# a* values to follow lycopene concentration during ripening and storage of tomato (cv. Caruso)

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## Abstract

The ripening of tomato fruit is a highly regulated process during which colour, flavour, aroma and texture change in a coordinated manner. This research work aims to correlate the colour changes measured objectively with the lycopene concentration in tomatoes during ripening at room temperature (21 and 26°C). These results were compared with colour and lycopene content of pink and light red tomatoes stored at 14°C, temperature used to prevent ripening and therefore extend the shelf life of the fruits. The duration of heat treatment at 100ºC was previously optimized in order to release the maximum lycopene from chromoplasts during extraction. An a* value of 20 for the peel corresponds to an increase of lycopene content of *Caruso* tomato from 9 to 43 mg/100 g TSS, at room temperature. The shelf life of pink and light red tomatoes can be extended to two weeks at 14ºC without loss of lycopene content, presenting the same content as green tomatoes ripened at room temperature for one week.

**Keywords**: Tomato, *Lycopersicon esculentum* Mill., lycopene, colour, ripening, storage

## Introduction

The composition of tomato (*Lycopersicon esculentum* Mill.) in carotenoids differs qualitatively and quantitatively with factors such as cultivar or varietal differences, stage of maturity, climatic or geographic effects, part of the plant, conditions during production, postharvest handling, processing and storage (Delia and Rodriguez-Amaya, 2003).

Carotenoids are responsible for the pleasing yellow, orange or red colour of many foods. The role of some of these compounds as provitamin A precursors has been known for years. Other beneficial effects to human health which have been more recently attributed to carotenoids are of enhancement of the immune response and reduction of the risk of degenerative diseases such as cancer, cardiovascular diseases, cataract and mascular degeneration, these actions not being restricted to the provitamin A (Olson, 1999). Since the human body is unable to synthesize carotenoids from endogenously produced biochemicals, the body is totally dependent on dietary sourced (exogenous) carotenoids.

The principal carotenoids in tomato are beta-carotene, beta-cryptoxanthin, lycopene, lutein and zeaxanthin. Lycopene is inactive vitamin A but is a more efficient antioxidant than beta-carotene (Di Mascio *et al.*, 1989). It has been linked with reduction of the risk of cancer, especially lung, stomach and prostate cancer (Giovannucci, 1999). In nature, carotenoids are protected by the cellular structure of the plant tissues. On the other hand, this natural protection lowers bioavailability.

The intactness of the cellular matrix determines the bioavailability of different nutrients (Van het Hof *et al.*, 2000). Heat treatment can have a deleterious effect on the micronutrient content of vegetables but at the same time the bioavailability of some nutrients can increase. There exist a lot of different opinions whether processed tomatoes contain a higher lycopene concentration or not. Food processing may improve lycopene bioavailability by breaking down cell walls, which weakens the bonding forces between lycopene and tissue matrix, thus making lycopene more accessible (Delia and Rodriguez-Amaya, 2003; Dewanto *et al.*, 2002; Gärtner *et al.*, 1997; Stahl and Sies, 1992; Thompson *et al.*, 2000). Other authors also established heating at 100°C to release the maximum lycopene from the chromoplasts (Sharma and Le Maguer, 1996). In other studies, heating of tomato products under different processing conditions may cause degradation of lycopene (Cole and Karpur, 1957; Klaui and Bauernfeind, 1981; Miki and Akatsu, 1970; Noble, 1975).

Data on the effects of processing on the carotenoid content of foods are therefore conflicting. The way of calculating the lycopene concentration is most important. When heating tomato pulp the lycopene content in pulp increases. However, this increase is directly related to the increase in tomato total soluble solids (TSS) concentration during heating. In fact, lycopene content in pulp may decrease (Sharma and le Maguer, 1996) when expressed per mass of TSS. In several of published papers (Gärtner *et al.*, 1997; Stahl and Sies, 1992; Thompson *et al.*, 2000; Khachik *et al.*, 1992; Nguyen and Schwartz, 1999; Nicoli *et al.*, 1999), the authors did not take into account the increase of tomato TSS. In consequence, it seemed that the lycopene level increased.

Tomato ripening is a regulated process during which colour, flavour, aroma and texture change in a coordinated manner. Tomato fruit colour is an important quality attribute, and it is the initial aspect evaluated by the consumers. Fruit colour is

related to the lycopene and chlorophyll content changes during the ripening process (Bramley, 1997).

