JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Lycopene, Vitamin C, and Antioxidant Capacity of Tomato Juice as Affected by High-Intensity Pulsed Electric Fields Critical Parameters

ISABEL ODRIOZOLA-SERRANO, INGRID AGUILÓ-AGUAYO, ROBERT SOLIVA-FORTUNY, VICENTE GIMENO-AÑÓ, AND OLGA MARTÍN-BELLOSO*

Department of Food Technology, UTPV-CeRTA, University of Lleida, Rovira Roure 191, 25198 Lleida, Spain

The effects of high-intensity pulsed electric field (HIPEF) treatment variables (frequency, pulse width, and pulse polarity) on the lycopene, vitamin C, and antioxidant capacities of tomato juice were evaluated using a response surface methodology. An optimization of the HIPEF treatment conditions was carried out to obtain tomato juice with the highest content of bioactive compounds possible. Samples were subjected to an electric field intensity set at 35 kV/cm for 1000 μs using squared wave pulses, frequencies from 50 to 250 Hz, and a pulse width from 1 to 7 μs, in monopolar or bipolar mode. Data significantly fit ($P < 0.001$) the proposed second-order response functions. Pulse frequency, width, and polarity significantly affected the lycopene, vitamin C, and antioxidant capacities of HIPEF-treated tomato juice. Maximal relative lycopene content (131.8%), vitamin C content (90.2%), and antioxidant capacity retention (89.4%) were attained with HIPEF treatments of a 1 μs pulse duration applied at 250 Hz in bipolar mode. Therefore, the application of HIPEF may be appropriate to achieve nutritious tomato juice.

KEYWORDS: High-intensity pulsed electric field; tomato juice; bioactive compounds; vitamin C; carotenoids and antioxidant capacity

INTRODUCTION

Reactive oxygen species (ROS) could cause a number of human diseases, including coronary heart diseases and cancers. It has been suggested that a high intake of fruits and vegetables, the main source of antioxidants in the diet, could decrease the potential stress caused by ROS (*1*). The tomato is a widely consumed vegetable, either fresh or industrially processed as juices, pastes, purees, sauces, and soups (*2*). In addition, the tomato is rich in health-related compounds as it is a good source of carotenoids (in particular, lycopene) and ascorbic acid (*3*). However, processed fruits and vegetables have been considered to have lower nutritional value than their respective fresh commodities due to the loss of antioxidant compounds during processing (*4*). Thermal processing is the most common method used to extend the shelf life of tomato juices by inactivating microorganisms and enzymes that curb the product's quality during storage (*5*), but this treatment can induce undesirable changes in color, flavor, and nutritional value (*6*). The increased demand for freshlike and highly nutritious products has raised the concern of the food industry for the development of milder preservation technologies that can replace traditional pasteurization methods. High-intensity pulsed electric fields (HIPEF) is a nonthermal technology in which the food industry is increasingly interested because it can lead to high inactivation levels of both spoilage and pathogenic microorganisms while maintaining the quality and freshness of juices (*7*). Up to now, most of the studies evaluating the effects of HIPEF processing conditions on juices have been focused on microbial and enzyme inactivation. Process parameters such as electric field strength, pulse width, frequency, pulse polarity, and treatment time are important to optimize the inactivation of microorganisms (*8–11*) and enzymes (*12–14*). In comparison to the extensive research devoted to the destruction of microorganisms and enzymes by HIPEF, there are very few works about the effect of HIPEF treatment parameters on the bioactive compounds of juices. Cortés et al. (*15*) reported that electric field strength and treatment time had a significant effect on orange juice carotenoids. In this way, Torregrosa et al. (*16*) observed that HIPEF processing caused a significant rise in the carotenoids content as the treatment time increased in orange–carrot juice. Elez-Martínez and Martín-Belloso (*17*) concluded that vitamin C retention in orange juice and gazpacho depended on processing factors such as pulse polarity, electric field strength, treatment time, pulse frequency, and pulse width. However, little research has been carried out evaluating the effect of HIPEF critical parameters on bioactive compounds of tomato juice. In addition, at the stage of development of

* Author to whom correspondence should be addressed. Phone: +34 973 702593. Fax: + 34 973 702596. E-mail: omartin@tecal.udl.es.

10.1021/jf0709101 CCC: $37.00
Published on Web 10/02/2007

Table 1. Analytical Characteristics of Tomato Juice

parameters[a]	tomato juice
pH	4.38 ± 0.01
soluble solids (°Brix)	4.52 ± 0.1
color L*	22.41 ± 0.07
a*	6.98 ± 0.12
b*	5.26 ± 0.06
electrical conductivity (S/m)	0.67 ± 0.02

[a] Results are the mean ± SD of three measurements.

HIPEF technology, evaluating the influence of process variables on the antioxidant properties of tomato juice is a key factor in defining treatment conditions necessary to prevent undesirable changes in these properties.

On the other hand, the use of an adequate experimental design is particularly important in assessing the effect of HIPEF treatment on health-related compounds. The response surface methodology consists of mathematical and statistical procedures to study the relationships between one or more responses (*18*). The multivariate approach reduces the number of experiments, improves statistical interpretations, and indicates whether parameters interact (*19*).

The aim of this research was to study the effect of pulse width, frequency, and polarity on the main bioactive compounds (lycopene and vitamin C) as well as on the antioxidant capacity of HIPEF-treated tomatoes. Moreover, an optimization of HIPEF-processing critical parameters was carried out in order to obtain tomato juice with a high retention of bioactive compounds and antioxidant capacity.

