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Analytical Methods

Carotenoid and phenolic profile of tomato juices processed by high intensity pulsed electric fields compared with conventional thermal treatments

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ABSTRACT

The effect of high intensity pulsed electric fields (HIPEF) processing (35 kV/cm for 1500 μs of overall treatment time with bipolar pulses of 4-μs at 100 Hz) and heat pasteurisation (90 °C for 30 s or 60 s) on carotenoids and phenolic compounds as well as on some quality attributes (pH, soluble solids and colour parameters) of tomato juice was evaluated and compared, having the untreated juice as a reference. Processing enhanced some carotenoids (lycopene, β-carotene and phytofluene) and the red colour of juices, whereas no significant changes in phenolic compounds, pH and soluble solids were observed between treated and untreated juices. A slight decrease in overall health-related compounds was observed over time, with the exception of some carotenoids (β-carotene and phytoene) and caffeic acid. However, HIPEF-processed tomato juices maintained higher content of carotenoids (lycopene, neurosporene and γ-carotene) and quercetin through the storage time than thermally and untreated juices. Hence, the application of HIPEF may be appropriate to achieve not only safe but also nutritious and fresh like tomato juice.

1. Introduction

Regular consumption of tomatoes and tomato based products has been associated with reduced incidence of some types of cancer and heart diseases (Clinton, 1998). These beneficial properties have been attributed in part to their content in various bioactive compounds such as carotenoids. Tomato and tomato products are the predominant source of lycopene, which exhibits a high oxygen-radical scavenging and quenching capacities, and β-carotene, which is the main carotenoid with provitamin A activity (Beecher, 1998). However, carotenoids are highly unsaturated compounds with an extensive conjugated double-bonds system and they are susceptible to oxidation, isomerisation and other chemical changes during processing and storage (Shi & Le Maguer, 2000). On the other hand, carotenoids are mainly responsible for the red colour of tomato which is the first quality factor that the consumer appreciates and has a remarkable influence on its acceptance (Abushita, Daood, & Biacs, 2000). In addition to carotenoids, other antioxidant compounds such as phenolics also contribute to the beneficial effects of tomato products. Phenolics possess reducing character, capacity of sequestering reactive oxygen species (ROS) and several electrophiles, tendency to self-oxidation and capacity to modulate the activity of some cell enzymes (Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999). Thus, consumption of tomato and tomato based product is being considered as a nutritional indicator of good dietary habits and healthy lifestyle.

Thermal processing is the most common method to extend the shelf-life of juices, by inactivating microorganisms and enzymes. However, heat treatments reduce the sensory and nutritional qualities of these products (Braddock, 1999). Therefore, high intensity pulsed electric fields (HIPEF) is being developed as a non-thermal emerging technology for the preservation of foods. Up to now, studies have suggested that HIPEF treatment is efficient enough to destroy microorganisms in fruit juices at levels equivalent to those achieved by heat pasteurisation without greatly affecting their nutritional and sensory properties (Min & Zhang, 2003; Yeom, Streaker, Zhang, & Min, 2000). In addition, the enzymes commonly present in fruit juices are partially or totally inactivated (Marsellés-Fontanet & Martín-Belloso, 2007). In this regard, Aguiló-Aguayo, Odriozola-Serrano, Quintão-Teixeira, and Martín-Belloso (2008a) reported complete POD inactivation in tomato juice after applying 5.5-μs bipolar pulses of 35 kV/cm for 2000 μs at 200 Hz. On the other hand, some studies have suggested that HIPEF processing may enhance the antioxidant properties of juices comparing to those untreated (Odriozola-Serrano, Aguiló-Aguayo, Soliva-Fortuny, Gimeno-Añó, & Martín-Belloso, 2007; Torregrosa, Cortés, Esteve, & Frígola, 2005). However, little is known about how HIPEF treatments can alter or modify the individual profile of health-related compounds in processed vegetable foods. Therefore, evaluating the influence of HIPEF processing and storage on individual carotenoids and phenolic compounds is a key factor to obtain

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tomato juices with antioxidant potential similar or higher than that of fresh juices.

The aim of the present work was to evaluate and compare the effects of HIPEF processing and heat pasteurisation on individual carotenoids and phenolic compounds of tomato juice. In addition, the effect of storage (4 °C) on the concentration of these bioactive compounds was investigated. Colour, pH and soluble solids content were also considered as quality parameter and related to health-related compounds.

2. Materials and methods

2.1. Tomato juice preparation

Tomatoes (*Lycopersium esculentum* Mill. cultivar Bodar) at commercial maturity were bought from a local supermarket, and kept at 4 °C before being processed. The fruits were washed, sorted and chopped and then filtered through a 2 mm diameter steel sieve to remove peel and seeds. pH (Crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), titratable acidity, soluble solids content (Atago RX-1000 refractometer; Atago Company Ltd., Tokyo, Japan), colour measurement (spectrophotocolorimeter Minolta CR-400; Konica Minolta Sensing, Inc., Osaka, Japan) and hardness of the mesocarp of whole tomato (TA-XT2 Texture Analyzer; Stable Micro Systems Ltd, Surrey, UK) were determined. The physico-chemical characteristics of tomato were: pH 4.32 ± 0.33, titratable acidity = 0.34 ± 0.01, soluble solids = 4.5 ± 0.1 °Brix, colour L^* = 42.2 ± 1.8, a^* = 21.8 ± 2.5 and b^* = 25.1 ± 1.9, hardness of mesocarp = 28.6 ± 1.3 N.

2.2. Pulsed electric fields equipment

Pulse treatments were carried out using a continuous flow bench scale system (OSU-4F, Ohio State University, Columbus, OH, USA), that provides squared-wave pulses within eight co-field flow chambers in series. Each chamber had a treatment volume of 0.012 cm^3, delimited by two stainless steel electrodes and separated by a gap of 0.29 cm. The flow rate of the process was adjusted to 60 mL/min and controlled by a variable speed pump (model 752210-25, Cole Palmer Instrument Company, Vermon Hills, IL, USA). The treatment temperature was kept below 40 °C using a cooling coil, which was connected before and after 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. HIPEF treatment was set up at 35 kV/cm for 1500 μs using bipolar squared-wave pulses of 4 μs and a frequency of 100 Hz.

2.3. Thermal treatment

In order to compare the effect of HIPEF treatment to that of the conventional thermal treatment, tomato juice was subjected to heat processes at 90 °C for 30 s (mild heat pasteurisation) or 90 °C for 60 s (high heat pasteurisation). These conditions were selected based on literature (Nagy, Chen, & Shaw, 1993). Tomato juice was thermally processed in a tubular stainless steel heat exchange coil of 2.2 mm of internal diameter and 11 m of length immersed in a hot water shaking bath (Universitat de Lleida, Lleida, Spain). A gear pump was used to maintain the desirable juice flow rate. After thermal processing, the juice was immediately cooled in a heat exchange coil immersed in an ice water-bath.

2.4. Packaging and storage conditions

HIPEF and thermal fluid handling system were sanitized prior to processing. Polypropylene sterile 100-mL bottles were filled directly from the outlet of the treatment systems leaving as less headspace as possible. Afterwards, the container was tightly closed and stored at 4 °C ± 1 °C for 56 days. Treatments were conducted in duplicate and two replicate analyses were carried out for each sample in order to obtain the mean value.