Tomato fruits are chilling sensitive and the recommended storage temperature varies with the maturity stage (Hardenburg *et al.*, 1986). Temperature management is critical to maintain quality. Ripe tomato fruits can be stored at 10°C, without visible symptoms of chilling injury, although flavour and aroma may be negatively affected (Maul *et al.*, 2000).

This research work aimed to correlate the colour changes measured objectively with the lycopene concentration in tomatoes during ripening at room temperature (21 and 26°C). An objective of the work was also to compare these results with colour and lycopene content of pink and light red tomatoes stored at 14°C, temperature used to extend the shelf life of the fruits (Hardenburg *et al.*, 1986).

## Materials and methods

**Plant material**: Tomato (cv. Caruso) at different stages of ripening (according to the experiment) were purchased at a local hypermarket (Matosinhos, Portugal).

**Heat treatment**: Red tomatoes (stage 5, light red, and stage 6, red, USDA, 1975) were cut into pieces and the seeds were removed. The tomato was blended in a plastic beaker covered with aluminium foil, to prevent the destruction of the lycopene by light. The blending was continued till a rough, smooth tomato pulp was formed. Afterwards the rough tomato pulp was further homogenized for 10 min with a homogenizer (Ultra-Turrax T25, Janke & Kunkel, IKA-Labortechnik, Germany). The homogenate was divided into two fractions. One fraction was kept to analyze the lycopene content in the fresh tomato and the other fraction was heated to 100°C with a magnetic stirrer at maximum speed. After starting to boil, the tomato pulp was left simmering for different periods of time (5 to 15 min) at 100°C. Both heated and non-heated samples were collected and 5 g from each sample were weighed in glass beakers covered with aluminium foil. Lycopene content was determined in these samples.

**Ripening experiment**: Tomatoes at the stage of breakers (stage 2, USDA, 1975) were exposed to light at room temperature (21 and 26°C) and left to ripen during 10 days. This colour stage was chosen because consumers may purchase tomatoes at this stage and leave them to ripen at room temperature.

Tomatoes were analysed for colour and lycopene content each day. Three replicates of three tomatoes were used for each day.

**Storage experiment**: Pink and light red tomatoes (stages 4, pink, and 5, light red, USDA, 1975) were stored at 14°C (this temperature is recommended to stop the ripening process and store tomatoes of stage 4 and stage 5) and a relative humidity 90-95% for two weeks (Hardenburg *et al.*, 1986). These colour stages were chosen because consumers usually buy tomatoes at these ripening stages. The colour and lycopene content were analysed during that time. Three replicates of three tomatoes were used for each colour stage and for each day.

**Colour determination**: The colours of peel and pulp were measured by using a hand-held tristimulus reflectance colorimeter (Minolta CR-300, Minolta Corp., Ramsey, New Jersey, USA).

Colour was expressed as the CIE-L* a* b* uniform colour space. L* indicates lightness, a* chromaticity on a green (-) to red (+) axis, b* the chromaticity on a blue (-) to yellow (+) axis. Numerical values of a* and b* were converted into Hue angle ($H° = \tan^{-1} b*/a*$) and chroma ($Chroma = (a*^2 + b*^2)^{1/2}$) (Francis, 1980). Ten measurements were performed on each tomato.

Tomato colour was also classified according to the USDA colour chart (USDA, 1975).

**Lycopene determination**: The method used for the determination of lycopene concentration was adapted from Goula *et al.* (2006). A sample of 5 g of homogenized tomato pulp (fresh or processed at 100°C) was mixed with acetone and petroleum ether and stirred in a glass beaker. The mixture was transferred to a Kitazato and connected to a vacuum pump. The extract was washed three times with distilled water. The acetone was then removed helping to prevent the formation of stable emulsions.

The absorbance was measured at 505 nm using a Perkin-Elmer Lambda 15 UV/VIS spectrophotometer (Shimadzu Corp., Tokyo, Japan) after calibration with petroleum ether. Lycopene determination was performed in triplicate. The extinction coefficient used in the calculations was 2820 for a 1% (w/v) solution (Pearson, 1976) and the lycopene content expressed in mg/ 100 g TSS.

**Total soluble solids determination**: The amount of total soluble solids was determined at room temperature by using refractometry (hand-held Atago refractometer model ATC1).

**Statistical analysis**: The descriptive analysis consisted of the calculation of mean and standard deviation for cardinal variables and of frequencies for ordinal variables. Kolmogorov-Smirnov test was used to verify the normality of the distribution of cardinal variables. The *Mann Whitney* test was used to compare medium orders of two independent samples and the *Kruskal-Wallis* to compare medium orders of three or more independent samples (non parametric tests).