MATERIALS AND METHODS

Reagents. Metaphosphoric acid and DL-1,4-dithiotreitol (DTT) were purchased from Acros Organics (New Jersey, U.S.A.); butylated hydroxytoluene (BHT), Unitated Stated Pharmacopeia (USP)-grade ethanol, hexane, acetone, ascorbic acid, sulphuric acid, methanol, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Scharlau Chemie, SA (Barcelona, Spain).

Tomato juices. Tomato fruits (*Licopersicon esculentum* Mill, cultivar Bodar) at commercial maturity were purchased from a local supermarket (Lleida, Spain). The fruits were chopped and then filtered through steel sieves 2 mm in diameter. The electric 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 (Minolta CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) of the tomato juice were determined (**Table 1**).

Pulsed Electric Fields Equipment. HIPEF treatments were carried out using a continuous-flow bench-scale system (OSU-4F, Ohio State University, Columbus, OH) that held positive monopolar and bipolar squared wave pulses. The treatment chamber device consists of eight collinear chambers disposed in series, each one containing two stainless steel electrodes separated by a gap of 0.29 cm with a treatment volume of 0.012 cm^3.

The treatment flow rate was 60 mL/min and was controlled by a variable-speed pump (model 752210–25, Cole Palmer Instrument Company, Vermon Hills, IL). Treated tomato juice was passed through a cooling coil connected between each pair of chambers and submerged in an ice-water shaking bath, so that the samples' temperatures never exceeded 40 °C. Tomato juice was treated using pulses of 35 kV/cm for 1000 μs at frequencies ranging from 50 to 250 and a pulse width between 1 and 7 μs in the monopolar or bipolar modes.

Bioactive Compounds. *Lycopene.* The total lycopene content was measured spectrophotometrically following the method proposed by Davis et al. (*20*). This method determines the content of lycopene and other derivates such as hydroxy lycopene and lycopene epoxides. Approximately 0.6 g of tomato juice was weighed and added to 5 mL of 0.05% (w/v) BHT in acetone, 5 mL of 95% USP-grade ethanol, and 10 mL of hexane. The homogenate was centrifuged at 320*g* for 15 min at 4 °C. After shaking, 3 mL of distilled water was 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-pathlength 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 (*21*). Lycopene was expressed as the percentage of lycopene content compared to that of the untreated samples.

Vitamin C. The extraction procedure was based on a method validated by Odriozola-Serrano et al. (*22*). A portion of 25 g of tomato juice was added to 25 mL of a solution containing 45 g of metaphosphoric acid and 7.2 g of DTT per liter. The mixture was centrifuged at 22100*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 and injected into the high-performace liquid chromatography system.

A Waters 600E multisolvent delivery system was used for the analysis. Samples were introduced onto the column via a manual injector equipped with a sample loop (20 μL). The separation of ascorbic acid was performed 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. Detection was performed with a 486 Absorbance Detector (Waters, Milford, MA) set at 245 nm. Identification of the ascorbic acid was carried out comparing the retention time and UV–visible absorption spectrum with those of the standards. Results were expressed as vitamin C concentration compared to the untreated sample.

Antioxidant Capacity. The antioxidant capacity was studied through the evaluation of the free-radical-scavenging effect on the DPPH radical. This determination was based on the method proposed by de Ancos et al. (*23*). An aliquot of 0.01 mL of tomato juice was mixed with 3.9 mL of methanolic DPPH solution (0.025 g/L) and 0.090 mL of distilled water. The homogenate was shaken vigorously and kept in darkness for 30 min. The absorption of the samples was measured with a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, U.K.) at 515 nm against a blank of methanol without DPPH. The results were expressed as the antioxidant capacity related to that of the untreated sample.

EXPERIMENTAL DESIGN

A response surface analysis was used to evaluate the effect of the different variables of the HIPEF treatment on the bioactive compounds and antioxidant capacity of tomato juice. A face-centered, central composite design with three factors was the proposed experimental design (*19*). The independent variables were frequency, pulse width, and polarity. The pulse repetition rate was set up from 50 to 250 Hz; each pulse had a duration between 1 and 7 μs, in monopolar or bipolar mode. Samples were treated at a field strength of 35 kV/cm for 1000 μs irrespective of the frequency, pulse width, and pulse mode applied according to previous studies where pasteurization levels were achieved in orange juice processed by HIPEF (*10, 11*). The levels for each independent parameter were chosen considering sample and equipment limitations. The selected responses were lycopene, vitamin C, and antioxidant capacity. The experimental design along with each experimental condition is shown in **Table 2**. A duplicate was performed, resulting in two blocks of experiments. The order of assays within each block was randomized and performed in triplicate. The effect of each HIPEF variable was modeled using a polynomial response surface. The second-order response function was predicted by eq 1, where Y is the dependent variable; β_0 is the constant; β_i, β_{i1}, and β_{ij} represent the coefficients of the linear, quadratic, and interactive effect, respectively; and X_i,