2.5. Carotenoids

2.5.1. Extraction

The extraction method was based on a procedure for extraction of carotenoids from thermally processed tomato products (Tonucci et al., 1995). First, a 2.5 g of magnesium carbonate and 2.5 g of Celite, used as filter aid, were added to 25 mL of tomato juice and 0.5 mg of internal standard (β-apo-8′-carotenal). The mixture was blended for 20 min in an Omni Mixer with 25 mL of tetrahydrofuran (THF) and then filtered through Whatman No. 1 filter paper using a Büchner funnel. The solid material was extracted two or three more times until it was devoid of red/orange colour. The THF extracts were combined, and the volume was reduced by about two-thirds under vacuum at 35 °C with a rotary evaporator. Components of the combined extract were portioned into 250 mL of methylene chloride and 150 mL of saturated sodium chloride water in a separatory funnel. The water layer was washed with methylene chloride until carotenoids were completely removed. The methylene chloride layer containing carotenoids was dried over anhydrous sodium sulphate and filtered through Whatman No. 42 filter paper. The volume of the filtrate was reduced under vacuum to approximately 25 mL and brought up to 50 mL with methylene chloride. Then the extracts were passed through a Millipore 0.45 μm membrane and injected into the HPLC system.

2.5.2. Chromatography conditions

Conditions for the HPLC separations were those reported by Khachik et al. (1992). The HPLC system was equipped with a 600 Controller and a 486 Absorbance Detector (Waters, Milford, MA) working at 470 nm. Samples were introduced into a reverse-phase C18 Spherisorb® ODS2 (5 μm) stainless steel column (4.6 mm × 250 mm) through a manual injector equipped with a sample loop (20 μl). The flow rate was fixed at 0.7 mL/min at room temperature. An isocratic elution of acetonitrile (85%), methanol (10%), methylene chloride (3%) and hexane (2%) was maintained from 0 to 10 min, followed by a linear gradient to acetonitrile (45%), methanol (10%), methylene chloride (23%) and hexane (22%) from 10 to 40 min. At the end of the gradient, the column was equilibrated under the initial conditions for 20 min. Carotenoids were quantified by comparison with external standards of lycopene, neurosporene, γ-carotene, ζ-carotene, β-carotene, phytofluene and phytoene. Results were expressed as milligrams of phenolic compounds in 100 mL of fw tomato juice.

2.5.3. Vitamin A quantification

Vitamin A was expressed as retinol equivalents (RAE), according to Eq. (1) (Trumbo, Yates, Schlicker-Renfro, & Suitor, 2003):

$$\mathrm{RAE} = \left[\frac{\beta\text{-carotene } (\mu g)}{12}\right] + \left[\frac{\gamma\text{-carotene } (\mu g)}{24}\right] \quad (1)$$

2.6. Phenolic compounds

2.6.1. Extraction and hydrolysis

The extraction was carried out following the method validated by Hertog, Hollman, and Venema (1992). Twenty millilitres of

62.5% aqueous methanol with 2 g/L of *tert*-bytlhydroquinone and 5 mL of 6 M HCl were carefully mixed with 2.5 g of freeze-dried tomato juice. After refluxing at 90 °C for 2 h with regular swirling, the extract was cooled and subsequently made up to 50 mL with methanol and sonicated for 5 min. The extract was then passed through a 0.45 μm filter prior to injection.

2.6.2. Chromatography conditions

HPLC system was equipped with a 600 Controller and a diode array detector (Waters, Milford, MA) which was set to scan from 200 to 600 nm. Separations were performed on a reverse-phase C18 Spherisorb® ODS2 (5 μm) stainless steel column (4.6 mm × 250 mm) at room temperature with a flow rate of 1 mL/min. A gradient elution was employed with a solvent mixture of 2.5% HCOOH in water (solvent A) and 2.5% HCOOH in acetonitrile (solvent B) as follows: linear gradient from 5% to 13% B, 0–15 min; linear gradient from 13% to 15% B, 15–20 min; linear gradient from 15% to 30% B, 20–25 min; isocratic elution 30% B, 25–28 min; linear gradient from 30% to 45% B, 28–32 min; isocratic elution 45% B, 32–35 min; linear gradient 45–90% B, 35–40 min; isocratic elution 90% B, 40–45 min; linear gradient to reach the initial conditions after 5 min; post-time 10 min before the next injection. Individual phenolics were quantified by comparison with external standards of chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, kaempferol and quercetin. The results were expressed as milligrams of phenolic compounds in 100 mL of fw tomato juice.

2.7. Quality attributes

The juice colour was measured using a Macbeth Colour-Eye 3000 colorimeter (Macbeth-Kollmorgen Inst Corp., Newburg, NY, USA) at room temperature. The CIE L^* (lightness), CIE a^* (red–green) and CIE b^* (yellow–blue) were read using a D_{75} light source and the observer angle at 10°. Hue angle ($h°$) was calculated using Eq. (2):

$$h^\circ = \tan^{-1}\frac{b^*}{a^*} \quad (2)$$

A temperature-compensated refractometer Atago RX-1000 (Atago Company Ltd., Tokyo, Japan) was used to determinate the soluble solids content of tomato juice. In addition, tomato juice pH was measured using a pH-meter (Crison Instruments SA, Alella, Barcelona, Spain).

2.8. Statistical analysis

Significance of the results and statistical differences were analysed using the Statgraphics Plus v.5.1 Windows package (Statistical Graphics Co., Rockville, Md). Data were analysed by multifactor analysis of variance. Duncan multiple-range test was employed to determine differences amongst means, with a level of significance of 0.05. Principal component analysis (PCA) was carried out to obtain correlations amongst variables. PCA is a multivariate statistical technique based on the calculation of linear combinations between the variables that explain the most variance of the data. As a result, data can be transformed to new coordinate system called principal components (PCs) that allow to explain the greatest capacity. A correlation matrix is used to standardise the variables which are not measured on the same scale. The loadings plot summarises the main relationship between variables and principal components and also highlights relationships between different variables themselves. Variables that appear close together in this plot correlated positively. On the other hand, the score plot represents the projection of each sample into PC, defining different groups.