The Spearman correlation coefficient was calculated to quantify the intensity between pairs of variables (non parametric correlation) (Finney, 1980). P level of critical significance was used to reject null hypothesis (rejected when *P*<0.05).

## Results and discussion

**Heat treatment**: The effect of different heating times was investigated. In the beginning, it became clear that the heating time optimum was between the 5 and 10 min heating. The maximum lycopene content seems to correspond to a heating time between 5 and 7 min (data not shown). For further experiments 6 min heating time was used in order to maximize the release of lycopene from tomato tissues.

**Ripening experiment**: At colour stage 1, tomatoes are still green, meaning, there is no red pigment, so there is no lycopene present. At colour stage 2 the level of lycopene is still very low. Only when tomato changed from colour stage 4 to stage 5, the lycopene level increases rapidly because during this change the tomato turns from pink to light red. Differences between lycopene concentration of level 4 and 5 were statistically significant (*P*<0.02) at both temperatures. Levels of around 40 mg/ 100 g

TSS were achieved for tomatoes at colour stages 5 and 6 (Table 1). Stage 5 was achieved after 7 days of storage while stage 6 was achieved after 10 days. Lycopene concentration did not increase from stage 5 to stage 6 (Table 1).

Table 1. Lycopene concentration in heated tomato samples during ripening at 21 and at 26ºC

| Ripening temperature (°C) | Colour stage (USDA, 1975) | Lycopene (mg/ 100 g TSS) | Standard deviation |
|---|---|---|---|
| 21 | 3 | 5.8 | 0.00 |
| | 4 | 9.0 | 2.38 |
| | 5 | 42.9 | 9.73 |
| | 6 | 40.6 | 22.79 |
| 26 | 2 | 6.4 | 0.00 |
| | 3 | 4.3 | 0.82 |
| | 4 | 15.4 | 10.05 |
| | 5 | 43.9 | 11.24 |
| | 6 | 28.9 | 0.00 |

A strong positive curvilinear relationship was found between the a* values of tomato peel and pulp and lycopene concentration at both temperatures (21 and 26ºC). Low lycopene contents correspond to low a* values. For a* values around 15 (pulp) and 20 (peel) the lycopene concentration increased abruptly, corresponding to the change from stage colour 4 to stage colour 5 (Figs. 1 and 2). For high values of a* (colour stage 5 and 6), the peel presented higher values than the pulp. This means that the red colour is more intense in the peel, probably because the peel contains higher lycopene concentration than the pulp. No correlation was found for the other colour parameters (L*, b*, hue and chroma) (data not presented).

**Storage experiment**: After two weeks of storage at 14°C pink and light red tomatoes presented no symptoms of chilling injury. No pitting or decay (Wills *et al.*, 1998) were observed which indicates that this temperature was adequate for storage of tomatoes at these maturity stages.

After two weeks, tomatoes at these stages of colour exhibited lycopene contents (Table 2) similar to the green tomatoes (stage 2) ripened to the same stages of colour (ripening experiment) after 6 and 7 days of storage, respectively. The same tendency was observed for the peel and pulp (Figs. 3 and 4).

Table 2. Lycopene concentration of heated tomato samples after two week storage at 14ºC

| Colour stage (USDA, 1975) | Lycopene (mg/ 100 g TSS) | Standard deviation |
|---|---|---|
| 4 | 14.6 | 3.80 |
| 5 | 41.7 | 12.35 |

Processing tomatoes is a value-added step in terms of lycopene bioavailability. By heating processed tomatoes (blended and simmered) at 100°C for 6 min, the bioavailability of lycopene seems to increase, due to a breakdown of the cell walls of the tomato pulp.

The a* parameter (CIE-L*a*b*) is a good parameter for quantifying the lycopene level in tomatoes. The evolution from colour stage 4 to colour stage 5 at room temperature (21 and 26°C), involves dramatic increase in the lycopene content from 9 mg 100g$^{-1}$ TSS to 43 mg 100g$^{-1}$ TSS. This happens for a* values around 15 for the pulp and 20 for the peel. The latter is a good
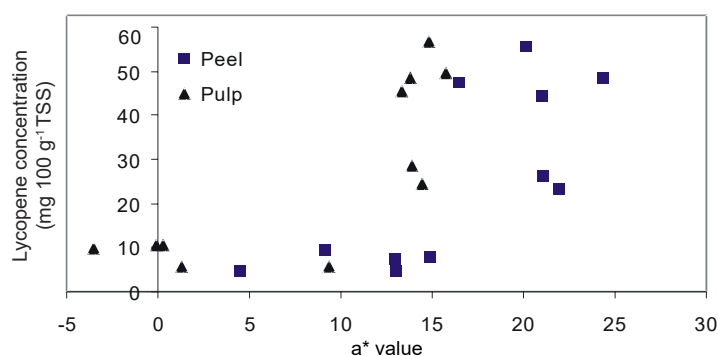


