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Changes of health-related compounds throughout cold storage of tomato juice stabilized by thermal or high intensity pulsed electric field treatments

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Abstract

The effect of high intensity pulsed electric fields (HIPEF) processing (35 kV/cm for 1500 μs in bipolar 4-μs pulses at 100 Hz, with an energy density of 8269 kJ/L) on the main bioactive compounds and antioxidant capacity of tomato juice was investigated and compared to heat pasteurization (90 °C for 1 min or 30 s) having the fresh juice as a reference. HIPEF and heat treated tomato juices showed higher lycopene and lower vitamin C levels than the untreated juice. However, no significant changes in the total phenolic content and antioxidant capacity were observed between treated and fresh juices just after processing. Lycopene, vitamin C and antioxidant capacity of both treated and untreated juices decreased exponentially during storage following a first order kinetics (R^2 = 0.763–0.987), whereas tomato juices maintained their initial phenolic content. HIPEF-treated tomato juice maintained higher lycopene and vitamin C content than the thermally treated juices during the storage time. Hence, the application of HIPEF may be appropriate to achieve nutritious and fresh like tomato juice.

Keywords: Thermal treatment; High intensity pulsed electric fields; Tomato juice; Bioactive compounds; Antioxidant capacity

Industrial relevance: HIPEF processing can lead to tomato juice with higher nutritional value than that thermally processed. HIPEF-treated (35 kV/cm for 1500 μs with 4-μs bipolar pulses at 100 Hz, energy input of 8269 kJ/L) tomato juice shows greater lycopene, vitamin C and antioxidant capacity just after the treatment and during the storage time than heat treated (90 °C−30 s and 90 °C−60 s) tomato juice. Therefore, HIPEF technology is a feasible alternative to thermal treatment to obtain tomato juice with a high presence of health-related compounds.

1. Introduction

Regular consumption of tomatoes and tomato based products has been associated with lower incidence of various forms of cancer, in particular prostate cancer, and heart diseases (Arab & Steck, 2000). These beneficial effects have been attributed to the antioxidant components tomato contains such as carotenoids, vitamin C and phenolic compounds (Dumas, Dadomo, Di Lucca & Grolier, 2003). The main carotenoid present in tomato, is lycopene which provides its color (Stahl & Sies, 1996). The ability of lycopene to act as a potent antioxidant is thought to protect cells against oxidative damage (Rao & Agarwal, 1999). On the other hand, vitamin C may prevent free radical-induced damage to DNA quenching oxidants (Fraga et al., 1991), that overcome cell dysfunction and decrease low-density lipoprotein induced leukocyte adhesion (Lehr, Frei, Olofsson, Carew & Arfors, 1995). Several studies demonstrated that a diet rich in phenolic compounds correlates with reduced risk of coronary heart diseases (Amiot, Fleuriet, Cheynier & Nicolas, 1997). This association was partially explained on the basis of the fact that phenols interrupt lipid peroxidation induced by reactive oxygen species (ROS). Although tomatoes are commonly consumed in fresh, over 80% of the tomato consumption comes from processed products such as tomato juices (Gould, 1992).

Thermal processing is the most common method for extending the shelf-life of tomato juices, by inactivating microorganisms and enzymes. However, heat treatments can reduce the sensory and nutritional qualities of juices (Braddock, 1999). Therefore, consumer demands for healthy and nutritious food products with a fresh-like appearance has raised the awareness of the food

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industry for the development of milder preservation technologies to replace the existing pasteurization methods (Linneman, Meerdink, Meulenberg & Jongen, 1999). High intensity pulsed electric fields (HIPEF) processing of liquid foods is being investigated to avoid the negative effects of heat pasteurization (Deliza, Rosenthal & Silva, 2003). HIPEF treatment is efficient enough to destroy microorganisms in fruit juices at levels equivalent to heat pasteurization (Yeom, Streaker, Zhang & Min, 2000a). In addition, the enzymes commonly present in fruit juices are inactivated (Espachs-Barroso, Barbosa-Cánovas & Martín-Belloso, 2003). Giner, Gimeno, Espachs, Elez, Barbosa-Cánovas and Martín (2000) reported a reduction of 93.8% of PME from tomato juice after applying a HIPEF treatment set up at 24 kV/cm with 400 0.02-ms pulses. Yeom, Streaker, Zhang and Min (2000b) and Ayhan, Zhang and Min (2002) reported that quality parameters of juices are kept after applying HIPEF treatments. However, knowledge about the effects of this emerging technology on the antioxidant potential of fruit juices is scarce and generally focused on orange juices (Min, Jin, Min, Yeom & Zhang, 2003a; Elez-Martínez, Soliva-Fortuny & Martín-Belloso, 2006). Min and Zhang (2003) and Min, Jin and Zhang (2003b) studied the evolution of some quality parameters and bioactive compounds of hot break tomato juice, which was treated for 2 min at 88 °C before HIPEF treatment. Nevertheless, no information is currently available about the effect of HIPEF pasteurization on bioactive compounds as well as antioxidant capacity of fresh tomato juice. In addition, little is known about the evolution of the antioxidant properties during the commercial shelf-life of HIPEF-processed juices. Therefore the aim of the present work was to evaluate and compare the effects of HIPEF processing and heat pasteurization on lycopene, vitamin C and phenolic content as well as the antioxidant capacity of tomato juice. In addition, the effect of storage (4 °C) on the concentration of bioactive compounds in the tomato juices was investigated.