3. Results and discussion

3.1. Effect of processing and storage on individual carotenoids

The effects of processing and storage time on total and individual carotenoid concentration of tomato juice are shown in Table 1. Considering total carotenoids, HIPEF-treated tomato juices showed the highest amount (14.7 mg/100 mL fw), whereas the lowest values were found in the fresh juice (14.1 mg/100 mL fw). Our results are in the range of those reported in other studies which vary from 5 to 17 to mg/100 g fw (Khachik et al., 2002; Podsędek, Sosnowska, & Anders, 2003). On the other hand, HIPEF-treated tomato juice maintained total carotenoids better than heat treatments during the storage period (Table 1). Total carotenoid content decreased over the time in mild and high thermally processed tomato juice from 14.4 to 7.3 mg/100 mL fw, after 56 days at 4 °C, without significant differences between treatments. Regarding the individual carotenoids, lycopene was found in higher concentration, reaching values from 7.13 to 7.84 mg/100 mL fw. Lycopene content was enhanced significantly (7.7–10%) after thermal or HIPEF processing compared to the untreated juice. In this way, other minor individual carotenoids which are lycopene precursors such as phytoene (1.83–2.07 mg/100 mL fw), phytofluene (1.29–1.37 mg/100 mL fw) and neurosporene (1.19–1.30 mg/100 mL fw) were affected by processing. However, ζ-carotene content of tomato juices (0.19–0.20 mg/100 mL fw) was similar between fresh and treated juices (Table 1). It has been reported that thermal treatment may imply an increase in some individual carotenoids, owing to greater stability, enzymatic degradation, and unaccounted losses of moisture, which concentrate the sample (Rodriguez-Amaya, 1997). Nguyen and Schwartz (1999) suggested that homogenisation and heat treatment disrupt cell membranes and protein–carotenoids complex, making carotenoids more accessible for extraction. However, the increase in lycopene just after processing coincided with a depletion of phytoene and neurosporene contents compared to the untreated juice (Table 1). Thus, HIPEF and heat treatments might stimulate the transformation of some carotenoids into lycopene. Phytoene undergoes a series of desaturation reactions, each of which creates a new double bond and extends the chromophore by two conjugated double bonds; the end product is lycopene, produced via the intermediates phytofluene, ζ-carotene and neurosporene (Britton & Hornero-Mendez, 1997). In accordance with our results, other authors have reported an enhancement of carotenoids after HIPEF processing of juices other than tomato (Cortés, Esteve, Rodrigo, Torregrosa, & Frígola, 2006; Sánchez-Moreno et al., 2005). In addition, Torregrosa et al. (2005) observed that the concentration of some carotenoids rose, whereas the content of other decrease when thermal pasteurisation and HIPEF treatments were applied to orange–carrot juice, reporting that conversion amongst carotenoids took place during processing. On the other hand, β-carotene and γ-carotene were found in the tomato juices in amounts ranging from 0.29 to 0.40 mg/100 mL fw and from 1.67 to 1.77 mg/100 mL fw, respectively (Table 1). Both carotenoids were affected by treatments so that significant differences in β-carotene and γ-carotene between treated and fresh tomato juices just after processing were observed. As can be seen in Table 1, β-carotene in treated tomato juice underwent a significant increase (31–38%), whereas γ-carotene content was depleted (3–6%) when HIPEF and thermal treatments were conducted. A plausible explanation for this fact is that γ-carotene may undergo cyclisation to form six membered rings at one end of the molecule, giving β-carotene as a product (Britton & Hornero-Mendez, 1997). A decrease in the amounts of all individual carotenoids was observed over time, with the exception of β-carotene and phytoene content which were maintained for 56 days, regardless of the

Table 1
Effects of high-intensity pulsed electric fields and heat pasteurisation on carotenoids of tomato juice throughout storage at 4 °C

Storage time (days)	Process	Individual carotenoids (mg/100 mL fw)							vitamin A (RAE/l)	TC[1] (mg/100 mL fw)
		Lycopene	Neurosporene	γ-Carotene	ζ-Carotene	β-Carotene	Phytofluene	Phytoene		
0	Fresh	7.13^{dA}	1.30^{aA}	1.77^{aA}	0.20^{aA}	0.29^{bA}	1.29^{bA}	2.07^{aA}	0.99^{bA}	14.1^{cA}
	HIPEF	7.84^{aA}	1.19^{cA}	1.70^{bA}	0.20^{aA}	0.40^{aA}	1.37^{aA}	1.98^{bB}	1.04^{aA}	14.7^{aA}
	MP	7.46^{cA}	1.27^{bA}	1.67^{bA}	0.19^{aA}	0.38^{aA}	1.37^{aB}	2.02^{bBC}	1.01^{abA}	14.4^{bA}
	HP	7.64^{bA}	1.23^{bcA}	1.71^{bA}	0.19^{aA}	0.39^{aA}	1.36^{aB}	1.83^{cC}	1.04^{aA}	14.4^{bA}
7	Fresh	6.59^{cB}	1.26^{aA}	1.34^{cB}	0.16^{aB}	0.28^{bA}	1.26^{bA}	1.97^{bB}	0.79^{cB}	12.9^{cB}
	HIPEF	7.39^{aB}	1.13^{bA}	1.68^{aA}	0.16^{aB}	0.37^{aB}	1.38^{aA}	1.95^{bB}	1.01^{aA}	14.1^{aB}
	MP	6.78^{bB}	1.17^{bB}	1.62^{bB}	0.17^{aA}	0.36^{aA}	1.43^{aA}	2.16^{aA}	0.98^{bAB}	13.7^{bB}
	HP	6.97^{bB}	1.24^{aA}	1.71^{aA}	0.16^{aB}	0.39^{aA}	1.40^{aA}	1.86^{cB}	1.04^{aA}	13.7^{bB}
14	Fresh	6.31^{aC}	1.24^{aA}	1.34^{bB}	0.08^{bC}	0.24^{cB}	0.92^{cB}	1.98^{abB}	0.75^{cB}	12.1^{cC}
	HIPEF	6.32^{aC}	1.11^{bA}	1.63^{aB}	0.15^{aB}	0.37^{aB}	1.37^{bA}	1.90^{bC}	0.97^{abB}	12.9^{abC}
	MP	6.37^{aC}	1.13^{bB}	1.63^{aB}	0.14^{aB}	0.33^{bB}	1.44^{aA}	2.05^{aB}	0.95^{bB}	13.1^{aC}
	HP	5.99^{bC}	1.24^{aA}	1.65^{aB}	0.14^{aC}	0.38^{aA}	1.40^{abA}	1.88^{cA}	1.00^{aA}	12.7^{bC}
21	HIPEF	6.23^{aC}	0.90^{bB}	1.69^{aA}	0.12^{aC}	0.37^{aB}	1.37^{bA}	1.98^{bB}	1.01^{aAB}	12.7^{aC}
	MP	6.23^{aC}	0.65^{cC}	1.46^{bC}	0.12^{aC}	0.33^{bB}	1.45^{aA}	2.11^{aA}	0.87^{bC}	12.3^{bD}
	HP	5.85^{bC}	1.15^{aB}	1.69^{aA}	0.13^{aC}	0.37^{aB}	1.37^{bB}	1.85^{cC}	1.02^{aA}	12.4^{bC}
28	HIPEF	5.87^{aD}	0.75^{aC}	1.64^{aB}	0.11^{aCD}	0.38^{aB}	1.38^{bA}	2.04^{bA}	1.00^{aAB}	12.2^{aD}
	MP	4.66^{bD}	0.47^{bD}	1.46^{bC}	0.11^{aC}	0.32^{bB}	1.43^{aA}	2.16^{aA}	0.87^{bC}	11.6^{bE}
	HP	5.24^{cD}	0.70^{aC}	1.70^{aA}	0.12^{aCD}	0.37^{aB}	1.33^{cC}	1.89^{cA}	1.02^{aA}	11.4^{bD}
35	HIPEF	5.81^{aD}	0.62^{aD}	1.60^{aC}	0.11^{aCD}	0.37^{aB}	1.36^{aA}	2.07^{aA}	0.97^{aB}	12.0^{aD}
	MP	4.46^{bD}	0.38^{bD}	1.47^{bC}	0.09^{bD}	0.31^{bB}	1.38^{aB}	2.01^{aBC}	0.88^{bC}	10.1^{bF}
	HP	4.38^{bD}	0.64^{aCD}	1.63^{aB}	0.11^{aD}	0.37^{aB}	1.26^{bD}	1.85^{bC}	0.99^{aB}	10.3^{bE}
42	HIPEF	5.20^{aE}	0.60^{aD}	1.65^{aB}	0.11^{aCD}	0.38^{aB}	1.33^{aB}	2.01^{aAB}	0.99^{aB}	11.3^{aE}
	MP	3.09^{bE}	0.15^{bE}	1.47^{cC}	0.09^{bD}	0.28^{bC}	1.38^{bB}	2.04^{aB}	0.84^{cC}	8.5^{bG}
	HP	2.56^{cE}	0.56^{aD}	1.57^{bC}	0.11^{aD}	0.37^{aB}	1.30^{aC}	1.82^{bD}	0.96^{bC}	8.3^{bF}
49	HIPEF	2.71^{aF}	0.60^{aD}	1.57^{aC}	0.10^{aD}	0.36^{aC}	1.21^{aC}	1.90^{bC}	0.95^{aC}	9.8^{aF}
	MP	2.70^{aF}	nd	1.40^{cD}	0.08^{bD}	0.26^{bC}	1.33^{bC}	2.07^{aB}	0.80^{bD}	8.2^{bG}
	HP	2.43^{bF}	0.54^{bD}	1.50^{bD}	0.10^{aD}	0.36^{aB}	1.24^{aD}	1.83^{cC}	0.93^{aD}	8.1^{bG}
56	HIPEF	2.71^{aF}	0.60^{aD}	1.44^{aD}	0.09^{aD}	0.36^{aC}	1.03^{aD}	1.96^{aB}	0.91^{aD}	8.2^{aG}
	MP	2.70^{aF}	nd	1.34^{bE}	0.07^{bD}	0.25^{bC}	0.99^{aD}	1.99^{aC}	0.77^{cD}	7.3^{bH}
	HP	2.43^{bF}	0.52^{bD}	1.37^{bE}	0.09^{aD}	0.36^{aB}	0.85^{bE}	1.81^{bD}	0.87^{bE}	7.4^{bH}