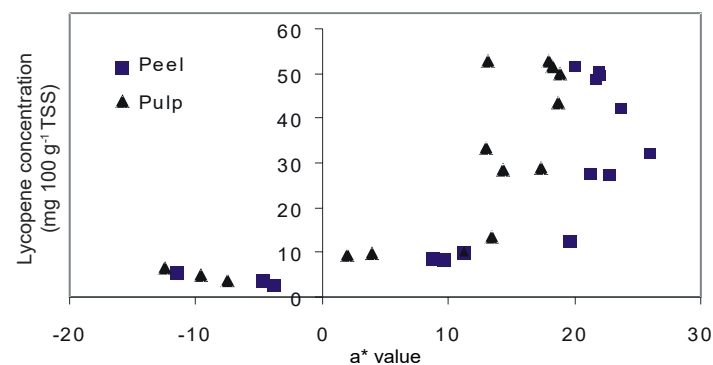Fig. 1. Lycopene concentration of heated tomato samples in function of a* value during ripening at 21ºC.



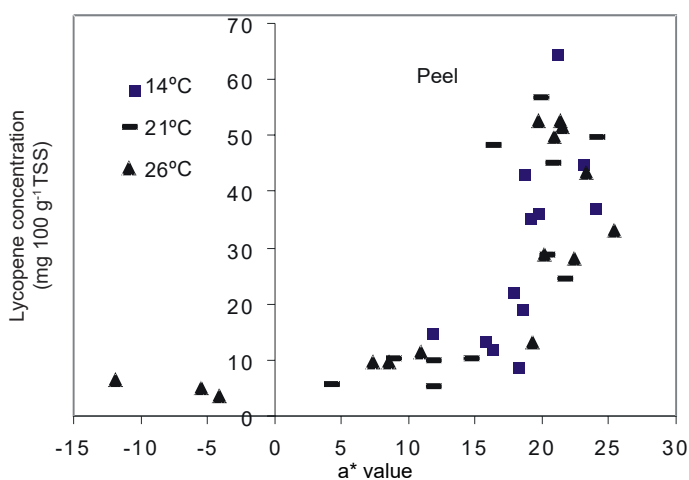Fig. 2. Lycopene concentration of heated tomato samples in function of a* value during ripening at 26ºC.



Fig. 3. Lycopene concentration of heated tomato samples in function of a* value of the peel during ripening at 21ºC and 26ºC and during storage at 14ºC.
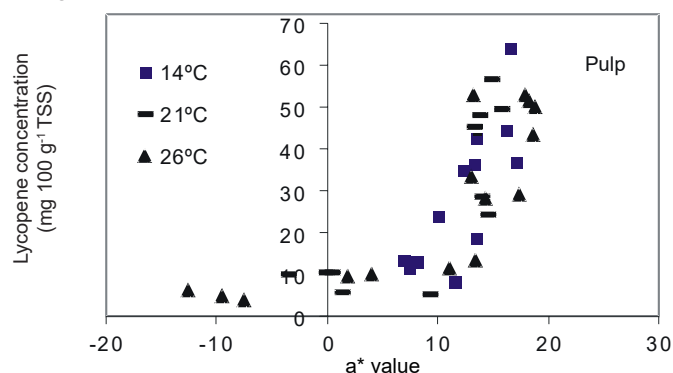


Fig. 4. Lycopene concentration of heated tomato samples in function of a* value of the pulp during ripening at 21ºC and 26ºC and during storage at 14 ºC.

indicator for the consumption of tomato (cv. Caruso) since it corresponds to high lycopene content and colour measurement of the peel by CIE- L*a*b* which is not a destructive method. The storage temperature (21 or 26ºC) does not influence the lycopene content for a specific stage of ripening.

Pink and light red tomatoes (colour stages 4 and 5), usually purchased by consumers, can be successfully stored for two weeks at 14°C, presenting lycopene contents similar to green tomatoes (colour stage 2) after being exposed to room temperature for one week, turning to light red (colour stage 5) tomatoes.

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