Table 2. Central Composite Response Surface Design for Bioactive Compounds and Antioxidant Capacity on Tomato Juice Treated under Different HIPEF Treatments

		variables							
				lycopene relative content (%)[a]		vitamin C retention (%)[a]		AC retention (%)[a]	
assay no.[b]	point type	frequency (Hz)	pulse width (μs)	monopolar	bipolar	monopolar	bipolar	monopolar	bipolar
1	factorial	50	1	5101.0 ± 0.2	103.2 ± 2.7	99.0 ± 0.1	98.3 ± 0.5	50.7 ± 1.1	66.5 ± 0.8
2	factorial	50	7	112.3 ± 2.6	114.2 ± 0.3	84.4 ± 1.1	82.1 ± 0.9	81.8 ± 2.5	86.1 ± 2.7
3	factorial	250	1	113.4 ± 1.1	129.1 ± 2.1	93.8 ± 0.9	86.1 ± 0.7	77.5 ± 0.2	90.4 ± 0.2
4	factorial	250	7	135.1 ± 3.0	146.2 ± 0.5	66.0 ± 0.9	60.6 ± 1.6	67.8 ± 0.2	75.9 ± 2.2
5	axial	50	4	110.2 ± 0.1	109.1 ± 0.4	92.2 ± 0.9	88.4 ± 0.7	62.3 ± 1.1	74.6 ± 0.5
6	axial	150	1	107.0 ± 1.5	118.7 ± 0.1	98.6 ± 0.3	89.2 ± 0.7	60.4 ± 0.9	80.1 ± 0.7
7	axial	150	7	118.9 ± 1.1	128.1 ± 0.3	80.2 ± 0.7	58.2 ± 0.7	78.3 ± 0.7	81.3 ± 0.1
8	axial	250	4	127.0 ± 0.4	142.7 ± 1.8	82.6 ± 0.4	78.8 ± 1.2	72.6 ± 0.3	81.0 ± 0.1
9	central	150	4	109.6[c] ± 0.5	119.4[c] ± 2.4	76.6[c] ± 1.2	74.8[c] ± 1.6	81.3[c] ± 1.1	92.3[c] ± 1.8

[a] Data shown are the mean ± SD of two treatment repetitions; each assay was performed in triplicate. AC = antioxidant capacity. [b] Order of the assays was randomized, and HIPEF treatment was set up at 35 kV/cm for 1000 μs. [c] Data shown are the mean of five repetitions.

X_i^2, and X_iX_j represent the linear, quadratic, and interactive effect of the independent variables, respectively.

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_1 + \sum_{i=1}^{3}\beta_{ii}X_i^2 + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij}X_iX_j \quad (1)$$

Three-dimensional surface plots and contour plots were drawn to illustrate the interactive effects of two factors on the dependent variable, while keeping the other variables constant. After carrying out an analysis of variance, the nonsignificant terms were deleted from the second-order polynomial model. Then, a new polynomial was recalculated to obtain the coefficients for the initial equation. The Design Expert 6.0.1 software (Stat Ease Inc., Minneapolis, MN) was used to generate models that fit the experimental data and to draw the response surface plots.

The optimization was done according to the method proposed by Derringer and Suich (*24*). All the individual desirability functions obtained for each response were combined into an overall expression, which is defined as the geometrical mean of the individual functions. The higher the desirability value, the more adequate is the system. In the present study, desirability functions were developed in order to obtain tomato juice with the highest levels of lycopene, vitamin C, and antioxidant capacity.

RESULTS AND DISCUSSION

Results of the analysis of variance (*F* test) for each dependent variable and their corresponding coefficients of determination (R^2) obtained by fitting the second-order response model to the experimental data are shown in **Table 3**.

Effect of HIPEF-Processing Critical Parameters on Lycopene. Lycopene is an isoprenoid compound which provides the red color to tomatoes and is used as an index of quality for tomato products (*25*). However, this compound is susceptible to oxidation in the presence of light, oxygen, and low pH (*2*). The effect of HIPEF processing on the concentration of lycopene in the tomato juice is shown in **Table 2**. Fresh tomato juice had a lycopene content of 7.5 mg/100 g. Higher lycopene concentrations were achieved in HIPEF-processed tomato juice than in the untreated. The increase ranged from 1.0% to 46.2% after applying different HIPEF treatments. The maximum lycopene relative content (146.2%) was observed when HIPEF treatment was carried out at 35 kV/cm for 1000 μs with bipolar pulses of 7 μs at 250 Hz. Sánchez-Moreno et al. (*26*) observed that the content of total carotenoids in "gazpacho", a cold vegetable soup where tomatoes are the major component, increased approximately 62% after applying bipolar 4-μs pulses of 35 kV/cm for 750 μs at 800 Hz. Torregrosa et al. (*16*) reported that HIPEF treatment set up at 25 or 30 kV/cm caused a significant enhancement in the content of carotenoids in orange–carrot juice. In addition, Cortés et al. (*15*) observed that the carotenoid concentration rose slightly after applying an intensive treatment (35 kV/cm) to orange juice. The lycopene in tomato juice treated by HIPEF might increase from other carotenoids in tomato juice such as phytoene, phytofluene, ζ-carotene, and neurosporene throughout desaturation, isomerization, and cyclization (*27*). The formation of lycopene is dependent on the temperature and takes place between 12 and 32 °C (*28*). As a result, HIPEF treatment conducted at temperatures lower than 40 °C might stimulate the conversion of some carotenoids into lycopene.

Table 3. Analysis of Variance of the Second-Order Models for Bioactive Compounds and Antioxidant Capacity

	F value		
source[a]	lycopene	vitamin C	antioxidant capacity
quadratic model	36.32[b]	11.26[b]	13.15[b]
F	160.50[b]	16.41[c]	7.05[c]
τ	52.94[b]	50.04[b]	6.36[c]
P	47.55[b]	6.92[c]	29.44[b]
F^2	10.85[c]	8.10[c]	13.34[c]
τ^2	0.12	1.32	6.27[c]
$F\tau$	5.16[c]	2.11	26.93[b]
Fp	12.17[c]	0.29	7.61×10^{-4}
τp	0.45	0.41	4.32
lack of fit	0.95	1.07	1.95
std. dev.	3.27	5.45	4.95
mean	117.73	80.27	78.95
coefficient of variation	2.78	6.79	6.27
R^2	0.9447	0.8413	0.8609
adj R^2	0.9187	0.7666	0.7954

[a] f = frequency, τ = pulse width, p = pulse polarity. [b] Significant at $p < 0.001$. [c] Significant at $p < 0.05$.

