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STABILITY OF LYCOPENE IN CV. SALADETTE TOMATOES (*Lycopersicon esculentum* Mill.) STORED UNDER DIFFERENT CONDITIONS

ESTABILIDAD DE LICOPENO EN TOMATES CV. SALADETTE (*Lycopersicon esculentum* Mill.) SUJETOS A DISTINTAS CONDICIONES DE ALMACENAMIENTO

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Abstract

The objectives of this study were to determine the concentration of lycopene in cv. Saladette tomato subjected to blanching (thermal treatment), to extract the carotenoid and to evaluate the stability of the pigment in solutions added with and without antioxidants, in conditions of darkness at 4, 20 and 60 °C, and in fluorescent light at 20 °C during 30 days. The concentration of lycopene in non blanched tomatoes was 79.20 μg/g, while in blanched tomatoes it was 75.25 μg/g, none presenting any significant difference. The degradation of lycopene in solution during storage in darkness, fluorescent light or different temperatures, followed a reaction of the first order. In conditions of darkened or lightened storing at 20 °C, the retention of the carotenoid in both experiments was greater for solutions containing lycopene extracted from blanched tomatoes than for solutions with pigment extracted from unblanched tomatoes after 30 days. When the pigment in solution with antioxidants added was stored at different temperatures, the greatest lycopene retention was in darkness at 4 °C. In contrast, pigment degradation was greater in conditions of light and 20 °C, than in darkness at 60 °C. The constant rate of lycopene degradation increased when elevating temperature.

Keywords: tomato, lycopene, blanching, storage stability, kinetics.

Resumen

Los objetivos de este estudio fueron determinar la concentración de licopeno en tomate cv. Saladette sometido a escaldado (tratamiento térmico), extraer el carotenoide y evaluar la estabilidad del pigmento en soluciones con y sin adición de antioxidantes, almacenadas en oscuridad a 4, 20 y 60 °C y en luz fluorescente a 20 °C durante 30 días. La concentración de licopeno en tomates sin escaldar fue de 79.20 μg/g mientras que para los tomates escaldados fue de 75.25 μg/g, no se presentó diferencia significativa. La degradación del licopeno en solución durante su almacenamiento en oscuridad, en luz fluorescente o a diferentes temperaturas siguió una reacción de primer orden. En condiciones de almacenamiento en oscuridad o en luz a 20 °C, la retención del carotenoide en ambos experimentos fue mayor para las soluciones con licopeno proveniente de tomates escaldados que para soluciones con pigmento de tomates no escaldados después de 30 días. Cuando el pigmento en solución con antioxidantes se almacenó a diferentes temperaturas, la mayor retención de licopeno fue en oscuridad a 4 °C. En condiciones de luz y 20 °C la degradación del pigmento fue mayor que en oscuridad y 60 °C. La constante de velocidad de degradación del licopeno se incrementó al elevar la temperatura.

Palabras clave: tomate, licopeno, escaldado, estabilidad en almacenamiento, cinéticas.

1. Introduction

Tomato (*Lycopersicon esculentum* Mill) is the principal vegetable that Mexico exports to the United States, with Sinaloa being the main producing and exporting state. The states of Baja California, Michoacán, San Luis Potosí, Baja California Sur and Sinaloa produced 68.5 % of the national production in 2005 (SIAP, 2007). Tomato exports were 683 and 668 thousand tons in 2004 and 2005, respectively (SNIIM, 2007). An increase in production of 5.2 % and 16.7 % was obtained during these two years

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when compared with the reported production in 2003 (SIAP, 2007); however, this overproduction caused a decrease in price and economical losses for producers. Therefore, an alternative to take advantage of excess tomatoes is to obtain the carotenoid pigments. Lycopene comprises between 80 and 90 % of those carotenoids present in the fruit and can be used as a natural colorant or as a nutritional complement (Shi and Le Maguer, 2000).

Natural colorants have acquired great importance in the food and pharmaceutical industries within the last few years due to toxicological problems associated with the use of synthetic colorants. Some natural colorants used in the food, pharmaceutical and cosmetic industries are anthocyanin, betanine, chlorophyl, curcumin and carotenoids such as bixin and crocetin (Bouvier *et al*., 2003; Delgado-Vargas and Paredes-López, 2003; Humphrey, 2004; Wrolstad, 2004).

Recent research acknowledges other benefits of lycopene present in tomatoes and its processed products, such as being a micronutrient with nutraceutical properties related to cancer prevention in different organs such as prostate, digestive tract and lungs; as well as in the decrease of heart related diseases (Boileau *et al*., 2002). These beneficial properties of this carotenoid are due to its acyclic polyene structure (eleven conjugated double bonds) which gives it the ability to protect against oxidative damage and to quench free radicals.

The all-*trans* isomer of lycopene, the most stable form, is most prevalent in fresh tomatoes. Nevertheless, it is liberated from the tissue matrix during its processing and spontaneously undergoes isomerization and oxidation which causes pigment degradation. A greater loss of lycopene occurs when various heat treatments such as blanching, sterilization and drying are applied (Bruno and Wetzel, 2004; Candelas-Cadillo *et al*., 2005, Goula *et al*., 2006).

Blanching is required to inactivate the pectinesterase and polygalacturonase enzymes, since these enzymes affect the quality of the processed tomato products and lycopene extraction (Schwartz, 1994; Takeoka *et al.,* 2001, Fellows, 2005). There are several blanching methods that differ in temperature, they can be applied either to the whole vegetable as well as to crushed tomatoes, such as the "hot break" (82-90 ºC) or the "cold break" (60-65 ºC) commonly used for tomatoes processing (Re *et al*., 2002; Fellows, 2005). In relation to the lycopene stability, Shi and Le Maguer (2000) reported that heat induces isomerization of the all-*trans* to *cis* forms. The *cis*-isomers increase with the temperature and processing time. Boileau *et al.* (2002) also reported that the lycopene *cis* isomers are more bioavailable than the all-*trans* due to the higher solubility of the *cis* isomers in the micelles of bile acid and its lesser tendency to aggregate. Lycopene in processed tomato products is also sensible to high temperature, prolonged processing time, light, oxygen, acids and some metallic ions such Cu^{2+} and Fe^{3+} that catalyze its oxidation (Shi and Le Maguer, 2000).