2. Materials and methods

2.1. Tomato juices

Tomato fruits (Licopersicon esculentum Mill, cultivar Bodar) were purchased at commercial maturity from a local supermarket. The fruits were ground and then filtered through 2-mm steel sieves. Electrical conductivity (Testo 240 conductivimeter; Testo GmBh & Co, Lenzkirch, germany), pH (crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), soluble solids content (Atago RX-1000 refractometer; Atago Company Ltd., Japan) and color measurement (Minolta CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) of tomato juice were determined (Table 1).

Table 1
Analytical characteristics of tomato juice

Parameters [a]	Tomato juice
pH	4.28 ± 0.02
Soluble solids (°Brix)	4.48 ± 0.1
Color	
L*	21.98 ± 0.08
a*	7.02 ± 0.22
b*	5.21 ± 0.05
Electrical conductivity (S/m)	0.65 ± 0.02

L*: lightness; a*: redness, b*: yellowness (the asterisk is a part of each color parameter).
[a] Results are the mean ± DS of three measurements.

2.2. Pulsed electric fields equipment

A continuous flow bench scale system (OSU-4F, Ohio State University, Columbus, OH, USA), that held bipolar squared wave pulses was used to treat tomato juice samples. The juice was pumped at a flow rate of 60 mL/min through a system of eight collinear chambers connected in series. Each chamber had a treatment volume of 0.012 cm^3 that was delimited by two stainless steel electrodes separated by a gap of 0.29 cm. The flow was controlled by a variable speed pump (model 752210-25, Cole Palmer Instrument Company, Vermon Hills, IL, USA). Treated tomato juice was passed through a cooling coil connected between each pair of chambers and submerged in an ice-water shaking bath. Thermocouples were attached to the surface of the stainless steel coils, 2.5 cm away from the HIPEF zones along the flow direction. The thermocouples were connected to temperature readers and isolated from the atmosphere with an insulation tape. The temperatures of the inlet and outlet of each pair of chambers were recorded every 0.1 s during HIPEF treatment and the samples never exceeded 40 °C. Tomato juice was subjected to HIPEF treatment consisted of bipolar square-wave pulses of 4 μs, with a frequency of 100 Hz, and 35 kV/cm field strength during 1500 μs, supplying an energy density of 8269 kJ/L. The main undesirable changes in juices are due to compounds produced by the growth of lactic acid bacteria (Lactobacillus and Leuconostoc) (Hendrix & Redd, 1995). Therefore treatment conditions were selected to reach a logarithmic reduction for *Lactobacillus brevis* of 5 log (data not shown).

2.3. Thermal treatment

Mild (90 °C, 30 s) and high (90 °C, 60 s) heat pasteurizations were applied as reference treatments that allow to compare the effectiveness of HIPEF treatments on bioactive compounds and antioxidant capacity. These conditions were selected based on literature, where typical heat treatments of juices vary from 95 °C to 90 °C for 15–60 s (Nagy, Chen & Shaw, 1993). Tomato juice was thermally processed in a tubular heat exchanger. A gear pump was used to maintain the juice flow rate through a stainless steel heat exchange coil, which was immersed in a hot water shaking bath (Universitat de Lleida, Lleida, Spain). After thermal processing, the juice was immediately cooled in a heat exchange coil immersed in an ice water-bath.

2.4. Sample packaging and storage

HIPEF and thermal fluid handling system was disinfected first with 4% NaOH and then with 10% chlorine and 20% ethanol solutions prior to processing. The first 200 ml treated liquid was discarded to ensure stationary treatment conditions. Polypropylene bottles of 100 ml previously sterilized at 121 °C for 30 min were used to store tomato juice. The juice was bottled

directly from the treatment system, leaving the minimum amount of headspace volume. Once filled, the receptacle was tightly closed and stored, up to analysis, under refrigeration at 4 °C in darkness until spoilage took place in samples.