The analysis of variance revealed that a second-order model adequately fitted the experimental data ($P < 0.001$). The determination coefficient (R^2) was 0.94, and the lack of fit was not significant (**Table 3**). Frequency, pulse width, and pulse polarity affected the lycopene content linearly, whereas only the quadratic term of frequency was significant (**Table 3**). The combined effects of frequency and pulse width, as well as frequency and pulse polarity, were included in the model as interaction terms. Pulse polarity was considered a categorical factor. As a result, the lycopene relative content in tomato juice using monopolar pulses can be fitted through eq 2, and in the case of bipolar pulses, the relative content can be modeled by

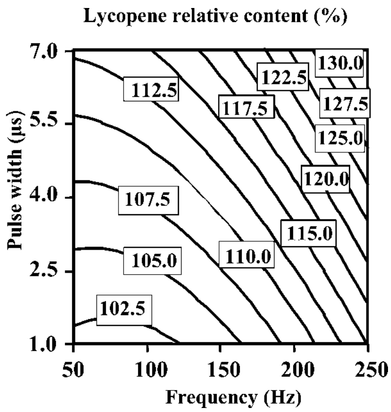

Figure 1. Counter plots for the combined effect of frequency and pulse width on the lycopene relative content of tomato juice treated at 35 kV/cm for 1000 μs in monopolar mode.

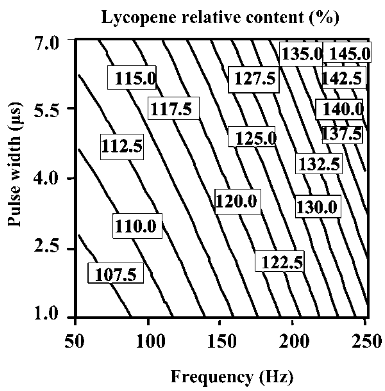

Figure 2. Counter plots for the combined effect of frequency and pulse width on the lycopene relative content of tomato juice treated at 35 kV/cm for 1000 μs in bipolar mode.

eq 3, where Lyc is lycopene relative content, f is the frequency (Hz), and τ is pulse width (μs).

$$\text{Lyc}(\%) = +98.007 - 0.05f + 1.472\tau + (4.766 \times 10^{-4})f^2 + (6.845 \times 10^{-3})f\tau \quad (2)$$

$$\text{Lyc}(\%) = +108.533 - 0.051f + 1.051\tau + (4.766 \times 10^{-4})f^2 + (6.845 \times 10^{-3})f\tau \quad (3)$$

Pulse polarity resulted in a marked effect on lycopene content ($P < 0.001$; **Table 3**). Bipolar treatments resulted in higher lycopene concentrations in tomato juices than monopolar treatments. A difference of 7.6% in lycopene was observed when using bipolar over monopolar pulses after applying a HIPEF treatment set up at 35 kV/cm for 1000 μs at 150 Hz and a 7 μs pulse width (**Figures 1** and **2**). On the other hand, frequency also had a significant effect ($P < 0.001$) on the lycopene content of tomato juice. The linear coefficient of the frequency variable was negative, meaning that the lycopene content was depleted with the increase of frequency (eqs 2 and 3). However, the linear negative effect of frequency may be masked by the positive effect of either the quadratic term of frequency or the interaction of frequency with pulse width (eqs 2 and 3). Pulse width also had an important effect on the lycopene content of tomato juice ($P < 0.001$; **Table 3**). Thus, the higher the pulse width, the greater the lycopene retention achieved in tomato juice. The lycopene content was affected by the combined effect of frequency and pulse width (eqs 2 and 3). The positive value of the interaction coefficient suggests that increasing the treatment frequency and pulse width resulted in a higher lycopene relative content. The simultaneous increase in the two variables from 50 Hz/1 μs to 250 Hz/7 μs resulted in an increment of lycopene of 43%, whereas when raising one variable and keeping the other constant, the lycopene content rose between 8.4% (50 Hz, 7 μs) and 26.4% (250 Hz, 1 μs) (**Figure 2**). In addition, interaction allowed combinations of frequency and pulse width for the monopolar (**Figure 1**) and bipolar (**Figure 2**) mode to achieve tomato juice with similar lycopene levels. As observed in **Figure 1**, tomato juice treated at 110 Hz using 7 μs monopolar pulses had the same lycopene relative content (115%) than that treated at 250 Hz with 1 μs pulses, also applied in the monopolar mode (**Figure 2**).

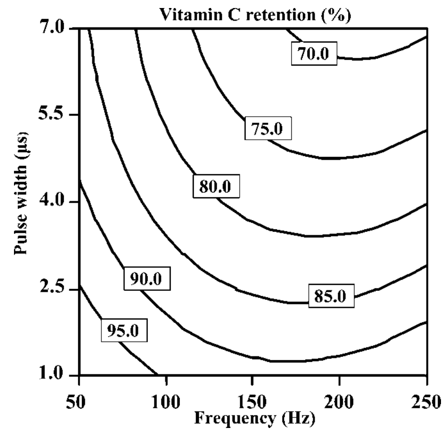

Figure 3. Counter plots for the combined effect of frequency and pulse width on the vitamin C retention of tomato juice treated at 35 kV/cm for 1000 μs in monopolar mode.