According to reported potential about the use of this carotenoid, the objectives of this study were to determine the concentration of lycopene in cv. Saladette tomato subjected to blanching (thermal treatment), to extract the carotenoid and to evaluate the stability of the pigment in solutions with and without antioxidants added in conditions of darkness at 4, 20 and 60 °C, and in fluorescent light at 20 °C during 30 days.

2. Materials and methods

'Saladette' tomatoes used for these experiments were harvested in the production area of Los Mochis, Sinaloa and purchased at the Central de Abasto main market in Mexico City. Tomatoes were ripe red (Hue 0.569 ± 0.04), uniform in size, firm and undamaged; with a reducing sugar content of 1.870 ± 0.09 %, total soluble solids of 4.90 ± 0.11 %, pH of 4.2 ± 0.02, containing 0.237 ± 0.06 % of citric acid and 36.78 ± 2.1 mg/100g of ascorbic acid. Samples were divided into two groups of 24 tomatoes each. One group was subjected to the blanching treatment, leaving a non-blanched set as control (8 tomatoes for each repetition). The blanching treatment consisted of submerging the tomatoes in water at 93.5 ºC for 50 sec, followed by cooling under running water at room temperature. The blanched and unblanched tomatoes were cut and ground using a tissue homogenizer (M 133/1281-O Biospec Products Inc. Bartlesville, OK, USA) at 4 °C for 60 sec; the homogenized product was passed through a sieve after each repetition to remove peel and seeds. Color was immediately determined using a Hunter Lab colorimeter, CM 2002 (Hunter Associates Laboratory Inc. Reston, VA, USA), reporting the hue (Hue= $\tan^{-1}$b/a); afterwards, lycopene extraction and quantification was performed.

2.1 Physicochemical analysis

In order to perform the physicochemical analysis, 100 g of the homogenate was centrifuged at 4000 rpm for 20 min at 20 ºC using a IEC Centra CL3/CL3R centrifuge (Thermo Electron Corporation, Waltham, MA). The supernatant was collected and subjected to pH, total soluble solids content and titratable acidity measurement; pH was measured using a Beckman ϕ 50 pH-Meter (Beckman Fullerton, CA).

Acidity was measured by titration to a final pH of 8.1-8.2 with NaOH 0.1N (Mencarelli and Saltveit, 1988). The total soluble solids content was measured directly in the supernatant with an Atago refractometer (Atago, Tokyo, Japan, AOAC, 1996). Reducing sugars content was determined by the Nelson-Somogy method (Nelson, 1944), using a

glucose concentration pattern curve of 0-600 μg/mL in water and reading at 520 nm. Ascorbic acid was determined by the Robinson and Stotz method (1954) using an ascorbic acid concentration curve of 0-200 μg /mL in metaphosphoric acid and reading at 520 nm.

2.2 Lycopene extraction

Lycopene was extracted from blanched and unblanched tomato homogenates according to a modification of the method described by Sadler *et al.* (1990) and Thompson *et al.* (2000). Ten grams of the sample were mixed with 100 mL of the solvent system (hexane:acetone:ethanol, 50:25:25, v/v/v), in a 250 mL Erlenmeyer flask covered with aluminum foil to prevent photooxidation; and stirred for 15 min at 20 ºC using an orbital shaker (Lab Line Instruments, Inc., Melrose Park, Ill). In order to study the effects of commercial antioxidants on lycopene retention, half of the samples (blanched and unblanched) were mixed with BHA:BHT at 0.025 % (Sigma Chemicals, St. Louis, MO) (Takeoka *et al.,* 2001). Fifteen mL of water were added to all the samples and the mixture was stirred for 5 min to separate the upper layer of hexane which contains the lycopene from the water: ethanol lower layer. The lycopene hexane phase was collected with a pipette. The residual lycopene in the water:ethanol phase was separated using the same procedure. The two lycopene:hexane phases were mixed in a total volume of 100 mL per extraction flask. The pigment solution was concentrated in a vacuum steamer (Buchi, Geneva, Switzerland) at 35 ºC. Hexane was later added for a total volume of 250 mL.

2.3. Lycopene analysis in HPLC

The pigment was identified in the lycopene-hexane samples which were previously filtered through 0.22 μm filters (Gelman Acrodis TM GHP No.13, Pall Corporation, Exton, PA). The lycopene concentration was determined by injecting filtered samples and pattern solutions prepared from a lycopene standard of 90 – 95 % (Sigma Chemical, St. Louis, MO), into an HPLC (Thermo Separation Products, ConstaMetric 3200Bio, Riviera Beach, FL) to relate the peak areas and retention times. The HPLC conditions were: isocratic runs, with a flow rate of 0.5 mL/min; an injection volume of 20 μL and a wave length detection of 470 nm. A 250 mm x 4.6 mm C_{18} reverse phase column (particle size was 5 μm, Spherisorb ODS, Waters Associates, Milford, MA) was used, the mobile phase consisted of acetonitrile:methanol:dichloromethane (43.3:43.3:13.4, v/v/v) previously degassed and filtered though a 0.45 μm nylon Millipore ™ (Millipore, Bedford, MA) solvent resistant membrane (Thompson *et al.,* 2000).