2.5. Lycopene

Total lycopene content was measured spectrophotometrically following the method proposed by Davis, Fish and Perkins-Veazie (2003). Approximately 0.6 g of sample were weighed with precision from each cultivar and added to a mixture consisting of 5 ml of 0.05% (w/v) butylated hydroxytoluene in acetone, 5 ml of 95% USP grade ethanol and 10 ml of hexane. The homogenate was centrifuged at 320 ×*g* for 15 min on ice. Afterwards, 3 ml of deionized water were added. The vials were then agitated for 5 min and left at room temperature to allow phase separation. The absorbance of the upper hexane layer, was measured in a 1 cm path length quartz cuvette at 503 nm blanked with hexane. The lycopene content of each sample was estimated using the absorbance at 503 nm and the sample weight (Fish, Perkins-Veazie & Collins, 2002). Results were expressed as mg of lycopene per 100 g of tomato juice.

2.6. Vitamin C

Vitamin C content in tomato juice was analysed by HPLC. The extraction procedure was based on a method validated by Odriozola-Serrano, Hernández-Jover and Martín-Belloso, (2007). A sample of 25 g of juice was mixed with 25 ml of a solution containing 45 g of metaphosforic acid and 7.2 g of DL-1,4-dithiotreitol per at 22,100 ×*g* for 15 min at 4 °C. The supernatant was vacuum-filtered through Whatman No. 1 paper. The sample was then passed through a Millipore 0.45 μm membrane. An aliquot of 20 μs was injected into the HPLC system using a reverse-phase C18 Spherisorb® ODS2 (5 μm) stainless steel column (4.6 mm×250 cm). The mobile phase was a 0.01% solution of sulphuric acid adjusted to pH=2.6. The flow rate was fixed at 1.0 ml/min at room temperature. Detection was performed with a 486 Absorbance Detector (Waters, Milford, MA) set at 245 nm. The quantification of vitamin C was carried out comparing the samples with a calibration line built with 0, 5, 10, 15, 30 and 50 mg ascorbic acid/100 g (Scharlau Chemie, SA., Barcelona, Spain). Results were expressed as mg of vitamin C per 100 g of tomato juice.

2.7. Total phenolic content

Total phenols were determined by the colorimetric method of Singleton, Orthofer and Lamuela-Raventos (1999) using the Folin-Ciocalteu reagent. Samples of tomato juice were centrifuged at 6000 ×*g* for 15 min at 4 °C (Centrifuge Medigifer; Select, Barcelona, Spain) and filtered through Whatman No 1 filter paper. Afterwards, 0.5 ml of the extract was mixed with 0.5 ml of Folin-Ciocalteu reagent and 10 ml of saturated Na_2CO_3 solution. Samples were allowed to stand for 1 h at room temperature before the absorbance at 725 nm was measured. Concentrations were determined by comparing the absorbance of the samples with a calibration line built with 0, 10, 20, 30, 40 and 50 mg gallic acid/100 g (Scharlau Chemie, SA.,Barcelona, Spain). Results were expressed as milligrams of gallic acid per 100 g of tomato juice.

2.8. Antioxidant capacity

The antioxidant capacity was studied through the evaluation of free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The determination was based on the method proposed by De Ancos, Sgroppo, Plaza and Cano (2002). Samples of tomato juice were centrifuged at 6000 ×*g* for 15 min at 4 °C (Centrifuge Medigifer; Select, Barcelona, Spain) and aliquots of 0.01 ml of the supernatant were mixed with 3.9 ml of methanolic DPPH (0.025 g/l) and 0.090 ml of distilled water. The homogenate was shaken vigorously and kept in darkness for 30 min. Absorption of the samples was measured with a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) at 515 nm against a blank of methanol without DPPH. Results were expressed as percentage decrease with respect to the absorption value of a reference DPPH solution.

2.9. Statistical analysis

Treatments were conducted in duplicate and two replicate analyses were carried out for each sample in order to obtain the mean value (n=4). Significance of the results and statistical differences were analyzed using the Statgraphics Plus v.5.1 Windows package (Statistical Graphics Co., Rockville, Md). Analysis of variance (ANOVA) was performed to compare treatment mean values. The least significant difference test was employed to determine differences between means at a 5% significance level. Correlations between antioxidant capacity and the studied bioactive compounds were evaluated with Pearson's test.

Experimental data were fitted to a first-order kinetic model (Eq. 1) to describe the evolution of the health-related compounds as well as the antioxidant capacity during cold storage.

$$C = C_o e^{(-k \cdot t)} \quad (1)$$

where C is the residual content of bioactive compound (mg/100 g) or antioxidant capacity (%), C0 is the initial content of the bioactive compound (mg/100 g) or antioxidant capacity (%), k is a first-order rate constant ($days^{-1}$) and t the storage time (days).