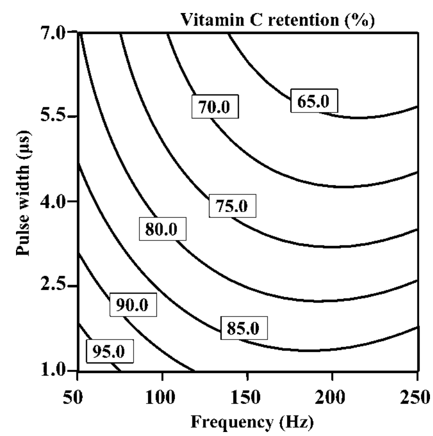

Figure 4. Counter plots for the combined effect of frequency and pulse width on the vitamin C retention of tomato juice treated at 35 kV/cm for 1000 μs in bipolar mode.

Effect of HIPEF-Processing Critical Parameters on Vitamin C. The vitamin C content of untreated tomato juice was 15.2 mg/100 g. The concentration of vitamin C expressed as vitamin C retention ranged between 58.2% and 99.0% in HIPEF-treated tomato juice (**Table 2**). Consistently, Torregrosa et al. (*29*) reported vitamin C retentions between 87.5 and 97% in orange–carrot juice treated at different electric field strengths (25, 30, 35, and 40 kV/cm) for different treatment times (from 30 to 340 μs) using 2.5 μs bipolar pulses. Vitamin oxidation and loss during processing and cooking is of great concern for nutritionists, processors, and consumers. Vitamin C is used as an index of the health-related quality of fruits, since, as compared to other beneficial compounds, it is more sensitive to degradation by processing (*30*). The analysis of variance

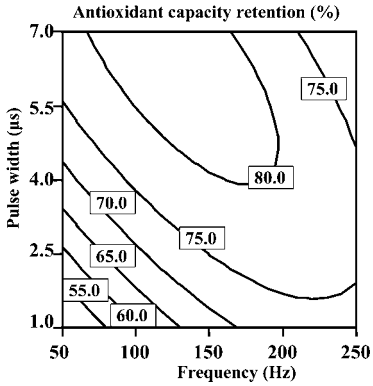

Figure 5. Counter plots for the combined effect of frequency and pulse width on the antioxidant capacity retention of tomato juice treated at 35 kV/cm for 1000 μs in monopolar mode.

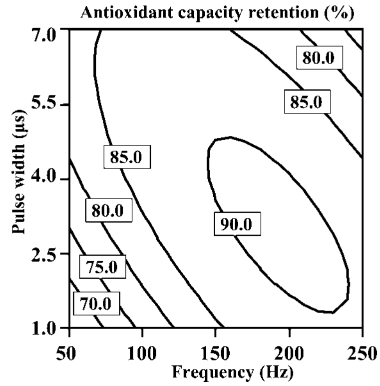

Figure 6. Counter plots for the combined effect of frequency and pulse width on the antioxidant capacity retention of tomato juice treated at 35 kV/cm for 1000 μs in bipolar mode.

showed that the second-order regression model adequately fitted vitamin C retention ($P < 0.001$) explaining 84.13% of the total variation without a significant lack of fit (**Table 3**). The vitamin C content was affected by the linear terms of frequency and pulse width, as well as by pulse mode and the quadratic term of frequency. HIPEF treatments applied at the same frequency and pulse width maintained more vitamin C when performed in monopolar than in bipolar mode. Vitamin C retention in bipolar mode was 7.3% lower than in monopolar mode for the HIPEF treatment set up at 35 kV/cm for 1000 μs at 250 Hz and a 4 μs pulse width (**Figures 3** and **4**). Vitamin C retention is represented by the following polynomial quadratic equations for monopolar (eq 4) and bipolar (eq 5) mode:

$$\text{VitC}(\%) = +121.099 - 0.292f - 3.710\tau + (7.616 \times 10^{-4})f^2 \quad (4)$$

$$\text{VitC}(\%) = +115.478 - 0.292f - 3.710\tau + (7.616 \times 10^{-4})f^2 \quad (5)$$

where VitC is vitamin C retention, f is the frequency (Hz), and τ is the pulse width (μs).

The lineal terms of frequency and pulse width are negative; thu,s the higher the frequency or pulse width, the lower the content of vitamin C is. Consistently, Elez-Martínez and Martín-Belloso (*17*) reported that vitamin C retention in orange juice and gazpacho increased with a decrease in pulse frequency or pulse width.

The high pulse width coefficient in the fitted model indicates that this parameter is more relevant for vitamin C retention than frequency. A difference of about 15% was observed between HIPEF treatments applying 1 and 7 μs monopolar pulses at 35 kV/cm for 1000 μs at 50 Hz (**Figure 3**). In contrast, higher variation ($\approx$ 30%) was obtained between 1 and 7 μs bipolar pulse treatments applied at 250 Hz (**Figure 4**). Consequently, the differences in vitamin C retention of tomato juice treated using different pulse widths depended on both frequency and polarity mode. On the other hand, the positive value of the quadratic term ($P < 0.05$) indicated that vitamin C retention reached a minimum as the frequency rose. In fact, increasing the frequency beyond 150 (monopolar mode) and 200 Hz (bipolar mode) provided decreasing vitamin C retentions than using lower frequencies (**Figures 3** and **4**).