2.4 Lycopene stability

The stability of the pigment was studied in the carotenoid solutions obtained from the blanched and unblanched tomatoes under different conditions and levels: antioxidant (none and BHA/BHT at 0.025 %); storage in darkness and fluorescent light at a temperature of 20 ºC. Temperature conditions of 4 ºC, 20 °C and 60 ºC were also applied to samples stored in darkness. The solutions were prepared under the following conditions: 10 mL portions of lycopene solution were placed in glass vials (50 % headspace), covered with aluminum foil and stored in darkness at 4 ºC, 20 ºC and 60 ºC. Other samples were placed in transparent vials and stored at 20 ºC under fluorescent light of 4186 lux, distance to luminous source was 30 cm (CIR LX-101 luxometer, Cole Parmer Instrument Company, Chicago, IL) to promote photooxidation (Gouldson and Warthesen, 1999). Storage times were of 0, 3, 6, 12, 20 and 30 days. After that, samples were stored at –20 ºC until further analysis. The lycopene concentration was determined by using a HPLC, as previously mentioned. Six experiments with 3 replicates were made under conditions shown in Table 1.

Table 1. Design of experiments

Experiment	Experimental unit	Condition (levels)
Ia	Lycopene solutions (blanched tomatoes)	Illumination (light and darkness) and time (0, 3, 6, 12, 20 and 30 days).
Ib	Lycopene solutions (unblanched tomatoes)	Illumination (light and darkness) and time (0, 3, 6, 12, 20 and 30 days).
IIa	Lycopene solutions (blanched tomatoes) with antioxidants at 20 °C	Illumination (light and darkness) and time (0, 3, 6, 12, 20 and 30 days).
IIb	Lycopene solutions (blanched tomatoes) without antioxidants at 20 °C	Illumination (light and darkness) and time (0, 3, 6, 12, 20 and 30 days).
IIIa	Lycopene solutions (blanched tomatoes) with antioxidants	Temperature (4, 20 and 60 °C) and time (0, 3, 6, 12, 20 and 30 days).
IIIb	Lycopene solutions (blanched tomatoes) without antioxidants	Temperature (4, 20 and 60 °C) and time (0, 3, 6, 12, 20 and 30 days).

2.5 Statistical analysis

Lycopene retention data were statistically analyzed using ANOVA for two factors designs with three repetitions: blanching-time, illumination-time, temperature-time, and Tukey's multiple comparisons tests ($\alpha \leq 0.05$), corresponding the main factors and interaction.

Exponential models were adjusted to lycopene retention through time using curvilinear regression (equivalent to a model of simple linear regression applying natural logarithm to the exponential function) and the slopes or degradation rates were compared through the t-Student test. Times of 50 % lycopene retention were calculated in the adjusted models. The program was SPSS for Windows 16.0 software (SPSS Inc., Chicago, Ill., USA). NCSS for Windows 2001 software was used to substantiate errors normality (NCSS Inc., Kaysville, Ut.,USA).

3. Results and discussion

3.1. The effect of blanching on the lycopene content in whole tomatoes

This experiment considered the fact that a blanching treatment was necessary to inactivate the pectinesterase and polygalacturonase enzymes found in tomatoes since they affect the quality of the processed products causing a quick diminishment of viscosity; as well as other natural enzymes which might decompose pigments (Schwartz, 1994; Barringer, 2004). The heat treatment may be applied using different methods, and in this case the whole tomato was submerged in water at 93.5 ºC for 50 sec. The intensity of the lycopene red color is used as a quality indicator of the tomato products and also when it is used as food colorant.

The mean lycopene concentration was higher (79.20 ± 1.94 μg/g) in the unblanched samples than the mean pigment concentration for blanched samples (75.25 ± 2.09 μg/g), the difference was not significant ($p \leq 0.05$); therefore, 95.01 % of the lycopene was retained after the tomatoes were blanched. It is possible that the blanching method applied to the whole fruit with skin reduced the autooxidation rate during heating. Reduction of lycopene during blanching was also reported by other researchers, for instance: Akanbi and Oludemi (2004), registered lycopene concentrations of 59.50 μg/g and 47.60 μg/g in Roma VF and lb local tomatoes that had not been treated with heat, but after steam blanching of the entire fruits, the lycopene concentrations were 58.40 μg/g and 46.30 μg/g, in that order. However, other authors that applied severe heat treatments in combination with a mechanical disruption of the fruit observed greater pigment degradation. Thompson *et al.* (2000) reported higher lycopene variations between the concentration for cultivars FL7765 and FL7655 of 67.10 μg/g and 57.12 μg/g, but after 4 min cooking time the lycopene concentration was 61.11 μg/g and 57.17 μg/g, respectively. Mayeaux *et al*. (2006) demonstrated that the extend of lycopene degradation varies according to the heating temperature and time, as only 36.6 % and 35.5 % of lycopene was retained after frying at 145 ºC and 165 ºC for 1 min, respectively. However mild thermal processing could increase lycopene concentration in tomato products by increasing the free and bioaceesible lycopene form. Nevertheless, other researchers such as Lin and Chen (2005) reported higher lycopene concentrations in tomatoes that underwent "hot-break" (82 ºC and 2 min, 57.7 μg/g) than in tomatoes that did not receive the heat treatment (51.2 μg/g). On the other hand, prolonged heating or high storage temperature has been reported to promote non-enzyme darkening, including Maillard reactions and ascorbic acid oxidation, even though these changes could be masked by the color of lycopene (Anese *et al.,* 2002). Nguyen and Schwartz (1999) reported that non-enzyme darkening and the loss of carotenoid color are the principal causes of the color changes in tomato products. This color change in lycopene is due to the isomerization of the pigment from the all-*trans* form into the *cis* form according to Anguelova and Warthesen (2000) and Hackett *et al.* (2004).

3.2. Experiments Ia and Ib (table 1): Kinetics of lycopene degradation (from blanched and unblanched tomatoes) in solutions under conditions of darkness and light during storage at 20 °C

Two experiments were made, the first for lycopene extracted from blanched tomatoes (Ia) and the second for lycopene extracted from unblanched tomatoes (Ib). The main factors to each two designs were illumination and time. In both designs illumination and time factors, as well as the interaction illumination*time were significant in lycopene retention ($p \leq 0.001$).