HIPEF = high intensity pulsed electric fields at 35 kV/cm for 1000 μs; bipolar 4-μs pulses at 100 Hz; HP: 90 °C for 60 s; MP: 90 °C for 30 s.
Different lower case letter in the same column for each day indicate significant differences amongst treatments ($p < 0.05$).
Different capital letters in the same column for each treatment correspond to significant differences with time ($p < 0.05$).
[1] TC: Total carotenoids quantified by HPLC. The values are the result of the sum of each component. nd: not detected.

treatment applied (Table 1). Lycopene content decreased much more considerably than other carotenoids through the storage period, leading to levels of 2.43–2.71 mg/100 mL fw at 56 days of storage. Vitamin A content is related to the amount of β-carotene and γ-carotene in the samples (Rodriguez-Amaya, 1997). Vitamin A in tomato juices also underwent a slight depletion, reaching contents in HIPEF and thermally processed juices between 0.77 and 0.91 RAE/L, at 56 days of storage at 4 °C (Table 1). The major cause of carotenoid losses in vegetable products is the oxidation of the highly unsaturated carotenoid structure (Kidmose, Edelenbos, Nøbæk, & Christensen, 2002). Oxidation may occur by autooxidation, which is a spontaneous free-radical chain reaction in the presence of oxygen, or by photooxidation produced by oxygen in the presence of light. These oxidative reactions may result in carotene bleaching, which is the cause of formation of colourless end-products (Gross, 1991). During autooxidation of carotenoids, alkylperoxyl radicals are formed and these radicals attack the double bonds resulting in formation of epoxides. The severity of oxidation depends on the structure of carotenoids and the environmental conditions, and the compounds being formed depend on the oxidation process and the carotenoids structure (Ramakrishnan & Francis, 1980). On the other hand, HIPEF-processed tomato juices kept higher amounts of carotenoids (lycopene, neurosporene, and γ-carotene) and vitamin A than juices treated by mild and high heat pasteurisation for 56 days at 4 °C. However, non significant differences in ζ-carotene and β-carotene content were observed between high thermally and HIPEF-treated tomato juices (Table 1). In this way, some authors (Cortés et al., 2006; Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2008) studied the evolution of some carotenoids in HIPEF-treated juices through the storage time, reporting higher maintenance of these health-related compounds in comparison to thermally pasteurised juices.

3.2. Effect of processing and storage on individual phenolic compounds

The initial total phenolic content of tomato juices ranged from 8.9 to 9.1 mg/100 mL fw (Table 2). In this way, Odriozola-Serrano et al. (2008) and Podsędek et al. (2003) reported a concentration of total phenolic between 26.8 to 52.3 mg/100 g in different processed tomato juices. The higher phenolic concentrations found in the above-mentioned studies compared to those obtained in the present work, could be attributed to the analytical method used to determine these compounds. The Folin-Ciocalteu reagent usually overestimates the content of phenolic compounds compared with the sum of the individual phenolics, since other reducing agents present in food, such as ascorbic acid can interfere (Martínez-Valverde, Periago, Provan, & Chesson, 2002). On the other hand, no significant differences in total phenolic content were observed amongst tomato juices just after processing. However, HIPEF-processed and mild pasteurised tomato juices showed higher total phenolic compounds throughout the storage period than those treated at 90 °C for 60 s (Table 2). In accordance with

Table 2
Effects of high-intensity pulsed electric fields and heat pasteurisation on phenolic compounds of tomato juice throughout storage at 4 °C