Effect of HIPEF-Processing Critical Parameters on Antioxidant Capacity. Table 2 shows the antioxidant capacity of HIPEF-processed tomato juices treated under the studied experimental conditions. The maximum antioxidant capacity retention, 92.3%, was achieved with a treatment of 35 kV/cm for 1000 μs at 150 Hz applying 4 μs bipolar pulses. In contrast, the combination of 50 Hz and a 1 μs monopolar pulse width resulted in the least antioxidant capacity retention (50.7%).

The analysis of variance test revealed that a second-order model fits well the antioxidant capacity results (**Table 3**). The determination coefficient, R^2, was 0.86, and the lack of fit was not significant, indicating that the model is sufficiently accurate for predicting the response. **Table 3** indicates that the linear terms of frequency, pulse width, and pulse polarity affected the antioxidant capacity. The quadratic terms of frequency and pulse width, as well as the interaction of frequency with pulse width, also exerted a significant influence on the antioxidant capacity of HIPEF-treated tomato juice. The antioxidant capacity was represented by polynomial quadratic equations in terms of the studied HIPEF parameters. Equation 6 fits the relative antioxidant capacity using monopolar pulses. The antioxidant capacity can be calculated using eq 7 in the case of bipolar pulses.

$$\text{AC}(\%) = +20.398 + 0.390f + 11.414\tau - (7.688 \times 10^{-4})f^2 - 0.586\tau^2 - 0.030f\tau \quad (6)$$

$$\text{AC}(\%) = +38.841 + 0.389f + 9.436\tau - (7.688 \times 10^{-4})f^2 - 0.586\tau^2 - 0.030f\tau \quad (7)$$

where AC (%) is the relative antioxidant capacity, f is frequency (Hz) and τ is pulse width (μs).

Pulse polarity was the most important variable ($P < 0.001$) affecting antioxidant capacity retention in HIPEF-treated tomato juice. As can be seen in **Figures 5** and **6**, tomato juices treated with bipolar pulses had greater antioxidant capacity than those processed using monopolar treatment. On the other hand, frequency and pulse width coefficients are positive, meaning that an increase in frequency or pulse width results in a lower antioxidant capacity (eqs 6 and 7). However, the expected behavior of frequency and pulse width was modified by their negative quadratic terms. The negative coefficients of the quadratic terms of frequency and pulse width indicate a rise in antioxidant capacity when frequency and pulse width are slightly increased above 50 Hz and 1 μs, respectively, although the antioxidant capacity might decrease if the frequency or pulse width increased further. In addition, an increment in either frequency or pulse width resulted in lower increments in antioxidant capacity (**Figures 5** and **6**). Antioxidant capacity retention rose from 68.1 to 75.8% when the frequency was increased from 50 to 250 Hz, applying 4 μs monopolar pulses at 35 kV/cm for 1000 μs. Nevertheless, the maximal antioxidant capacity retention (80.2%) under these conditions was reached at approximately 175 Hz. When bipolar pulses were applied, the maximum was observed also at 175 Hz (**Figure 6**).

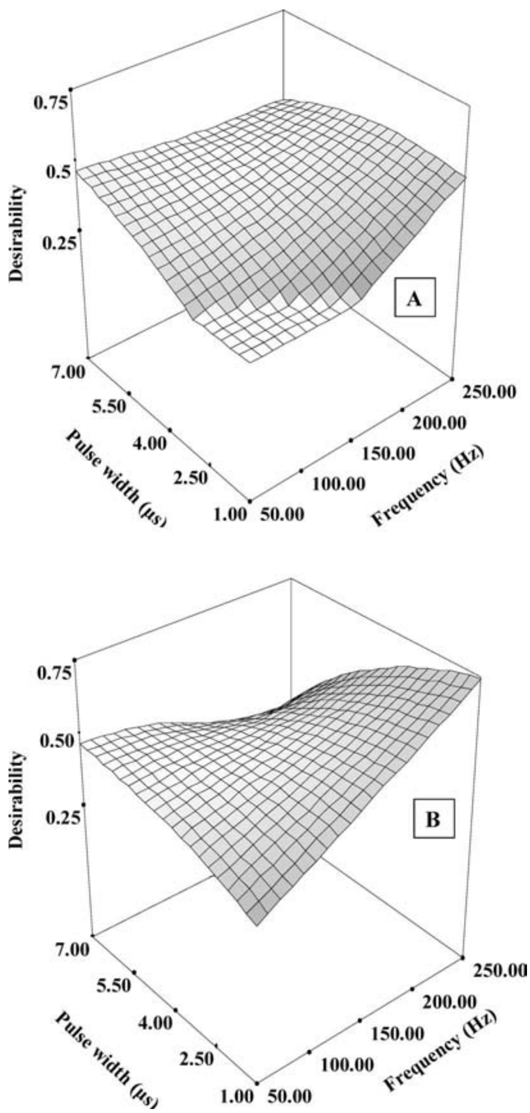

Figure 7. Response surface of desirability of HIPEF-treated tomato juice as a function of maximal lycopene, vitamin C, and antioxidant capacity retention for monopolar (A) and bipolar mode (B).

The coefficient sign of the interaction frequency with pulse width is negative, thus indicating that these factors act in opposite directions. An increase of both parameters may lead to a decrease in antioxidant capacity. Moreover, it is possible to exchange different combinations of the variables frequency and pulse width to achieve the same antioxidant capacity of tomato juice (**Figures 5** and **6**). The maximum values of antioxidant capacity in the monopolar mode were obtained by combining frequencies between 100 and 200 Hz with pulse widths higher than 4 μs, whereas frequencies higher than 150 Hz and pulse widths lower than 5 μs achieved the maximum values of antioxidant capacity in the bipolar mode.