When comparing both designs, it was found that the greater lycopene retention was observed in darkness with a lycopene retention of 81.0 % for solutions from blanched tomatoes and 66.78 % for solutions made from unblanched tomatoes. In conditions of light, the mean was 65.39 % for lycopene solutions made from blanched tomatoes and 59.43 % for those from unblanched tomatoes.

Regarding the interactions illumination*time in both experiments, it was found that day 3 presented the greatest retention of lycopene in conditions of darkness (the mean was 90.33 % for lycopene solutions from blanched tomatoes and 77.85 % for lycopene solutions from unblanched tomatoes) and the significantly lesser than others mean was at 30 days in conditions of light (mean was 31.6 % for lycopene solutions from blanched tomatoes and 19.42 % for lycopene solutions from unblanched tomatoes). It must be pointed out that the

heat treatment (blanching) allowed greater pigment retention in solutions than in those with lycopene from unblanched tomatoes during storage period. It was also found that, in conditions of light, the pigment was degraded faster. It is possible that blanching affords greater stability to the pigment during storage due to the fact that this heat treatment inactivates the oxidative enzymes responsible of color deterioration and therefore allows pigment retention. This enzymes and oxygen accelerate pigment oxidation in the case of the unblanched samples (Macrae *et al*., 1993; Schwartz, 1994; Britton, 1996; Ishida *et al*., 2007). Because of the aforesaid, it is convenient to scald tomatoes when extracting lycopene and to store them under conditions of darkness.

On the other hand, Lee and Chen (2002) and Goula *et al*., (2006) reported that kinetics of reaction of lycopene degradation followed a kinetic model of the first order:

$$C = C_0\, exp(-kt)\ equivalent\ to\ lnC = lnC_0 - kt \quad (1)$$

Where C_o is the initial lycopene concentration, C is lycopene concentration at time t; k is the reaction rate constant. The adjustment to the exponential model for the % of lycopene retained (% L) through time for the experiments of the pigment solutions from blanched and unblanched tomatoes in conditions of darkness and light at 20 °C was made based on these criteria and considering that initial lycopene concentration was 100 %. The estimated models are indicated in Table 2.

In the adjusted models for lycopene solutions from blanched tomatoes (Ia), the reaction rate constant was significantly lower ($p \leq 0.05$) in conditions of darkness (A) than in light (B) (0.018 and 0.040), meaning that pigment degradation was lower during the 30 days of storage (R^2 = 0.933 and 0.933). This same behavior was observed in the models adjusted for lycopene solutions from unblanched tomatoes (Ib), the rate constants were 0.037 in darkness (A') and 0.055 in light (B')(R^2 = 0.87 and 0.98), (Fig. 1). Considering the lower reaction rate constant and that initial lycopene concentration as 100 %, the adequate storage conditions for the carotenoid were in lycopene solutions from blanched tomatoes in darkness (A). This was confirmed as after 38 days half of the initial lycopene was retained (Table 2). Spagna *et al* (2005) reported that tomato polyphenol oxidase is responsible to the color changes, as darkening and lycopene degradation, thus it is highly recommended to inactivate the polyphenol oxidase in order to maintain the lycopene stability.

The following experiments were made with lycopene proceeding from blanched tomatoes.

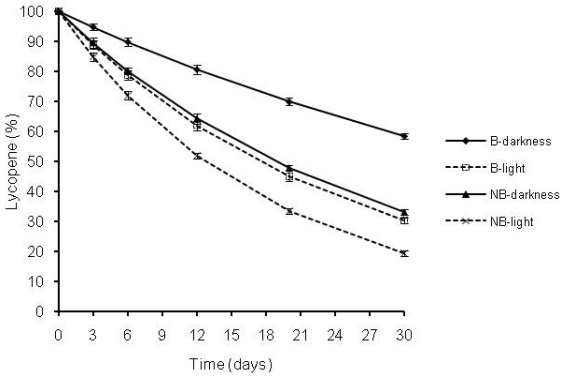

Fig. 1. Kinetics of lycopene degradation in solutions (from blanched and unblanched tomatoes) in conditions of darkness and light at 20 °C (adjusted models). Key: -♦- blanching-darkness; -□- blanching-light; -▲- non-blanching-darkness; -x- non-blanching-light.

3.3 Experiments IIa and IIb (table 1): Kinetics of lycopene degradation in solutions with and without antioxidants in darkness and light during storage at 20 °C

Physical and chemical factors such as high temperatures, light exposure, oxygen, extreme pH and acids degrade the carotenoids present in processed tomato products. In particular, lycopene is highly reactive to oxygen and light because of its double bond structure (Nguyen and Schwartz, 2000; Calvo *et al.,* 2007).

Table 2. Adjusted models for lycopene retention in solutions (from blanched and unblanched tomatoes) in conditions of darkness and light at 20 °C.

Case	Blanching	Illumination	Adjusted model	R^2	50 % lycopene retention time (days)
A	Yes	Darkness	% L = 100 exp (- 0.018t)	0.933	38
B	Yes	Light	% L = 100 exp (- 0.040t)	0.933	17
A'	No	Darkness	% L = 100 exp (- 0.037t)	0.873	15
B'	No	Light	% L = 100 exp (- 0.055t)	0.985	12

Statistical differences observed among cases:
A with B presented significant difference in the rate constants.
A' with B' presented significant difference in the rate constants.
A with A' presented significant difference in the rate constants.
B with B' presented significant difference in the rate constants.
In all cases $p \leq 0.05$

Lycopene can convert itself into peroxyl radicals capable of acting as prooxidants and undergo autooxidation just by the presence of oxygen in the air. Shi *et al.* (2004) proposed that the pathway towards oxidative degradation can take place by two mechanisms depending on how oxygen is introduced into lycopene: 1) Oxidation of a methyl or methylene group, and 2) Addition to a carbon-carbon double bond; the oxidative degradation can be present at either end of the carotenoid structure. The final products of lycopene degradation are the result of the direct oxidative scission within the double bonds present in the molecule (Shi and Le Maguer, 2000; Shi *et al.*, 2003).