3. Results and discussion

3.1. Lycopene

The effects of processing and storage time on the concentration of lycopene in tomato juices are shown in Fig. 1. Lycopene concentration was enhanced significantly after thermal or HIPEF processing compared to the untreated juice. The increase in lycopene content ranged from 4.67% in high thermally processed to 7.6% in HIPEF-treated tomato juice. It has been

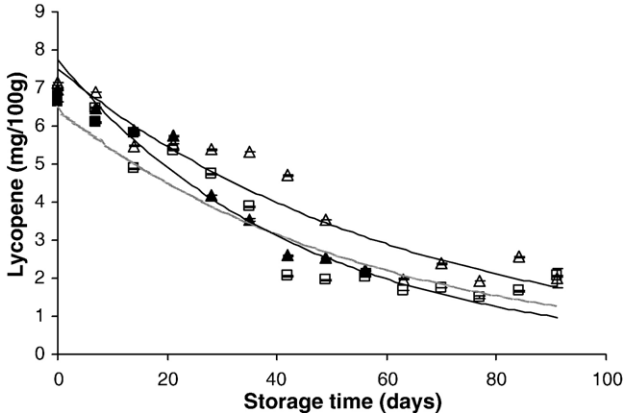

Fig. 1. Effects of HIPEF treatment and heat pasteurization on lycopene content of tomato juice throughout storage at 4 °C. Tomato juices: (■) untreated, (Δ,—) HIPEF, (▲,---) heat pasteurized at 90 °C for 30 s and (□,……) heat pasteurized at 90 °C for 60 s. Data shown are mean ± standard deviation.

reported that food processing such as cooking or grinding might improve lycopene bioavailability by breaking down cell walls (Gartner, Stahl & Sies, 1997). Nguyen and Schwartz (1999) suggested that homogenization and heat treatment disrupt cell membranes and protein–carotenoids complex, making carotenoids more accessible for extraction. Nevertheless further investigations are still needed to explain this enhancement in lycopene content of tomato juice treated by HIPEF. Some authors reported higher levels of carotenoids in juices processed with HIPEF compared to the untreated tomato juice. For instance, Sánchez-Moreno et al. (2005a) observed that in "gazpacho" a soup where tomato is the major component, the content of total carotenoids increased after applying 35 kV/cm in bipolar mode, 800 Hz pulse frequency, 4 μs pulse width during 750 μs in comparison to the untreated juice. Consistently, Torregrosa, Cortés, Esteve and Frígola (2005) and Cortés, Esteve, Rodrigo, Torregrosa, and Frígola (2006) reported a significant rise in some carotenoids in HIPEF-treated juices.

The concentration of lycopene in tomato juices depleted with storage time irrespective of the treatment applied (Fig. 1). Lycopene content decreased in high thermally and HIPEF processed tomato juice from 6.94 to 2.05 mg/100 g and from 7.15 to 2.02 mg/100 g, respectively, after 91 days at 4 °C. This trend is in accordance with that observed by other authors. Min et al. (2003b) reported significant losses of lycopene in hot break tomato juice treated by HIPEF (40 kV/cm for 57 μs) and thermally processed (92 °C for 90 s) over 112 days of study. As can be seen in Fig. 1, the lycopene content of the tomato juice processed with HIPEF was 2.21 mg/100 g at 56 days. At the same day, thermally processed juice showed a lycopene concentration of 2.03 and 2.20 mg/100 g for high and mild pasteurization, respectively. These changes in lycopene content throughout the storage might be due to the oxygen availability in the headspace of the bottles during the early storage period. Rodriguez-Amaya (1993) found that the stability of lycopene in foods greatly depends on oxygen availability and packaging conditions. Shi and Le Maguer (2000) observed that carotenoids are susceptible to oxidation in the presence of light, oxygen and low pH. On the other hand, HIPEF-treated tomato juice had higher lycopene content than the thermally treated during the storage time. Cortés et al. (2006) reported that the decrease in the concentration of total carotenoids was greater in untreated and pasteurized juice (90 °C, 20 s) than in HIPEF-treated (30 kV/cm for 100 μs) orange juice during cold storage. However, Min et al. (2003b) did not observe differences in the lycopene content between thermally and HIPEF processed hot break tomato juices, which was heat processed (88 °C for 2 min) before HIPEF treatment, during the storage period. The experimental data values of lycopene content as a function of storage time were described by a first-order kinetic model. The regression parameters of the fitted model ($P<0.05$) are given in Table 2, which shows that the first-order kinetic model displayed high determination coefficients (R^2) and fits well the experimental data. The first-order model rate constant ranged from 1.59×10^{-2} (HIPEF processing) to 2.27×10^{-2} (mild pasteurization), indicating higher destruction of lycopene in thermally treated than in HIPEF-treated tomato juice with storage time. Comparing the intercepted point of the first-order kinetic models, the lycopene content just after applying the HIPEF treatment was 0.5–1.6 mg/100 ml higher than that treated at 90 °C, 60 s (Table 2). In addition, an increment over 14% of lycopene was obtained for juices treated by HIPEF compared to high heat treated juice, during the storage period (Table 2). No significant differences in lycopene concentration were observed between HIPEF and mild heat treated juices immediately after the treatments as well as during the first week of storage.