Storage time (days)	Process	Individual phenolic (mg/100 mL fw)						Total phenolic[1] (mg/100 mL fw)
		Chlorogenic acid	Ferulic acid	*p*-Coumaric acid	Caffeic acid	Quercetin	Kaempferol	
0	Fresh	4.41^{aA}	0.88^{aA}	0.64^{aA}	0.43^{aA}	2.05^{aA}	0.57^{aA}	9.0^{aA}
	HIPEF	4.42^{aA}	0.86^{aA}	0.62^{a}	0.44^{aC}	2.01^{aA}	0.57^{aA}	8.9^{aA}
	MP	4.43^{aB}	0.89^{aAB}	0.64^{a}	0.43^{aD}	1.97^{aA}	0.56^{aA}	9.1^{aA}
	HP	4.44^{aA}	0.87^{aA}	0.62^{a}	0.45^{aB}	1.98^{aA}	0.57^{aA}	9.0^{aA}
7	Fresh	4.34^{bB}	0.82^{bB}	0.65^{aA}	0.43^{aA}	2.04^{aA}	0.46^{cB}	9.0^{aA}
	HIPEF	4.32^{bB}	0.87^{bA}	0.63^{a}	0.44^{aC}	1.85^{abB}	0.57^{aA}	8.7^{aB}
	MP	4.53^{aA}	0.92^{aA}	0.63^{a}	0.43^{aD}	1.67^{bC}	0.54^{bAB}	8.7^{aB}
	HP	4.16^{cB}	0.86^{bA}	0.58^{b}	0.45^{aB}	1.73^{bB}	0.54^{bB}	8.4^{bB}
14	Fresh	4.04^{cC}	0.77^{cC}	0.58^{bB}	0.44^{cA}	1.89^{aB}	0.46^{cB}	8.1^{bB}
	HIPEF	4.43^{abA}	0.85^{bA}	0.61^{a}	0.50^{aB}	1.72^{bC}	0.55^{aA}	8.5^{aB}
	MP	4.57^{aA}	0.90^{aA}	0.62^{a}	0.46^{bC}	1.62^{cC}	0.52^{bB}	8.7^{aB}
	HP	4.18^{bcB}	0.86^{bA}	0.57^{b}	0.47^{bAB}	1.63^{cC}	0.55^{aB}	8.3^{bB}
21	HIPEF	4.42^{aA}	0.80^{aB}	0.61^{a}	0.43^{aC}	1.77^{aC}	0.51^{aB}	8.5^{aC}
	MP	4.53^{aA}	0.85^{aB}	0.61^{a}	0.47^{aBC}	1.62^{bC}	0.52^{aB}	8.5^{aB}
	HP	4.09^{bBC}	0.88^{aA}	0.54^{b}	0.44^{aB}	1.60^{bC}	0.54^{aB}	8.0^{bC}
28	HIPEF	4.26^{aC}	0.83^{aB}	0.53^{b}	0.42^{bC}	1.67^{aD}	0.51^{aB}	8.2^{aD}
	MP	4.09^{bD}	0.86^{aB}	0.61^{a}	0.48^{aB}	1.67^{aC}	0.54^{aAB}	8.1^{aC}
	HP	4.13^{bB}	0.83^{aB}	0.51^{b}	0.43^{bB}	1.68^{aBC}	0.55^{aB}	8.2^{aBC}
35	HIPEF	4.21^{aC}	0.76^{aC}	0.52^{a}	0.52^{aB}	1.74^{aC}	0.50^{bB}	8.3^{aD}
	MP	4.26^{aC}	0.79^{aC}	0.52^{a}	0.50^{aB}	1.75^{aB}	0.50^{bB}	8.2^{aC}
	HP	4.12^{bB}	0.81^{aB}	0.50^{a}	0.50^{aA}	1.73^{aB}	0.57^{aA}	8.2^{aBC}
42	HIPEF	4.29^{aB}	0.79^{aBC}	0.49^{a}	0.57^{aA}	1.82^{aB}	0.52^{aB}	8.5^{aC}
	MP	4.10^{bD}	0.80^{aC}	0.49^{a}	0.55^{aA}	1.75^{bB}	0.51^{aB}	8.1^{bC}
	HP	4.08^{bBC}	0.77^{aC}	0.49^{a}	0.50^{bA}	1.62^{cC}	0.47^{bC}	8.1^{bBC}
49	HIPEF	4.10^{aD}	0.63^{bD}	0.51^{a}	0.55^{aA}	1.70^{aD}	0.48^{aC}	8.0^{aE}
	MP	3.84^{aE}	0.70^{aD}	0.52^{a}	0.54^{aA}	1.74^{aB}	0.42^{bC}	7.8^{bD}
	HP	3.92^{aC}	0.67^{aD}	0.51^{a}	0.51^{aA}	1.57^{bD}	0.44^{bC}	7.7^{bC}
56	HIPEF	3.78^{aE}	0.59^{aD}	0.34^{a}	0.57^{aA}	1.64^{aE}	0.47^{aC}	7.4^{aF}
	MP	3.74^{aE}	0.64^{aE}	0.32^{a}	0.55^{abA}	1.58^{bD}	0.40^{cC}	7.2^{aE}
	HP	3.49^{bD}	0.61^{aE}	0.34^{a}	0.51^{bA}	1.32^{cE}	0.43^{bC}	6.8^{bD}

HIPEF = high intensity pulsed electric fields treatment at 35 kV/cm for 1000 μs; bipolar 4-μs pulses at 100 Hz.
HP: 90 °C for 60 s.
MP: 90 °C for 30 s.
Different lower case letter in the same column for each day indicate significant differences amongst treatments ($p < 0.05$).
Different capital letters in the same column for each treatment correspond to significant differences with time ($p < 0.05$).
[1] Total phenolic quantified by HPLC. The values are the result of the sum of each component.

our results, Dewanto, Wu, Adom, and Liu (2002) did not find significant changes in total phenolic content in either thermally treated or fresh tomato purees. As expected, chlorogenic acid was the main hydroxycinnamic acid derivative in tomato juices, obtained in concentrations of 4.4 mg/100 mL fw (Table 2). Chlorogenic acid was also found to be in greater concentrations over the time in tomato juices treated by mild and HIPEF processes than by high pasteurisation. In addition, tomato juices underwent a substantial loss of chlorogenic acid from 4.4 to 3.5–3.8 mg/100 mL fw at 56 days of storage at 4 °C. Peroxidase is involved in the oxidative degradation of phenolic compounds (Amiot, Fleuriet, Cheynier, & Nicolas, 1997). Thus, the degradation of phenolic compounds during storage might be associated to the residual activity of peroxidase. It has been demonstrated that both thermal and HIPEF treatments could partially inhibit peroxidase in tomato juices. Aguiló-Aguayo, Soliva-Fortuny, and Martín-Belloso (2008b) reported residual peroxidase activities of 10% and 21% in tomato juices treated at 90 °C for 60 s and 30 s, respectively. In this product, a reduction of 96.87% of the initial POD activity of tomato juice was also obtained after applying a HIPEF treatment similar to that used in the present study. Tomato juices have been found to be a rich source of flavonoids, obtaining as the main flavonols quercetin and kaempferol (Stewart et al., 2000). The initial concentrations of quercetin in the studied tomato juices were 1.98–2.05 mg/100 mL fw, whereas kaempferol was found at concentrations of 0.56–0.57 mg/100 mL fw just after processing. Stewart et al. (2000) observed that tomato juice had high levels of quercetin, which ranging from 2.8 to 3.7 mg/100 mL fw. In this way, Martínez-Valverde et al. (2002) reported concentrations of kaempferol between 0.12 and 0.21 mg/100 g for different commercial cultivars. Quercetin content of tomatoes varies according to fruit cultivar, country of origin, harvesting seasons and growing condition (Crozier, Lean, McDonald, & Black, 1997). Quercetin and kaempferol content depleted significantly throughout the storage of tomato juices irrespective of the treatment conducted, thus reaching values of 1.32–1.64 mg/100 mL fw and 0.40–0.47 mg/100 mL fw, respectively (Table 2). HIPEF-treated tomato juices showed significantly greater quercetin content than thermally treated juices during storage for 56 days at 4 °C. Changes in minor phenolic acids such as ferulic acid (0.86–0.89 mg/100 mL fw), *p*-coumaric acid (0.62–0.64 mg/100 mL fw) and caffeic acid (0.43–0.45 mg/100 mL fw) are shown in Table 2. Tomato juices underwent a substantial depletion of *p*-coumaric acid during storage, since values of 0.32–0.34 mg/100 mL fw at 56 days of cold storage, which may be a consequence of its conversion to caffeic acid. As can be seen in Table 2, the caffeic acid content was slightly enhanced during the storage time, regardless of the processing treatment, reaching maximal values (0.51–0.57 mg/100 mL fw) at 56 days. Hydroxylation of *p*-coumaric acid into caffeic acid takes place in food as a consequence of the introduction of a second hydroxyl group into *p*-coumaric acid, probably catalysed by monophenol monooxygenases (Macheix, Fleuriet, & Billot, 1990). Therefore, the increase of caffeic acid in tomato juices after 28 days of storage may be directly associated with residual hydroxylase activities which convert *p*-coumaric acid in caffeic acid. Nevertheless, further research would be needed to know more about the effect of HIPEF treatments on this kind of enzymes.