Two experiments were made, the first for lycopene solutions added with antioxidants (IIa) and the second for lycopene solutions without antioxidants (IIb). The main factors for each two experiments design were illumination and time. In both experiments, the two main factors (illumination and time) and their interaction (illumination*time) were significant ($p \leq 0.001$) in lycopene retention.

When comparing both experiments, it was observed that, in conditions of darkness the greater lycopene retention was detected in lycopene solutions containing antioxidants, being of 81.07 % and 65.28 % the lycopene retention for solutions added with and without antioxidants, respectively. In conditions of light, the mean was 66.92 % for carotenoid solutions with antioxidants and 55.05 % for lycopene solutions not containing antioxidants.

Regarding illumination*time interactions, it was found in both experiments that lycopene retention in days 3 and 6 in conditions of darkness presented the highest means and did not present significant difference among them ($p \leq 0.05$). The pigment means with antioxidants in those days were 90.8 % and 85.4 % respectively and the means for lycopene without antioxidants were 70.41 % and 66.21 % respectively. The significantly lesser mean was in conditions of light at 30 days for lycopene solutions with antioxidants as well as for pigment solutions without antioxidants (31.10 % and 26.91 % respectively). According to these results, the antioxidants allowed greater lycopene retention in solutions in both conditions of light and darkness. It was also found that light degrades the pigment quicker. These data demonstrate the sensitivity of the pigment to light, as it had been shown by other researchers (López-Hernández *et al*., 2001; Lee and Chen, 2002; Shi *et al*., 2003). Britton (1996) claims that this effect is mainly due to the combination of light and the presence of singlet oxygen ($^1O_2$). Lee and Chen (2002) as well as Cvetkovic and Markoviv (2008) reported a decrease of all-*trans* lycopene when light exposure time was increased. These researchers also pointed that lycopene and its *cis* isomers isomerization and degradation can proceed simultaneously. However, mild processing temperatures such as 50 ºC, isomerization is followed by degradation, while at 100ºC degradation proceeded faster than isomerization; the presence of light also induces isomerization as the main reaction. The autooxidation of lycopene is irreversible and will lead to fragmentation of the molecule, producing acetone, methylheptenone, laevulinic aldehyde and probably glyoxal, which cause apparent color loss (Xianquan *et al*., 2005). Whereas, Anguelova and Warthesen (2000) have reported that the principal product of photooxidation is lycopene-5,6-diol (5,6-dihydroxy-5,6-dihydrolycopene). Hackett *et al*. (2004) found that pigment stability decreased with time when stored under fluorescent light at 25 and 50 °C.

Considering that the kinetics of lycopene degradation reaction followed a kinetic model of the first order (equation 1) and considering that initial lycopene concentration was 100 %, the adjustment to the exponential model of the % of lycopene retained (% L) through time was made for the experiments with the pigment in solutions with and without antioxidants in conditions of darkness and light (IIa and IIb respectively). The estimated models are indicated in Table 3.

Table 3. Adjusted models for lycopene retention in solutions with and without antioxidants in conditions of darkness and light at 20 °C.

Case	Antioxidants	Illumination	Adjusted model	R^2	50 % lycopene retention time (days)
A	Yes	Darkness	% L = 100 exp (- 0.018t)	0.922	38
B	Yes	Light	% L = 100 exp (- 0.038t)	0.898	18
A’	No	Darkness	% L = 100 exp (- 0.037t)	0.579	19
B’	No	Light	% L = 100 exp (- 0.062t)	0.763	11

Statistical differences observed among cases:
A with B presented significant difference in the rate constants.
A’ with B’ presented significant difference in the rate constants.
A with A’ presented significant difference in the rate constants.
B with B’ presented significant difference in the rate constants.
In all cases $p \leq 0.05$

In the adjusted models for lycopene in solutions with antioxidants (IIa), the reaction rate constant was significantly lesser ($p \leq 0.05$) in conditions of darkness (A) than in light (B)(0.018 and 0.038), meaning that pigment degradation was lesser (R^2 = 0.922 and 0.898). This same behavior was presented in the adjusted models for lycopene in solutions without antioxidants (IIb), the rate constants were 0.037 in darkness (A') and 0.062 in light (B') (R^2 = 0.579 and 0.763) (Fig. 2). According to the lower reaction rate constant and considering that initial lycopene concentration was 100 %, the adequate conditions to store the carotenoid were lycopene in solutions with antioxidants in darkness at 20 °C (A). These data are similar to those data reported by Ferreira and Rodriguez-Amaya (2008), who studied lycopene model aqueous systems under darkness and light conditions under ambiental temperature. These researchers claimed that the carotenoid degradation under light and darkness conditions followed a first-order-kinetics, although the presence of light accelerates pigment degradation.

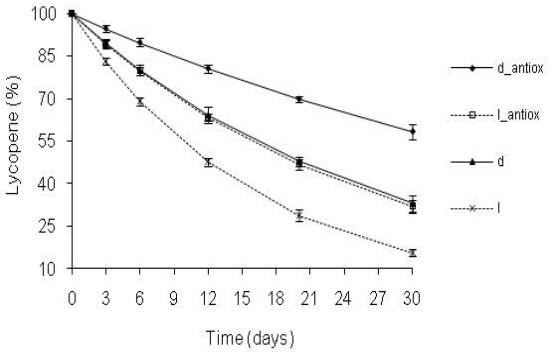

Fig. 2. Kinetics of lycopene degradation in solutions with and without antioxidants in conditions of darkness and light at 20 °C (adjusted models). Key: -♦- darkness with antioxidants; -□- light with antioxidants -▲-darkness; -x- light.