Table 2
First-order kinetic rate constants (k) and determination coefficients (R^2) for the degradation of the health-related compounds and antioxidant capacity of treated tomato juice during storage at 4 °C

Parameters	Treatments	C_0	k ($days^{-1}$)	R^2
Lycopene (mg/100 g)				
	HIPEF	7.50±0.27	$1.59\times10^{-2}\pm1.11\times10^{-3}$	0.866
	High pasteurization	6.45±0.28	$1.77\times10^{-2}\pm1.42\times10^{-3}$	0.834
	Mild pasteurization	7.74±0.21	$2.27\times10^{-2}\pm1.30\times10^{-3}$	0.959
Vitamin C (mg/100 g)				
	HIPEF	10.37±0.18	$2.41\times10^{-2}\pm7.43\times10^{-4}$	0.968
	High pasteurization	9.07±0.40	$3.04\times10^{-2}\pm2.70\times10^{-3}$	0.987
	Mild pasteurization	11.68±0.27	$3.75\times10^{-2}\pm1.54\times10^{-3}$	0.968
Antioxidant Capacity (%)				
	HIPEF	7.17±0.37	$1.32\times10^{-2}\pm1.46\times10^{-3}$	0.901
	High pasteurization	7.49±0.39	$1.37\times10^{-2}\pm1.50\times10^{-3}$	0.906
	Mild pasteurization	8.14±0.65	$1.22\times10^{-2}\pm2.96\times10^{-3}$	0.763

HIPEF= high intensity pulsed electric fields treatment at 35 kV/cm for 1000 μs; bipolar 4-μs pulses at 100 Hz.
High pasteurization=treatment at 90 °C for 1 min.
Mild pasteurization= treatment at 90 °C for 30 s.

Nevertheless, the difference in lycopene content between HIPEF- and mild heat treated tomato juice rose when storage increase. In this way, tomato juices treated by HIPEF had 0.8–1.7% more lycopene presence the day 7 of storage than that treated by mild heat, whereas this difference increased to 26.7–29.5% at 56 storage days (Table 2).