Optimization of HIPEF-Processing Critical Parameters. Optimal conditions for tomato juice HIPEF-processing were determined to obtain juices with a high content of bioactive compounds as well as high antioxidant capacity. To this end, the same priority was assigned to each variable, seeking maximum levels of lycopene, vitamin C, and antioxidant capacity. The desirability of each HIPEF treatment is shown in **Figure 7**. The maximal desirability was achieved at high frequencies and low pulse widths in the bipolar mode. An overall score of 0.748 was obtained when the treatment was carried out at 35 kV/cm for 1000 μs using 1 μs squared wave pulses at 250 Hz in the bipolar mode. At these optimal conditions, the predicted lycopene relative content, vitamin C, and antioxidant capacity retention were 131.8, 90.2, and 89.4%, respectively. On the other hand, the second highest desirability (0.528) was obtained when processing at 35 kV/cm for 1000 μs, using 2.75 μs monopolar pulses at 250 Hz, reaching a 120.3% predicted lycopene relative content, 85.7% vitamin C retention, and 75.9% antioxidant capacity retention. The results of the optimization of HIPEF critical parameters were validated by repeating the experiment at the conditions predicted. When tomato juices were treated at 250 Hz with a 1 μs pulse width in the bipolar mode, lycopene relative content, vitamin C, and antioxidant capacity retention were 129.3%, 90.2%, and 90.1%, respectively. Hence, no significant differences between the predicted and experimental results were observed.

CONCLUSIONS

Lycopene retention, vitamin C, and antioxidant capacity depend on the pulse frequency, width, and polarity mode applied. Higher frequency and pulse width result in a greater lycopene relative content, but lower vitamin C retention, although the effect of these variables is nonlinear. The use of bipolar pulses raises lycopene and decreases vitamin C in tomato juice more than monopolar treatments. The maximum values of antioxidant capacity are obtained by combining frequencies around 150 Hz with pulse widths higher than 6 μs in the monopolar mode or HIPEF treatments with frequencies higher than 150 Hz and pulse widths lower than 5 μs applying bipolar pulses. In addition, different combinations of frequencies and pulse widths lead to equivalent lycopene relative content and antioxidant capacity retention. The evaluation of the effects of HIPEF critical parameters on bioactive compounds as well as on the antioxidant capacity of tomato juice is useful to achieve the optimal processing conditions to obtain tomato juice with high nutritional quality. However, further research is required to determine the effect of other critical HIPEF parameters such as electric field strengths and treatment time on the bioactive compounds of tomato juice.

ABBREVIATIONS USED

HIPEF, high-intensity pulsed electric fields; *f*, frequency; τ, pulse width; *p*, pulse polarity.

LITERATURE CITED

(1) Pellegrini, N.; Riso, P.; Porrini, M. Tomato consumption does not affect the total antioxidant capacity of plasma. *Nutrition* **2000**, *16*, 268–271.
(2) Shi, J.; Le Maguer, M. Lycopene in tomatoes: Chemical and physicochemical properties affected by food processing. *Crit. Rev. Food Sci. Nutr.* **2000**, *40*, 1–42.
(3) Sahlin, E.; Savage, G. P.; Lister, C. E. Investigation of the antioxidant properties of tomatoes after processing. *J. Food Compos. Anal.* **2004**, *17*, 635–647.
(4) Murcia, M. A.; López-Ayerra, B.; Martinez-Tomé, M.; Vera, A. M.; García-Carmona, F. Evolution of ascorbic acid and peroxidase during industrial processing of broccoli. *J. Sci. Food Agric.* **2000**, *80*, 1882–1886.
(5) Vega-Mercado, H.; Martín-Belloso, O.; Qin, B. L.; Chang, F. J.; Góngora-Nieto, M. M.; Barbosa-Cánovas, G. V.; Swanson, B. G. Non-thermal food preservation: pulsed electric fields. *Trends Food Sci. Technol.* **1997**, *8*, 151–157.
(6) Qin, B. L.; Pothakamury, U. R.; Barbosa-Cánovas, G. V.; Swanson, B. G. Nonthermal pasteurization of liquid foods using high-intensity pulsed electric fields. *Crit. Rev. Food Sci. Nutr.* **1996**, *36*, 603–627.