3.4 Experiments IIIa and IIIb (Table 1): Kinetics of lycopene degradation in solutions with and without antioxidants in darkness during storage at 4, 20 and 60 °C

Two experiments were made, the first for lycopene solutions with antioxidants (IIIa) and the second for lycopene solutions without antioxidants (IIIb). The main factors for each were temperature and time. In both experiments, the main factors and their interaction (temperature*time) were significant ($p \leq 0.001$) with respect to lycopene retention. For both experiments, Tukey's multiple comparisons test revealed, regarding temperature, that the three means were significantly different ($p \leq 0.05$) and that, by increasing temperature, the lycopene retention mean diminished significantly. For the experiment with antioxidants, the pigment retention means were 85.58, 81.07 and 68.68 % at 4, 20 y 60 °C respectively. Regarding time, the same behavior was shown, all means were significantly different ($p \leq 0.05$) and by increasing time lycopene retention means diminished. In the case of the experiment without antioxidants, pigment retention means were 69.26, 65.28 and 60.84 % at 4, 20 y 60 °C respectively.

Regarding the interactions (temperature*time) for the experiment with antioxidants, the highest lycopene retention mean was 98.57 % on day 3 at 4 °C and presented significant difference ($p \leq 0.05$) with respect to the others. The lesser mean that presented significant difference from the rest was 36.18 % and was that of day 30 at 60 °C. For the experiment without antioxidants the highest lycopene retention mean was 74.43 % on day 3 at 4 °C, the significantly lesser ($p \leq 0.05$) mean was 35.82 % on day 30 at 60 °C. These significant differences are due to lycopene degradation which involves two stages: isomerization, to *cis* form isomers, and autooxidation of the unsaturated double bonds (Lee and Chen, 2001). These researchers also reported that lycopene mono-isomers *cis* degradation was greater when heating time was increased at 50°C. The main *cis* isomers found in tomatoes subjected to various heat treatment methods are 5-*cis* lycopene, 13-*cis* lycopene, 9-*cis* lycopene, 15-*cis* lycopene and di-*cis* lycopene (Lee and Chen, 2001; Hackett *et al*., 2004; Ishida and Chapman, 2004; Moraru and Lee, 2005).

For each of the three temperatures with and without antioxidants the models of the form: $\% L = C_o (\exp(-k t))E$ were adjusted, corroborating in each of them that the errors had normal distribution. The estimated models are indicated in Table 4.

In the models adjusted for lycopene in solutions with antioxidants (IIIa), the reaction rate constant was significantly lower ($p \leq 0.05$) at 4 °C (A) than at 60 °C (C) (0.014 and 0.033), meaning that pigment degradation was lesser (R^2 = 0.946 and 0.854). This same behavior was presented in the adjusted models for lycopene in solutions without antioxidants (IIIb), the rate constants were 0.030 at 4 °C (A') and 0.045 at 60 °C (C') (R^2 = 0.562 and 0.571), considering that initial lycopene concentration was 100 % (Fig. 3). In the case of 4 °C and 20 °C for solutions with antioxidants, the rate constant was significantly lower ($p \leq 0.05$) at 4 °C temperature (A) than at 20 °C (B) (0.014 and 0.018), meaning that pigment degradation was lesser (R^2 = 0.946 and 0.922). In the models adjusted for lycopene in solutions without antioxidants at 4 °C (A') and 20 °C (B') did not present significant difference either ($p \leq 0.05$). In the case of 4 °C for solutions with antioxidants (A) and without antioxidants (A'), the rate constant was significantly lesser ($p \leq 0.05$) for solutions with antioxidants than for solutions without antioxidants (0.014 and 0.030), meaning that pigment degradation was lesser (R^2 = 0.946 and 0.562). This same behavior was presented

Table 4. Adjusted models for lycopene retention in solutions with and without antioxidants in conditions of darkness at 4, 20 and 60 °C.

Case	Antioxidants	Temperature ° C	Adjusted model	R^2	50 % lycopene retention time (days)
A	Yes	4	% L = 100 exp (- 0.014t)	0.946	48
B	Yes	20	% L = 100 exp (- 0.018t)	0.922	38
C	Yes	60	% L = 100 exp (- 0.033t)	0.854	21
A'	No	4	% L = 100 exp (- 0.030t)	0.562	23
B'	No	20	% L = 100 exp (- 0.037t)	0.576	19
C'	No	60	% L = 100 exp (- 0.045t)	0.571	15

Statistical differences observed among cases:
A with B presented significant difference in the reaction rate constants.
A with C presented significant difference in the reaction rate constants.
B with C presented significant difference in the reaction rate constants.
A' with B' did not present significant difference in the reaction rate constants.
A' with C' presented significant difference in the reaction rate constants.
B' with C' did not present significant difference in the reaction rate constants.
A with A' presented significant difference in the reaction rate constants.
B with B' presented significant difference in the reaction rate constants.
C with C' did not present significant difference in the reaction rate constants.
In all cases $p \leq 0.05$

in the adjusted models for lycopene in solutions with antioxidants (B) and solutions without antioxidants (B') at 20 °C, the rate constants were 0.018 for the first model and 0.037 for the second model (R^2 = 0.922 and 0.576), considering that initial lycopene concentration was 100 %. The solutions with antioxidants (C) and solutions without antioxidants (C') at 60 °C did not present significant difference either ($p \leq 0.05$). Based on these results, the lesser reaction rate constant and the initial lycopene concentration of 100 %, the adequate conditions to store the pigment were in lycopene solutions with antioxidants in darkness at 4 °C (A). This was confirmed by needing 48 days to retain half of the initial lycopene (Table 4). These results are similar to those reported by Nguyen and Schwartz (1998), where lycopene in solution stored at 4 °C decreased formation of isomers; also, addition of BHT as antioxidant reduced the rate of lycopene isomerization.