3.3. Effect of processing and storage on quality attributes

The effects of HIPEF and heat treatments on lightness (L^*) and hue angle (h^*) of tomato juice as well as changes in these colour parameters during storage at 4 °C are shown in Table 3. Fresh tomato juice was statistically brighter than processed juices, but exhibited higher h° than the treated. Changes in the CIELab parameters between HIPEF and heat treatments appeared to be significant. Generally, HIPEF treatments preserved better the colour of tomato juice than heat treatments (Min & Zhang, 2003). HIPEF-treated tomato juice showed significantly higher L^* values compared to high pasteurised (90 °C, 60 s) tomato juices through the storage period. However, no changes in L^* values were observed between mild heat treated (90 °C, 30 s) and HIPEF-processed juices (Table 3). A decrease in L^* values in thermal treated juices is associated with the formation of dark colour compounds due to nonenzymatic browning reactions, thus reducing the acceptance of the juices (Klim & Nagy, 1988). Enzymatic browning of non-treated and HIPEF processed tomato juices may take place during storage, diminishing the brightness of juices. However, HIPEF treatment at 35 kV/cm for 1500 μs using 4 μs bipolar pulses at 100 Hz has been shown to deplete a 97% of POD activity compared to fresh tomato juice (Aguiló-Aguayo et al., 2008b). In this study, an increase in L^* values was observed during the storage at 4 °C irrespective of the treatment conducted. L^* value rose in mild thermally and HIPEF processed tomato juice from 22.5–22.6 to 24 after 56 days at 4 °C. At the same day, high thermally processed juice exhibited an L^* value of 23.6 (Table 3). According to these results, Aguiló-Aguayo et al. (2008b) reported an enhancement of brightness in

Table 3
Effects of high-intensity pulsed electric fields and heat pasteurisation on whiteness (L^*) and hue angle ($h°$) of tomato juice throughout storage at 4 °C

Storage time	L^*				$h°$			
	Fresh	HIPEF	MP	HP	Fresh	HIPEF	MP	HP
0	22.8^{aB}	22.6^{bD}	22.5^{bcE}	22.6^{bE}	38.1^{aA}	36.5^{bF}	37.1^{bE}	36.7^{bG}
7	22.7^{bB}	23.0^{aC}	22.5^{cE}	22.6^{cE}	37.2^{bcA}	36.6^{cF}	37.6^{bDE}	36.8^{cG}
14	23.1^{aA}	23.0^{bC}	22.9^{bD}	22.6^{cE}	37.7^{cA}	39.0^{aE}	38.6^{abCD}	38.2^{bcF}
21		23.3^{bB}	23.6^{aC}	22.9^{cD}		42.3^{aD}	38.6^{cCD}	39.9^{bE}
28		23.4^{abB}	23.7^{aBC}	23.1^{bC}		44.0^{aC}	38.9^{bC}	43.8^{aD}
35		23.7^{aA}	23.8^{aAB}	23.4^{bB}		47.5^{aB}	42.3^{bB}	46.3^{aC}
42		23.8^{aA}	23.9^{aA}	23.5^{bAB}		48.7^{aAB}	43.1^{bB}	48.2^{aB}
49		23.7^{aA}	23.9^{aA}	23.6^{aA}		49.8^{aAB}	43.3^{bB}	50.1^{aA}
56		24.0^{aA}	24.0^{aA}	23.6^{bA}		49.0^{aA}	45.0^{bA}	50.3^{aA}

HIPEF = high intensity pulsed electric fields treatment at 35 kV/cm for 1000 μs; bipolar 4-μs pulses at 100 Hz.
HP: 90 °C for 60 s.
MP: 90 °C for 30 s.
Different lower case letter in the same row indicate significant differences amongst treatments ($p < 0.05$).
Different capital letters in the same column correspond to significant differences with time ($p < 0.05$).

processed tomato juices after prolonged storage. In their study, statistically significant differences in colour parameters were also found between HIPEF and thermally treated tomato juice during storage at 4 °C. The kind of treatment applied led to significant differences on $h°$ during storage. Lower $h°$ values were obtained in tomato juices treated by mild heat pasteurisation compared to the HIPEF-treated and high thermally processed (Table 3). The $h°$ values rose dramatically from 36°–37° to 45°–50° throughout the storage at 4 °C in processed tomato juices. Thus, treated juices depleted their redness as storage time increased irrespective of the treatment applied. On the other hand, values of pH and soluble solids content for the different processed tomato juices are exhibited in Table 4. Processing had no significant effect on these physical properties of tomato juice. Little effect of HIPEF and thermal treatments on physical properties (pH and soluble solids) of orange juice was reported by Elez-Martínez, Soliva-Fortuny, and Martín-Belloso (2006). Yeom et al. (2000) observed that pH and soluble solids were neither affected by HIPEF (35 kV/cm for 59 μs) nor by heat treatment (94.6 °C for 30 s). No significant differences in pH values were observed through the storage period amongst the treated juices (Table 4). Although statistical differences between treatments on soluble solids content were obtained, pH values for HIPEF and thermally treated tomato juices were similar throughout the storage (Table 4). pH values of thermally and HIPEF-pasteurised tomato juice were slightly decreased during the storage from 4.38 to 4.32–4.34 (at 56 days). This depletion in pH may be related to microbial spoilage growth. Microorganisms cause fruit juice spoilage by reduction of acidity and organic acid fermentation (Sodeko, Izuagde, & Ukhun, 1987).

3.4. Correlation between health-related compounds and quality attributes

A principal components analysis (PCA) was performed on all samples and variables (total and individual carotenoids, total and individual phenolic compounds, pH, soluble solids and colour parameters) to obtain relationships amongst the studied parameters. Two principal components (PC1 and PC2) were obtained. They accounted for 74.17% of the variability in the original data (Fig. 1). The statistical analysis for the data showed a correlation between carotenoids and $h°$ and L^* variables. It is noted a positive correlation between $h°$ value and total carotenoids content of tomato juices, whereas a negative correlation was observed for lycopene or total carotenoids concentrations and L^*. On the contrary, vitamin A did not appear to correlate adequately with colour parameters, whereas there was an outstanding correlation between this