(7) Mertens, B.; Knorr, D. Developments of nonthermal processes for food preservation. *Food Technol.* **1992**, *46*, 124–133.
(8) Wouters, P. C.; Smelt, J. P. P. M. Inactivation of microorganisms with pulsed electric fields: potential for food preservation. *Food Biotechnol.* **1997**, *11*, 193–229.
(9) Ortega-Rivas, E.; Zarate-Rodríguez, E.; Barbosa-Cánovas, G. V. Apples juice pasteurization using ultrafiltration and pulsed electric fields. *Food Bioprod. Process.* **1998**, *76*, 193–198.
(10) Elez-Martínez, P.; Escolà-Hernández, J.; Soliva-Fortuny, R. C.; Martín-Belloso, O. Inactivation of Saccharomyces cerevisiae suspended in orange juice using high-intensity pulsed electric fields. *J. Food Prot.* **2004**, *67*, 2596–2602.
(11) Elez-Martínez, P.; Escolà-Hernández, J.; Soliva-Fortuny, R. C.; Martín-Belloso, O. Inactivation of Lactobacillus brevis in orange juice by high-intensity pulsed electric fields. *Food Microbiol.* **2005**, *22*, 311–319.
(12) Giner, J.; Gimeno, V.; Espachs, A.; Elez, P.; Barbosa-Cánovas, G. V.; Martín, O. Inhibition of tomato (Licopersicon esculentum Mill.) pectin methylesterase by pulsed electric fields. *Innovative Food Sci. Emerging Technol.* **2000**, *1*, 57–67.
(13) Espachs-Barroso, A.; Van-Loey, A.; Hendrickx, M.; Martín-Belloso, O. Inactivation of plant pectin methylesterase by thermal or high intensity pulsed electric field treatments. *Innovative Food Sci. Emerging Technol.* **2006**, *7*, 40–48.
(14) Elez-Martinez, P.; Aguiló-Aguayo, I.; Martín-Belloso, O. Inactivation of orange juice peroxidase by high-intensity pulsed electric fields as influenced by process parameters. *J. Sci. Food Agric.* **2006**, *86*, 71–81.
(15) Cortés, C.; Esteve, M. J.; Rodrigo, D.; Torregrosa, F.; Frígola, A. Changes of colour and carotenoids contents during high-intensity pulsed electric field treatment in orange juices. *Food Chem. Toxicol.* **2006**, *44*, 1932–1939.
(16) Torregrosa, F.; Cortés, C.; Esteve, M. J.; Frígola, A. Effect of high-intensity pulsed electric fields processing and conventional heat treatment on orange-carrot juice carotenoids. *J. Agric. Food Chem.* **2005**, *53*, 9519–9525.
(17) Elez-Martínez, P.; Martín-Belloso, O. Effects of high intensity pulsed electric field processing conditions on vitamin C and antioxidant capacity of orange juice and gazpacho, a cold vegetable soup. *Food Chem.* **2007**, *102*, 201–209.
(18) Diniz, F. M.; Martin, A. M. Use of response surface methodology to describe the combined effects of pH, temperature and E/S ratio on the hydrolysis of dogfish (Squalus acanthias) muscle. *Int. J. Food Sci. Technol.* **1996**, *31*, 419–426.
(19) Myers, R. H.; Montgomery, C. *Response surface methodology: process and product optimization using design experiments*; John Wiley & Sons: New York, 2002.
(20) Davis, A. R.; Fish, W. W.; Perkins-Veazie, P. A rapid spectrophotometric method for analyzing lycopene content in tomato and tomato products. *Postharvest Biol. Technol.* **2003**, *28*, 425–430.
(21) Fish, W. W.; Perkins-Veazie, P.; Collins, J. K. A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. *J. Food Compos. Anal.* **2002**, *15*, 309–317.
(22) Odriozola-Serrano, I.; Hernández-Jover, T.; Martín-Belloso, O. Comparative evaluation of UV-HPLC methods and reducing agents to determine Vitamin C in fruits. *Food Chem.* **2007**, *105*, 1151–1158.
(23) De Ancos, B.; Sgroppo, S.; Plaza, L.; Cano, M. P. Possible nutritional and health-related value promotion in orange juice preserved by high-pressure treatment. *J. Sci. Food Agric.* **2002**, *82*, 790–796.
(24) Derringer, G.; Suich, R. Simultaneous optimization of several response variables. *J. Qual. Technol.* **1980**, *12*, 214–219.
(25) Abushita, A. A.; Daood, H. G.; Biacs, P. Change in carotenoids and antioxidant vitamins in tomato as a function of varietal and technological factors. *J. Agric. Food Chem.* **2000**, *48*, 2075–2081.
(26) Sánchez-Moreno, P.; Cano, M. P.; De Ancos, B.; Plaza, L.; Olmedilla, B.; Granado, F.; Elez-Martínez, P.; Martín-Belloso, O.; Martín, A. Intake of Mediterranean soup treated by pulsed electric fields affects plasma vitamin C and antioxidant biomarkers in humans. *Int. J. Food Sci. Nutr.* **2005**, *56*, 115–124.
(27) Bramley, P.; Teuliers, C.; Blain, I.; Bird, C.; Schuch, W. Biochemical characterization of transgenic tomato plants in which carotenoids synthesis has been inhibited through the expression of antisense RNA to p TOM5. *Plant J.* **1992**, *2*, 343–349.
(28) Dumas, Y.; Dadomo, M.; Di Lucca, G.; Grolier, P. Review: Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. *J. Sci. Food Agric.* **2003**, *83*, 369–382.
(29) Torregrosa, F.; Esteve, M. J.; Frígola, A.; Cortés, C. Ascorbic acid stability during refrigerated storage of orange-carrot juice treated by high pulsed electric field and comparison with pasteurized juice. *J. Food Eng.* **2006**, *73*, 339–345.
(30) Davey, M. W.; Van Montagu, M.; Inze, D.; Sanmartin, M.; Kanellis, A.; Smirnoff, N.; Benzie, I. J. J.; Strain, J. J.; Favell, D.; Fletcher, J. Plant L-ascorbic: chemistry, function, metabolism, bioavailable and effects of processing. *J. Sci. Food Agric.* **2000**, *80*, 825–860.

Received for review March 29, 2007. Revised manuscript received August 21, 2007. Accepted September 5, 2007. This work was supported by the Ministerio de Ciencia y Tecnología (Spain) through Project ALI 2005-05768. This study has been carried out with financial support from the Commission of the European Communities, Framework 6, Priority 5 "Food Quality and Safety", Integrated Project NovelQ FP6-CT-2006-015710, as well. I.O.-S. thanks the Agència de Gestió d'Ajuts Universitaris i de Recerca of the Generalitat de Catalunya (Spain) and the European Social Fund for the predoctoral grant.

JF0709101