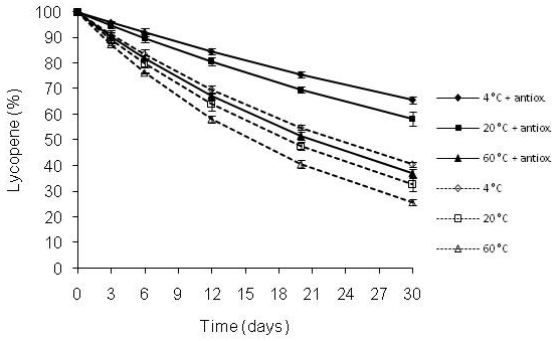

Fig. 3. Kinetics of lycopene degradation in solutions with and without antioxidants in conditions of darkness at 4, 20 and 60 °C (adjusted models). Key:-♦- 4 °C with antioxidants; -■- 20 °C with antioxidants; -▲- 60 °C with antioxidants; -◊- 4 °C; -□- 20 °C; -Δ- 60 °C.

The addition of antioxidants and storage under conditions of darkness are determining factors for pigment retention.

On the other hand, the greater lycopene loss was observed in lycopene solutions without antioxidants in darkness at 60 °C. In general, lycopene retention decreased as much as temperature and storage time were raised. This reduction is more probably due to oxidation than to isomerization since Lee and Chen (2002) reported that lycopene oxidation rate is higher than isomerization rate, when storage temperature is increased.

Conclusions

The retention of lycopene solutions depends on illumination, temperature and storage time, and whether the pigment is extracted from blanched or unblanched tomatoes.

The kinetics of lycopene degradation in solution follow a reaction of the first order with a reaction rate constant depending on temperature, addition of antioxidants and illumination.

The kinetic studies showed that pigment degradation was greater under light conditions and a temperature of 20 ºC than under conditions of darkness at 60 ºC during the same storage time.

Lycopene concentration from cv. Saladette tomatoes was greater when the fruit was blanched previous to the extraction since the heat treatment afforded greater pigment stability during storage at 4 ºC in the dark with the addition of antioxidants.

Lycopene from cv. Saladette tomatoes presents an adequate concentration and can be extracted from the blanched fruit and stored in darkness at 4°C with the purpose of usage as food additive or nutritious supplement once the toxicologic studies are carried out.

It is necessary to continue studies about the conversion mechanism of all-*trans* lycopene into its *cis* isomers in commercial foods systems for cv Saladette tomatoes.

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References

Akanbi, C.T. and Oludemi, F.O. (2004). Effect of processing and packaging on the lycopene content of tomato products. *International Journal of Food Properties 7*(1), 139-152.

Anese, M., Falcone, P., Fogliano, V., Nicoli, M.C. and Massini, R. (2002). Effect of equivalent thermal treatments on the color and the antioxidant activity of tomato purees. *Journal of Food Science 67*(9), 3442-3446.

Anguelova T. and Warthesen, J. (2000). Lycopene stability in tomato powders. *Journal of Food Science 65*(1), 67-70.

AOAC. (1996). *Official Methods of Analysis of AOAC International, sixteenth edition*, Vol. II. Association of Official Analytical Chemists, USA. Chapter 37, Pp. 5-6

Barringer, S. (2004). Frozen tomatoes. In: *Handbook of vegetable preservation and processing*, (Hiu, Y.H., Ghazala, S., Graham, D.M. and Nip, W.K.,eds.). Pp. 293-308. Marcel Dekker, Inc. New York,

Boileau, T.W.M., Boileau, A.C. and Erdman, J.W. (2002). Bioavailability of all-t*rans* and *cis* isomers of lycopene. *Experimental Biology and Medicine 227*(10), 914-919.

Bouvier, F., Dogbo, O. and Camara, B. (2003). Biosynthesis of the food and cosmetic plant pigment bixin (annatto). *Science 300*, 2089-2091.

Britton, G. (1996). Carotenoids. In: *Natural food colorants*, (Hendry GF Houghton JD, ed.). Pp. 197-243. Blackie Academic and Professional, Glasgow, Scotland.

Bruno, A.K. and Wetzel, C.M. (2004). The early light-inducible protein (ELIP) gene is expressed during the chloroplast-to-chromoplast transition in ripening tomato fruit. *Journal of Experimental Botany 55*(408), 2541-2548.

Calvo, M. M., Dado, D. and Santa-María, G. (2007). Influence of extraction with ethanol or ethyl acetate on the yield of lycopene, β-carotene, phytoene and phytofluene from tomato peel powder. *European Food Research and Technology 224*, 567-571.

Candelas-Cadillo, M.G., Alanís-Guzmán, M.G.J., Bautista-Justo, M., Del Río-Olague and García-Díaz, C. (2005). Contenido de licopeno en jugo de tomate secado por aspersión. *Revista Mexicana de Ingeniería Química 4*, 299-307.

Cvetkovic, D. and Markovic, D. (2008). Stability of carotenoids toward UV-irradiation in hexane soluction. *Journal of the Servian Chemical Society 73*(1), 15-27.

Delgado-Vargas F. and Paredes-Lopez, O. (2003). Carotenoids as food colors. In: *Natural colorants for food and nutraceutical uses*, (Delgado-Vargas F, Paredes-Lopez O. editors). Pp. 143-153. CRC Press, Boca Raton, Fla.

Fellows, P. (2005). Blanching. In: *Food processing technology: principles and practice*, (Fellows, P. ed.). Pp. 201-209. Ellis Horwood Ltd.,Publisher, London, England.

Ferreira, J.E.M. and Rodriguez-Amaya, D.B. (2008). Degradation of lycopene and β-carotene in model systems and in lyophilized guava during ambient storage: kinetics, structure, and matrix effects. *Journal of Food Science 78*(8), C589-C594.

Goula, A.M., Adamopoulos, K.G., Chatzitakis, P.C. and Nikas, V.A. (2006). Prediction of lycopene degradation during a drying process of tomato pulp. *Journal of Food Engineering 74*, 37-46.

Gouldson, M.J. and Warthesen, J.J. (1999). Stability and antioxidant activity de beta carotene in conventional and high oleic canola oil. *Journal of Food Science 64*(6), 996-999.