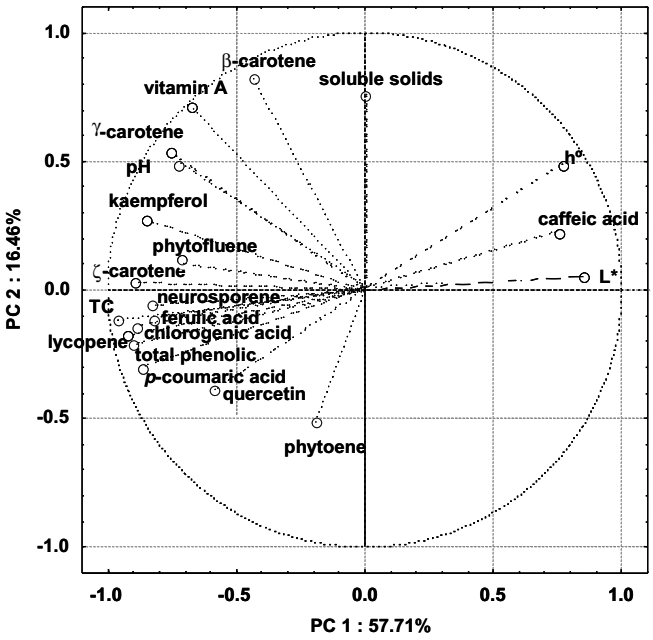

Fig. 1. Principal components plot of fresh, HIPEF-treated and heat pasteurised tomato juices stored for 56 days at 4 °C. TC: total carotenoids.

vitamin and pH. On the other hand, PC2 correlated well with soluble solids content, meaning that this quality attribute was positively related to β-carotene, but negatively associated to phytoene. Nevertheless, soluble solids were weakly related to total bioactive compounds, demonstrating that soluble solids value is a bad indicator of the nutritional quality of juices. The score plot of PC1 versus PC2 from the full-data PCA model plotted in Fig. 2 describes differences between treatments and storage days. It can be observed that the majority of the samples stored for up to 28 days are situated in the left part of the score plot, whereas those samples preserved for over 28 days appear on the right-hand side. Therefore, total health-related compounds content in tomato juice samples depleted as storage time increased. In addition, the plot allows to discriminate amongst differently processed tomato juices (Fig. 2). HIPEF-processed and high heat pasteurised samples located in the upper part of the plot are well related with soluble solids content, pH, $h°$, vitamin A and β-carotene, whereas fresh and mild heat pasteurised samples scored the lowest values of these parameters as they were located at the bottom of the plot (Fig. 2).

Table 4
Effects of high-intensity pulsed electric fields and heat pasteurisation on pH and soluble solids content of tomato juice throughout storage at 4 °C

Storage time	pH				Soluble solids content (°Brix)			
	Fresh	HIPEF	MP	HP	Fresh	HIPEF	MP	HP
0	4.38^{aA}	4.38^{aA}	4.38^{aA}	4.39^{aA}	5.8^{aA}	5.9^{aA}	5.9^{aAB}	5.9^{aA}
7	4.35^{bB}	4.38^{aA}	4.38^{aA}	4.38^{aA}	5.6^{bB}	5.9^{aA}	5.8^{aB}	5.9^{aA}
14	4.31^{bC}	4.38^{aA}	4.38^{aA}	4.38^{aA}	5.5^{cB}	5.9^{bA}	5.9^{aAB}	6.0^{bA}
21		4.37^{aB}	4.34^{aB}	4.38^{aA}		6.0^{aA}	6.0^{aA}	5.9^{aA}
28		4.36^{abB}	4.34^{bB}	4.38^{aA}		6.0^{aA}	6.0^{aA}	6.0^{aA}
35		4.36^{aB}	4.33^{aB}	4.37^{aAB}		6.1^{aA}	6.0^{aA}	5.9^{bA}
42		4.36^{aB}	4.33^{bB}	4.37^{aAB}		6.1^{aA}	5.8^{bB}	5.9^{bA}
49		4.36^{aB}	4.33^{aB}	4.35^{aB}		6.1^{aA}	5.8^{bB}	5.9^{bA}
56		4.34^{aC}	4.32^{aB}	4.35^{aB}		6.1^{aA}	5.8^{bB}	6.0^{aA}

HIPEF = high intensity pulsed electric fields treatment at 35 kV/cm for 1000 μs; bipolar 4-μs pulses at 100 Hz.
HP: 90 °C for 60 s.
MP: 90 °C for 30 s.
Different lower case letter in the same row indicate significant differences amongst treatments ($p < 0.05$).
Different capital letters in the same column correspond to significant differences with time ($p < 0.05$).

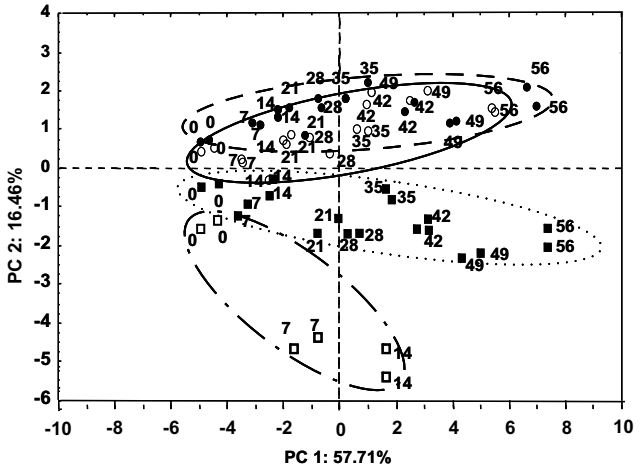

Fig. 2. Score plot of PC1 vs. PC2 of the samples for fresh and treated tomato juices stored during 56 days at 4 °C. Tomato juices: □ untreated, ○ HIPEF treatment (35 kV/cm for 1500 μs in bipolar mode using 4-μs pulses at 100 Hz), ■ mild heat pasteurised (90 °C for 30 s) and ● high heat pasteurised (90 °C for 60 s). Numbers next to each marker stand for the storage day.

4. Conclusions

HIPEF-treated tomato juices maintained better nutritional value than those thermally pasteurised just after processing and during the storage period. HIPEF (35 kV/cm for 1500 μs with 4 μs bipolar pulses at 100 Hz) and thermal treatments (90 °C–30 s and 90 °C–60 s) led to tomato juices with higher total and individual carotenoids (lycopene, β-carotene and phytofluene) and redder colour than fresh juices, demonstrating that processing may improve not only quality attributes but also the antioxidant properties of juices. However, this enhancement in health-related compounds was greater in HIPEF-processed tomato juices than in those heat-treated. The amounts of individual health-related compounds in fresh and treated tomato juices underwent a substantial loss during storage, with the exception of β-carotene, phytoene and caffeic acid content. However, HIPEF-processed tomato juices better maintained over time the individual carotenoids (lycopene, neurosporene and γ-carotene) and quercetin than thermally-treated and untreated juices. Therefore HIPEF technology could be an alternative to thermal treatments in order to obtain tomato juices with high antioxidant properties.

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References

Aguiló-Aguayo, I., Odriozola-Serrano, I., Quintão-Teixeira, L. J., & Martín-Belloso, O. (2008a). Inactivation of tomato juice peroxidase by high-intensity pulsed electric field as affected by process conditions. *Food Chemistry, 107*, 949–955.

Aguiló-Aguayo, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2008b). Comparative study on color, viscosity and related enzymes of tomato juice processed by pulsed electric fields or heat treatment. *European Food Research and Technology*, doi:10.1007/s00217-007-0761-2.

Abushita, A. A., Daood, H. G., & Biacs, P. (2000). Change in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors. *Journal of Agricultural and Food Chemistry, 48*, 2075–2081.

Amiot, M. J., Fleuriet, A., Cheynier, V., & Nicolas, J. (1997). Phenolic compounds and oxidative mechanisms in fruits and vegetables. In F. A. Tomás-Barberán & R. J. Robins (Eds.), *Phytochemistry of fruit and vegetables* (pp. 51–85). Oxford: Science Publications.

