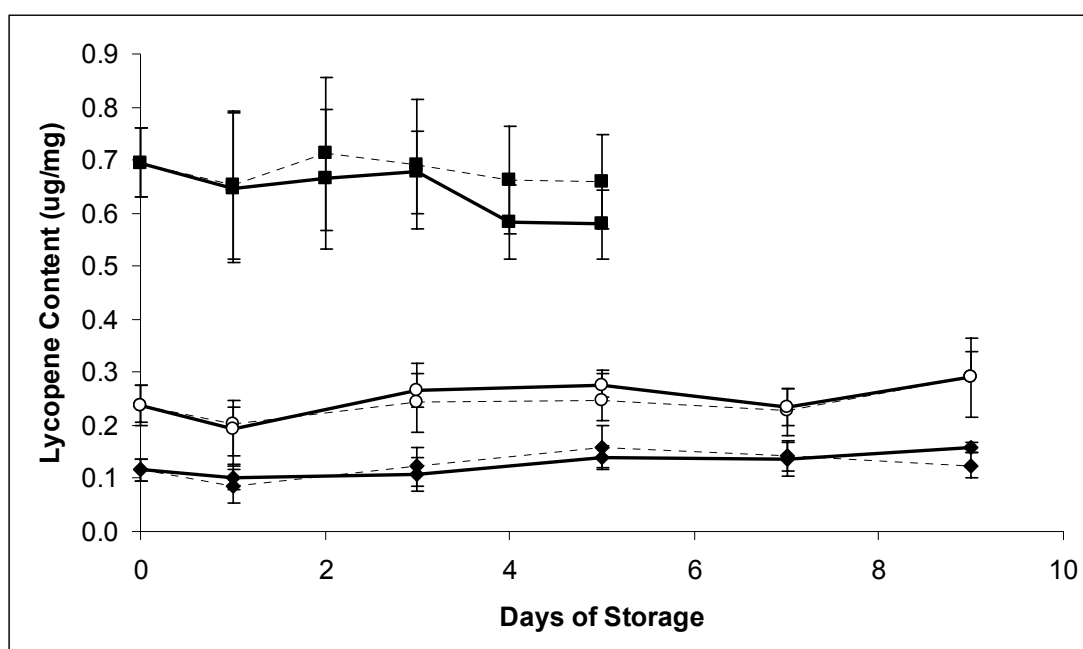


Fig. 1. Changes in lycopene concentration of sliced (dotted line) and intact tomato fruit (solid line) during storage at 5°C, when harvested at stages I (breaker) - ♦, II (pink) - ○, and IV (light red) ■.

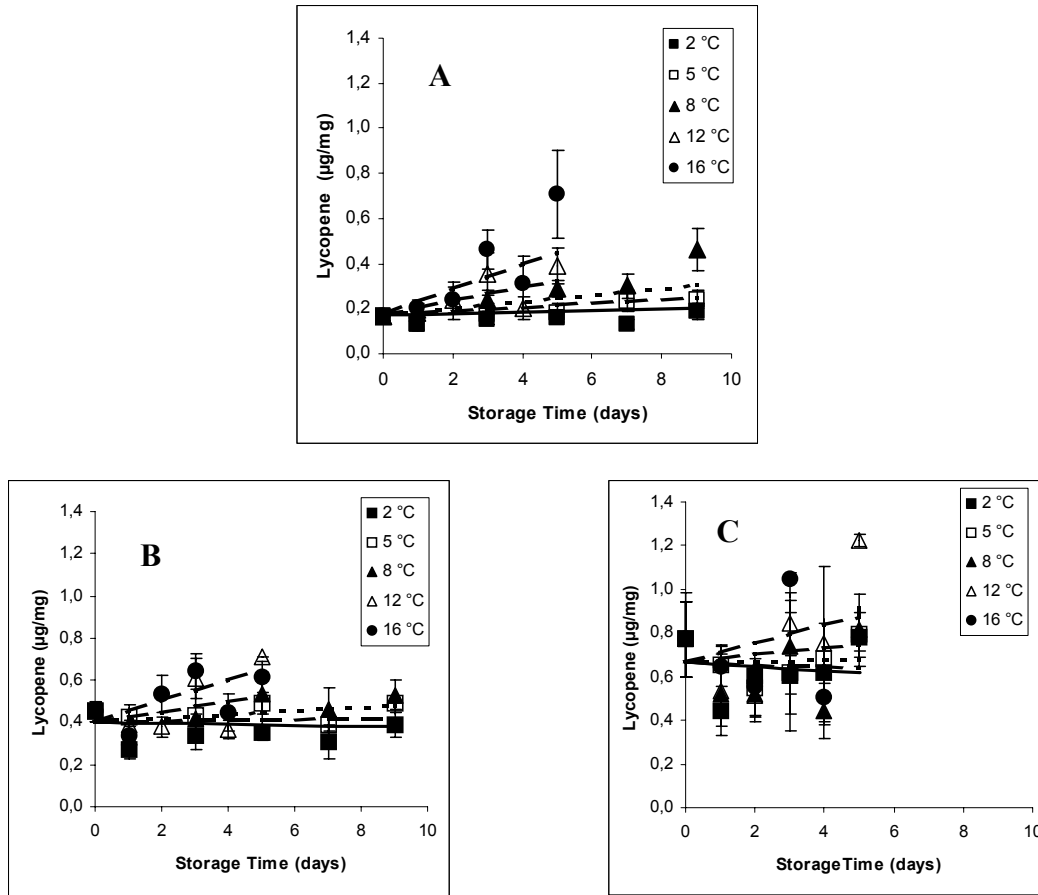


Fig. 2. Data and models fits of changes in lycopene concentration of fresh-cut tomato processed at stage I (A), stage II (B) and stage IV (C) during storage at different temperatures. Points are means + SD (n=5). Fit lines were obtained according to the kinetic model based upon pathway A.

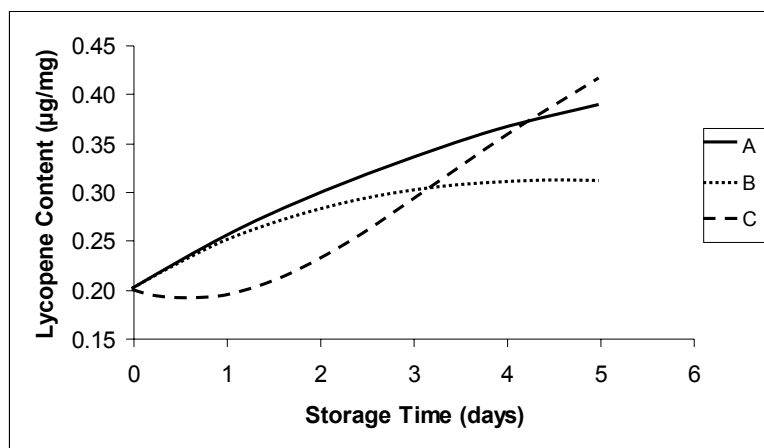


Fig. 3. Simulation of lycopene profiles in fresh-cut tomato slices during storage, using pathways A, B and C. (see text for details on pathways).