Hackett, M.M., Lee, J.H., Francis, D. and Schwartz, S.J. (2004). Thermal stability and isomerization of lycopene in tomato oleoresins from different varieties. *Journal of Food Science 69*(7), 536-541.

Humphrey, A.M. (2004). Chlorophyll as a color and functional ingredient. *Journal of Food Science* 69(5), 422-425.

Ishida, B.K. and Chapman, M.H. (2004). A comparison of carotenoid content and total antioxidant activity in catsup from several commercial sources in the United States. *Journal of Agricultural and Food Chemistry 52*(26), 8017-8020.

Ishida, B.K., Roberts, J.S., Chapman, M.H. and Burri, B.J. (2007). Processing tangerine tomatoes: effects on lycopene-isomer concentrations and profile. *Journal of Food Sci*ence *72*(6), 307-312.

Lee, M. T. and Chen B.H. (2001). Separation of lycopene and its cis isomers by liquid chromatography. *Chromatographia 54*, 613-617.

Lee, M. T. and Chen B.H. (2002). Stability of lycopene during heating and illumination in a model system. *Food Chemistry 78*, 425-432.

Lin, C.H. and Chen, B.H. (2005). Stability of carotenoids in tomato juice during processing. *European Food Research and Technology 221*, 274-280.

López-Hernández, E., Ponce-Alquicira, E., Cruz-Sosa, F. and Guerrero-Legarreta, I. (2001). Characterization and stability of pigments extracted from *Terminalia catappa* leaves. *Journal of Food Science 66*(6), 832-836.

Macrae, R., Robinson, R.K. and Sadler, M.J. (1993). Colours. In: *Encyclopaedia of Food Science, Food Technology and Nutrition*, vol. 2, Pp. 1164-1180. Academic Press, London, England.

Mayeaux, M., Xu, Z., King, J.M. and Prinyawiwatkul, W. (2006). Effects of cooking condictions on the lycopene content in tomatoes. *Journal of Food Science 71*(8), C461-C464.

Mencarelli F. and Saltveit, M. (1988). Ripening of mature-green tomato fruits slices. *Journal of the American Society for Horticultural Science 113*, 742-745.

Moraru, C. and Lee, T.C. (2005). Kinetic studies of lycopene isomerization in a tributyrin model system at gastric pH. *Journal of Agricultural and Food Chemistry 53*(23), 8997-9004.

Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucosa. *Journal of Biological Chemistry 153*(2), 375-378.

Nguyen, M. L. and Schwartz, S. J. (1998). Lycopene stability during food processing. *Proceedings of the Society for Experimental Biology and Medicine 218*, 101-105.

Nguyen, M.L. and Schwartz, S.J. (1999). Lycopene: chemical and biological properties, *Food Technolgy* 53(2), 38-45.

Nguyen, M.L. and Schwartz, S.J. (2000). Lycopene. In: *Natural food colorants,* (Lauro, J.G., Francis, F.J., editors). Pp. 153-192. Marcel Dekker, New York.

Re, R., Bramley, P.M. and Rice-Evans, C. (2002). Effects of Food processing on flavonoids and lycopene status in a Mediterranean tomato variety. *Free Radical Research 36*(7), 803-810.

Robinson W.B. and Stotz E. (1954). The indophenol-xileno extraction method for ascorbic acid. *Journal of Biological Chemistry 160*, 217-221.

Sadler, G., Davis, J. and Dezman, D. (1990). Rapid extraction of lycopene and β-caroteno from reconstituted tomato paste and pink grapefruit homogenates. *Journal of Food Science 55*(5), 1460-1461.

Schwartz, S.T. (1994). *Pigment analysis. In: Nielsen, S. Introduction to the chemical analysis of food*. Pp. 261-271. Jones and Bartlett Publishers International. New York.

Servicio de Información y Estadística Agroalimentaria y Pesquera (SIAP). (2007). Anuario estadístico de la producción agrícola, 2005. Ciudad de México, México: SIAP-SAGARPA. p 63-66.

Shi, J. and Le Maguer, M. (2000). Lycopene in tomatoes: chemical and physical properties affected by food processing. *Critical Reviews in Food Science and Nutrition 40*(1), 1-42.

Shi, J., Le Maguer, M., Bryan, M. and Kakúda, Y. (2003). Kinetics of lycopene degradation in tomato puree by heat and light irradiation. *Journal of Food Process Engineering 25*, 485-498.

Shi, J., Qu, Q., Kakúda, Y., Yeung, D. and Jiang, Y. (2004). Stability and synergistic effect of antioxidative properties of lycopene and other active components. *Critical Reviews in Food Science and Nutrition 44*, 559-573.

Sistema Nacional de información e Integración de Mercados (SNIIM). (2007). Consumo nacional aparente de productos agrícolas seleccionados, 2007. Ciudad de México, México: SNIIM-SE. 3p.

Spagna, G., Barbagallo, R.N., Chisari, M. and Branca, F. 2005. Characterization of a tomato polyphenol oxidase and its role in browning and lycopene content. *Journal of Agricultural and Food Chemistry 53*, 2032-2038.

Takeoka, G.R., Dao, L., Flessa, S., Gillespie, D.M., Jewell, W.T., Huebner, B., Bertow, D. and Ebeler, S.E. (2001). Processing effects on lycopene content and antioxidant activity of tomatoes. *Journal of Agricultural and Food Chemistry* 49(8), 3713-3717.

Thompson, K.A., Marshall, M.R., Sims, C.A., Wei, C.I., Sargent, S.A. and Scott, J.W. (2000). Cultivar, maturity, and heat treatment on lycopene content in tomatoes. *Journal of Food Science* 65(5), 791-795.

Wrolstad, R.E. (2004). Anthocyanin pigments: bioactivity and coloring properties. *Journal of Food Science* 69(5), 419-421.

Xianquan, S., Shi, J., Kakuda, Y. and Yueming, J. (2005). Stability of lycopene during food processing and storage. *Journal of medicinal food* 8(4), 413-422.