Beecher, G. R. (1998). Nutrient content of tomatoes and tomato products. *Proceedings of the Society for Experimental Biology and Medicine, 218*, 98–100.

Braddock, R. J. (1999). *Handbook of citrus by-products and processing technology*. New York: John Wiley & Sons. pp. 53–83.

Britton, G., & Hornero-Mendez, D. (1997). Carotenoids and colour in fruit and vegetables. In F. A. Tomas-Barberan & R. J. Robins (Eds.), *Phytochemistry of fruit and vegetables* (pp. 11–27). Oxford: Science Publications.

Clinton, S. K. (1998). Lycopene: Chemistry, biology, and implications for human health and disease. *Nutrition Reviews, 56*, 35–51.

Cortés, C., Esteve, M. J., Rodrigo, F., Torregrosa, F., & Frígola, A. (2006). Changes of colour and carotenoids contents during high-intensity pulsed electric field treatment in orange juices. *Food and Chemical Toxicology, 44*, 1932–1939.

Crozier, A., Lean, M. E. J., McDonald, M. S., & Black, C. (1997). Quantitative analysis of the flavonoid content of commercial tomatoes, onions, lettuce, and celery. *Journal of Agricultural and Food Chemistry, 45*, 590–595.

Dewanto, V., Wu, X., Adom, K., & Liu, R. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing the total antioxidant activity. *Journal of Agricultural and Food Chemistry, 50*(10), 3010–3014.

Elez-Martínez, P., Soliva-Fortuny, R. C., & Martín-Belloso, O. (2006). Comparative study on shelf-life of orange juice processed by high intensity pulsed electric fields or heat treatments. *European Food Research and Technology, 222*(3–4), 321–329.

Gross, J. (1991). *Pigments in vegetables. Chlorophylls and carotenoids*. New York: Van Nostrand Reinold.

Hertog, M., Hollman, P., & Venema, D. (1992). Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *Journal of Agricultural and Food Chemistry, 40*, 1591–1598.

Khachik, F., Goli, M. B., Beecher, G., Holden, J., Lusby, W. R., Tenorio, M. D., et al. (1992). Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. *Journal of Agricultural and Food Chemistry, 40*, 390–398.

Khachik, F., Carvalho, L., Bernstein, P. S., Muir, G. J., Zhao, D. Y., & Katz, N. B. (2002). Chemistry, distribution, and metabolism of total carotenoids and their impact on human health. *Experimental Biology and Medicine, 227*, 845–851.

Kidmose, U., Edelenbos, M., Nøbæk, R., & Christensen, L. P. (2002). Colour stability in vegetables. In D. B. MacDougall (Ed.), *Colour in food: Improving quality* (pp. 179–232). Florida, Boca Raton: CRC Press.

Klim, M., & Nagy, S. (1988). An improved method to determine nonenzymic browning in citrus juices. *Journal of Agricultural and Food Chemistry, 36*, 1271–1274.

Macheix, J. J., Fleuriet, A., & Billot, J. (1990). *Fruit phenolics*. Florida, Boca Raton: CRC Press.

Marsellés-Fontanet, A. R., & Martín-Belloso, O. (2007). Optimization and validation of PEF processing conditions to inactivate oxidative enzymes of grape juice. *Journal of Food Engineering, 83*, 452–462.

Martínez-Valverde, I., Periago, M. J., Provan, G., & Chesson, A. (2002). Phenolic compounds, lycopene and antioxidant capacity in commercial varieties of tomato (*Lycopersicum esculentum*). *Journal of the Science of Food and Agriculture, 82*, 323–330.

Min, S., & Zhang, Q. H. (2003). Effects of commercial-scale pulsed electric field processing on flavour and color of tomato juice. *Food Chemistry and Toxicology, 68*, 1600–1606.

Nagy, S., Chen, C. S., & Shaw, P. E. (1993). *Fruit juice processing technology*. Auburndale, Florida: Agscience.

Nguyen, M. L., & Schwartz, S. J. (1999). Lycopene: Chemical and biological properties. *Food Technology, 53*(2), 38–45.

Odriozola-Serrano, I., Aguiló-Aguayo, I., Soliva-Fortuny, R., Gimeno-Añó, V., & Martín-Belloso, O. (2007). Lycopene, vitamin C, and antioxidant capacity of tomato juice as affected by high-intensity pulsed electric fields critical parameters. *Journal of Agricultural and Food Chemistry, 55*, 9036–9042.

Odriozola-Serrano, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2008). Changes of health-related compounds throughout cold storage of tomato juice stabilized by thermal or high intensity pulsed electric field treatments. *Innovative Food Science and Emerging Technologies, 9*, 272–279.

Podsędek, A., Sosnowska, D., & Anders, B. (2003). Antioxidative capacity of tomato products. *European Food Research and Technology, 217*(4), 296–300.

Ramakrishnan, T. V., & Francis, F. J. (1980). Autooxidation of carotenoids and their relative polarity. *Journal of Food Quality, 3*, 25–34.

Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P., & Glover, W. (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry, 66*, 401–436.

Rodriguez-Amaya, D. B. (1997). *Carotenoids and food preservation: The retention of provitamin A carotenoids in prepared, processed and stored food*. USAID, OMNI Project.

Sánchez-Moreno, C., Cano, M. P., de Ancos, B., Plaza, L., Olmedilla, B., Granado, F., et al. (2005). Intake of mediterranean soup treated by pulsed electric fields affects plasma vitamin C and antioxidant biomarkers in humans. *International Journal of Food Sciences and Nutrition, 56*, 115–124.

Shi, J., & 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.

Sodeko, O. O., Izuagde, Y. S., & Ukhun, M. E. (1987). Effect of different preservative treatments on the microbial population of Nigerian orange juice. *Microbios, 51*, 133–143.

Stewart, A. J., Bozonnet, S., Mullen, W., Jenkins, G. I., Lean, M. E. J., & Crozier, A. (2000). Occurrence of flavonols in tomatoes and tomato-based products. *Journal of Agricultural and Food Chemistry, 48*, 2663–2669.

Tonucci, L. H., Holden, J. M., Beecher, G. R., Khachik, F., Davis, C. S., & Mulokozi, G. (1995). Carotenoid content of thermally processed tomato-based food products. *Journal of Agricultural and Food Chemistry, 43*, 579–586.

Trumbo, P. R., Yates, A. A., Schlicker-Renfro, S., & Suitor, C. (2003). Dietary reference intakes: Revised nutritional equivalents for folate, vitamin E and provitamin A carotenoids. *Journal of Food Composition and Analysis, 16*, 379–382.

Torregrosa, F., Cortés, C., Esteve, M. J., & Frígola, A. (2005). Effect of high-intensity pulsed electric fields processing and conventional heat treatment on orange–carrot juice carotenoids. *Journal of Agricultural and Food Chemistry, 53*, 9519–9525.

Yeom, H. W., Streaker, C. B., Zhang, Q. H., & Min, D. B. (2000). Effects of pulsed electric fields on the quality of orange juice and comparison with hat pasteurization. *Journal of Agricultural and Food Chemistry, 48*, 4597–4605.