3.2. Vitamin C

Vitamin C content of treated and untreated tomato juice measured directly after treatment ranged from 10.17 to12.8 mg/100 g (Fig. 2). The highest content of this vitamin was observed in fresh tomato juice. The results obtained in the present work for vitamin C content are in the range of those published in literature (Davey et al., 2000; Sánchez-Moreno, Plaza, de Ancos & Cano, 2006). Vitamin C content of both HIPEF and thermally processed tomato juices decreased drastically after being treated compared to untreated juices. However, HIPEF-treated tomato juice showed higher concentration of vitamin C than the thermally processed juice (Fig. 2). Vitamin C retention just after treatment in heat-treated tomato juice was 79.2% (high pasteurization) and 80.4% (mild pasteurization), whereas in HIPEF-treated tomato juice a 86.5% retention was attained. This is in agreement with results observed by Elez-Martínez and Martín-Belloso (2007), who reported a vitamin C retention of 84.3% in gazpacho treated under HIPEF conditions similar to those used in the present work. Most differences between HIPEF and heat treatments can be explained through the temperatures reached through processing. Ascorbic acid is a heat-sensitive bioactive compound in the presence of oxygen. Thus, high temperatures during processing can greatly affect the rates of its degradation through an aerobic pathway. The maximum temperature achieved during HIPEF processing was 40 °C. Therefore the higher retention of Vitamin C of HIPEF-treated tomato juice compared to the thermally processed samples might be due to the lower processing temperatures of the HIPEF treatment. Different studies have proven the effectiveness of HIPEF in achieving higher vitamin C retention in comparison with heat treatments in juices. Min et al. (2003b) did not find difference between fresh and HIPEF processed tomato juice treated at 40 kV/cm for 57 μs with bipolar pulses of 2-μs and a maximum temperature of 45 °C. These authors observed similar vitamin C retention in thermally and HIPEF-treated hot break tomato juices, which might be due to the heat pasteurization (88 °C for 2 min) applied before HIPEF treatment. The concentration of vitamin C in thermally processed, HIPEF-processed and untreated juices decreased as storage time increased (Fig. 2). However, tomato juice treated by HIPEF retained more vitamin C than thermally processed tomato juice for 91 days at 4 °C. Vitamin C retentions in HIPEF and high thermally processed tomato juices were 8.9% and 5.0%, respectively, at 91 days of storage at 4 °C. Consistently, Min et al. (2003b) observed significantly higher contents of vitamin C in HIPEF-treated than in thermally pasteurized hot break tomato juice. Vitamin C retention has been used as indicator of shelf-life for chilled orange juices. It has been considered that juices with 50% of the initial vitamin C are at the end of its shelf-life (Shaw, 1992). As can be seen in Fig. 2, the concentration of vitamin C in the tomato juice treated by HIPEF or mild pasteurization was reduced about 50% after 28 days of storage at 4 °C. However, the losses of vitamin C were grater than 50% in juices subjected to high pasteurization after 21 days of storage. Min et al. (2003b) reported a reduction of 50% of vitamin C in HIPEF and heat treated hot break tomato juices during the first 30 days of the storage period. The great reduction of vitamin C within the first month of storage might be due to the presence of oxygen in the head space of the packages. Atmospheric oxygen is responsible for most losses during long-term storage. Vitamin C is usually degraded by oxidative processes which are stimulated in the presence of light, oxygen, heat peroxides and enzymes (especially ascorbate oxidase and peroxidase) (Davey et al., 2000). The rate of vitamin C depletion during storage depends on type of processing, storage conditions and packaging (Ayhan, Yeom, Zhang & Min, 2001). The contents of vitamin C as a function of the storage time were described by a first-order kinetic model. The model fitted well the experimental data ($R^2 \geq 0.968$) (Table 2) and the first-order model rate constant values varied from 2.41×10^{-2} (HIPEF processing) to 3.75×10^{-2} (mild pasteurization). Significantly higher vitamin C content (15.1–59.8%) was obtained in HIPEF-treated tomato juice compared to that high heat treated during the storage period. Although during the first week of storage the concentration of vitamin C was greater in tomato juice treated by mild pasteurization than that treated by HIPEF, no significant differences were found between 7 and 21 days of storage and lower content of vitamin C (15.9–49.4%) were observed in the former juice compared to HIPEF-treated juice after 21 days of storage at 4 °C (Fig. 2).

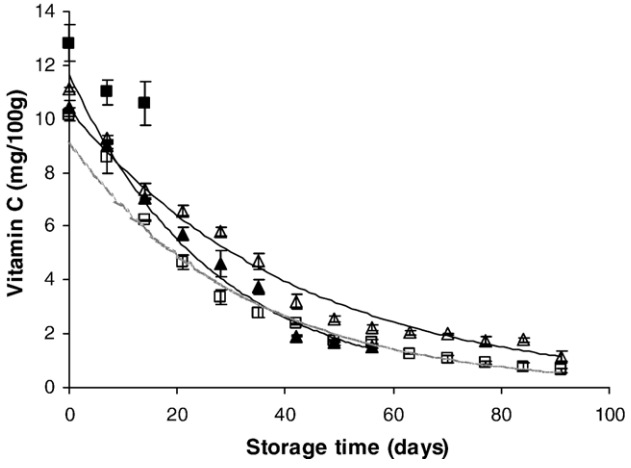

Fig. 2. Effects of HIPEF treatment and heat pasteurization on vitamin C retention of tomato juice throughout storage at 4 °C. Tomato juices: (■) untreated, (Δ,—) HIPEF, (▲,---) heat pasteurized at 90 °C for 30 s and (□,.....) heat pasteurized at 90 °C for 60 s. Data shown are mean ± standard deviation.

3.3. Total Phenolic Compounds

The concentration of total phenolic compounds in tomato juices varied from 31.1 mg/100 g (high pasteurization) to 32.8 mg/100 g (untreated) (Fig. 3). These values are within the

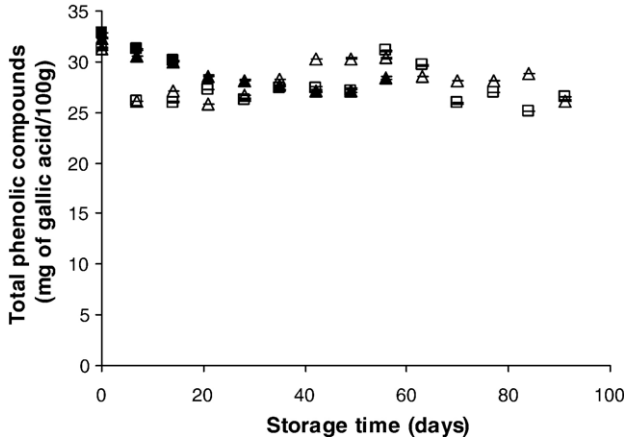

Fig. 3. Effects of HIPEF treatment and heat pasteurization on total phenolic content of tomato juice throughout storage at 4 °C. Tomato juices: (■) untreated, (Δ) HIPEF (▲) heat pasteurized at 90 °C for 30 s and (□) heat pasteurized at 90 °C for 60 s. Data shown are mean ± standard deviation.

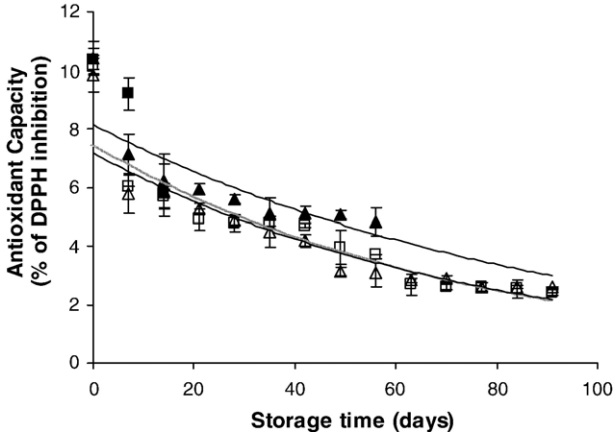

Fig. 4. Effects of HIPEF treatment and heat pasteurization on antioxidant capacity of tomato juice throughout storage at 4 °C. Tomato juices: (■) untreated, (Δ,—) HIPEF (▲,---) heat pasteurized at 90 °C for 30 s and (□,……) heat pasteurized at 90 °C for 60 s. Data shown are mean ± standard deviation.

range observed in other studies. Podsędek, Sosnowska and Anders (2003) reported a concentration of phenolic content between 26.77 to 52.26 mg/100 g in different tomato juices. On the other hand, no significant differences were observed in the phenolic content of processed and unprocessed tomato juices. In accordance with our results, Dewanto, Wu, Adom and Liu (2002) did not find significant changes in total phenolic content between thermally treated and fresh tomato puree. As can been seen in Fig. 3, tomato juice treated at 90 °C for 30 s retained the initial total phenolic content for a period of 56 days at 4 °C. In the same way, the tomato juices subjected to both high thermal treatment and to HIPEF-processing retained their initial phenolic content during 91 days. Tomato juices showed phenolic compounds values of 26.2 mg/100 g (HIPEF) and 26.5 mg/100 g (high pasteurization) at 91 days of storage. As far as we know, there are no published works quantifying total phenolic content throughout storage of tomato juices. However, Pérez-Vicente, Serrano, Abellán and García-Viguera (2004) reported insignificant changes in total phenolic compounds of pomegranate juices stored during 160 days at 18 °C. The maintenance of total phenolic compounds during storage might be due to the inactivation of the enzymes responsible for its degradation. Peroxidase is the main enzyme implicated in reactions that are associated with loss of quality in tomato juice. In addition, this enzyme is involved in the oxidative degradation of phenolic compounds (Amiot et al., 1997). It has been demonstrated that both thermal and HIPEF treatments could inhibit peroxidase in juices. Anthon, Sekine, Watanabe and Barret (2002) observed a residual peroxidase activity of 10% after applying a thermal treatment (72 °C–30 s) in tomato juices. A reduction of 96.87% of the initial POD activity of tomato juice were obtained after applying a HIPEF treatment similar to that used in the present study (Aguiló-Aguayo, Soliva-Fortuny & Martín-Belloso, 2007). In this way, Aguiló-Aguayo, Elez-Martínez, Soliva-Fortuny and Martín-Belloso (2006) reported a 65% inactivation of peroxidase activity in "gazpacho" soup, where tomato is the main component, treated under HIPEF conditions similar to the used in the present work.

3.4. Antioxidant Capacity

The antioxidant capacity, measured on the basis of the DPPH stable radical, of fresh and processed tomato juices ranged from 9.8 to 10.4% of DPPH inhibition (Fig. 4), with no significant differences between treated and untreated products. Therefore the treatment did not affect the DPPH inhibition of tomato juices versus the fresh juice. Elez-Martínez and Martín-Belloso (2007) reported that HIPEF treatments of 15 or 35 kV/cm for 1000 μs at 200 Hz applying bipolar or monopolar 4-μs pulses led to similar levels of antioxidant capacity in orange juice and gazpacho with respect to the thermal treatment. On the other hand, Sánchez-Moreno, Plaza, Elez-Martínez, de Ancos, Martín-Belloso and Cano (2005b) observed that the antioxidant capacities of untreated and HIPEF-treated orange juices were not significantly different. It has been demonstrated that heat processing causes no change in the antioxidant capacity of fruit and vegetables due to the formation of novel compounds such as Maillard reaction products having antioxidant activity (Manzocco, Calligaris, Mastrocola, Nicoli & Lerici, 2001). The antioxidant capacity of tomato juice depleted with storage time at 4 °C irrespective of the treatment applied (Fig. 4). Antioxidant capacity of tomato juice subjected to mild heat treatment was 46.52% of the initial value at 56 days, whereas antioxidant capacity decreased to 29.6% and 35.7% of the initial DPPH inhibition for HIPEF-treated and high pasteurized juice respectively, at the same storage day. No significant differences

Table 3
Correlation coefficients among different bioactive compounds and antioxidant capacity of tomato juice subjected to different treatments and storage at 4 °C

	Lycopene	Vitamin C	Phenolic compounds	Antioxidant capacity
Lycopene	–	0.913 [a]	0.344	0.808 [a]
Vitamin C	0.913 [a]	–	0.467 [a]	0.859 [a]
Phenolic compounds	0.344	0.467 [a]	–	0.524 [a]
Antioxidant capacity	0.808 [a]	0.859 [a]	0.524 [a]	–

[a] significant differences at $P<0.001$, using Pearson's correlation coefficients.

in antioxidant capacity were observed between tomato juice treated by HIPEF and high heat pasteurization. The antioxidant capacity is related to the amount and composition of bioactive compounds present in food (Sánchez-Moreno et al., 2005b). Eberhardt, Lee and Liu (2000) indicated that most antioxidant capacity comes from the natural combination of different phytochemicals. Vitamin C and phenols are reported to be the major antioxidant components in tomato (Takeoka et al., 2001). However, the magnitude of the changes in the antioxidant capacity could not be associated with the maintenance of the total phenolic compounds during storage ($R^2=0.524$) (Table 3). There was a significant correlation between antioxidant capacity and vitamin C ($R^2=0.859$) as well as between lycopene content ($R^2=0.808$) and antioxidant capacity (Table 3). These correlations seem to indicate that changes in antioxidant capacity might be modulated by vitamin C and lycopene. The decrease of antioxidant capacity during refrigeration could be attributed to vitamin C and lycopene losses. Sánchez-Moreno et al. (2006) observed that vitamin C and lycopene were the main compounds responsible for the changes of antioxidant capacity of tomato juices during the storage time. As can be seen in Table 3, there was a strong correlation ($R^2=0.913$) between lycopene and vitamin C content. Thus, the higher the lycopene content of tomato juice, the greater the vitamin C concentration was.

The first-order kinetic model predicted well the antioxidant capacity as indicated by the obtained good determination coefficients ($R^2=0.763$–0.906). Nevertheless, the model did not describe adequately the fast decrease in antioxidant capacity that occurred during the first 2 days of storage at 4 °C. First-order kinetic rates took values from 1.22×10^{-2} to 1.37×10^{-2} (Table 2). No references about modeling the effect of the storage time on the antioxidant capacity of heat or HIPEF-treated tomato juice through the first-order kinetic model have been published up now.

4. Conclusions

HIPEF processing can produce tomato juice with higher nutritional value than conventional thermal processing. HIPEF-treated (35 kV/cm for 1500 μs with 4-μs bipolar pulses at 100 Hz, energy input of 8269 kJ/L) tomato juice shows higher lycopene and vitamin C content just after the treatment and during the storage time than thermally treated (90 °C–30 s and 90 °C–60 s) tomato juice. A rise in lycopene content and a depletion of vitamin C is observed after HIPEF and thermal treatments compared to the fresh tomato juice. Nevertheless no significant differences are observed in phenolic content and antioxidant capacity between treated and untreated samples just after processing. In addition, storage time shows a significant effect on the studied compounds. Lycopene, vitamin C and antioxidant capacity deplete with time irrespective of the treatment applied, whereas the initial content of total phenolic compounds is kept during storage. Therefore HIPEF technology could be an alternative to thermal treatment to obtain tomato juice with high nutritional quality. Further research is needed in order to know more about the effects of HIPEF on the mechanisms involving bioactive changes.

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