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Effects of high-intensity pulsed electric field processing conditions on lycopene, vitamin C and antioxidant capacity of watermelon juice

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ABSTRACT

Watermelon juice was subjected to high-intensity pulsed electric fields (HIPEF). The effects of process parameters including electric field strength (30–35 kV/cm), pulse frequency (50–250 Hz), treatment time (50–2050 μs), pulse width (1–7 μs) and pulse polarity (monopolar/bipolar) on lycopene, vitamin C and antioxidant capacity were studied using a response surface methodology. Lycopene content was measured spectrophotometrically, vitamin C was determined by HPLC and antioxidant capacity through the inhibition of DPPH· (1,1-diphenyl-2-picrylhydrazyl) radical. Watermelon juice exhibited high retention of lycopene and antioxidant capacity when high electric field strengths, frequencies and pulse widths were applied. However, severe HIPEF treatments reduced vitamin C content. Maximal relative lycopene content (113%), vitamin C (72%) and antioxidant capacity retention (100%) were obtained when HIPEF treatments were set up at 35 kV/cm for 50 μs using 7 μs bipolar pulses at 200 Hz.

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1. Introduction

Epidemiological studies have demonstrated that high consumption of fruits and vegetables can provide health benefits due to their antioxidant constituents including carotenoids, flavonoids, phenolic compounds and vitamins (Gardner, White, McPhail, & Duthie, 2000; Sánchez-Moreno, Plaza, De Ancos, & Cano, 2003; Tibble, 1998; Williamson, 1999). Watermelon contains small amounts of phenolics as well as low vitamin C content compared with other fruits (Gil, Aguayo, & Kaer, 2006). On the other hand, watermelon is a rich natural source of lycopene, a compound responsible for its red colour (Perkins-Veazie, Collins, Pair, & Roberts, 2001). Intake of lycopene containing-products has been associated with a reduced incidence of coronary heart disease and some types of cancer (Fraser & Bramley, 2004; Giovannucci, 2002).

Thermal preservation is believed to be responsible for a depletion of naturally occurring antioxidants in food. The overall antioxidant properties of foods may be affected by processing in several ways, including losses of naturally occurring compounds, improvement of their antioxidant properties, as well as formation of novel compounds by Maillard or other reactions that affect antioxidant activity (Nicoli, Anese, & Parpinel, 1999). The main causes of lycopene degradation during thermal processing of tomatoes are oxidation and isomerisation (Shi & Le Maguer, 2000). Vitamin C is also high susceptible to oxidation in the presence of oxygen (Davey et al., 2000).

High-intensity pulsed electric field (HIPEF) treatments is one of the non-thermal emerging technologies being studied as an alternative to thermal treatments not only to ensure safety and extend shelf-life of fruit juices but also to provide fresh-like products with high antioxidant potential (Elez-Martínez & Martín-Belloso, 2007; Odriozola-Serrano, Aguiló-Aguayo, Soliva-Fortuny, Gimeno-Añó, & Martín-Belloso, 2007; Sánchez-Moreno et al., 2005). Studies evaluating the effects of HIPEF-processing conditions on watermelon juices have been demonstrated that HIPEF treatments were effective in reducing the population of pathogenic microorganisms and inactivating spoilage enzymes (Aguiló-Aguayo, Soliva-Fortuny, & Martín-Belloso, 2008; Mosqueda-Melgar, Raybaudi-Massilia, & Martín-Belloso, 2007). Populations of *Salmonella enteritidis*, *E. coli* and *L. monocytogenes* were reduced up to 3.71, 3.70 and 3.56 log units, respectively, when watermelon juice was submitted to a HIPEF treatment set up at 35 kV/cm for 1727 μs using 4-μs bipolar pulses at 188 Hz (Mosqueda-Melgar et al., 2007). However, no information has been found in literature regarding the effects of HIPEF treatments on antioxidant potential of watermelon juice. In orange, tomato and strawberry juices, vitamin C retention depends on process parameters, such as electric field strength, treatment time, pulse frequency, width and polarity (Elez-Martínez & Martín-Belloso, 2007; Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2009; Odriozola-Serrano, Aguiló-Aguayo, et al., 2007). However, the influence of process parameters on the overall antioxidant properties of fruit juices needs to be deeply studied in order to define adequate treatment conditions to obtain juices with high antioxidant potential.

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Therefore, the objective of this research was to study the combined effect of electric field strength, pulse frequency, treatment time, pulse width and polarity on lycopene, vitamin C and antioxidant capacity of HIPEF-treated watermelon juice. We aimed to select the most appropriate HIPEF treatment to obtain juices with high antioxidant potential.

2. Materials and methods

2.1. Watermelon juice

Watermelon fruits (*Citrullus lanatus* var. “Seedless”) were purchased from a local supermarket (Lleida, Spain). The fruits were washed, drained, chopped and filtered using a steel sieve with an approximate mesh of 2 mm.

2.2. Pulsed electric fields equipment

HIPEF treatments were performed using a continuous flow bench scale system (OSU-4F, Ohio State University, Columbus, OH, USA), that produces monopolar and bipolar squared wave pulses. The treatment flow rate was 60 mL/min and it was controlled by a variable speed pump (model 752210-25, Cole Palmer Instrument Company, Vermon Hills, IL, USA). The treatment chamber device consists of eight co-linear 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 temperature was kept below 40 °C, using a cooling coil, which was connected between each pair of chambers and submerged in an iced water 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. The characteristics of the electric pulses delivered such as shape, polarity, width, difference of potential as well as the electric current generated across the electrodes and the pulse frequency were monitored using a digital oscilloscope (model THS720, Tektronix Inc., Beaverton, OR, USA).

Watermelon juice was treated at electric field strength ranging from 30 to 35 kV/cm for 50–2050 μs at frequencies between 50 and 250 Hz, applying 1–7 μs pulses in monopolar or bipolar mode.

2.3. Vitamin C

Vitamin C in watermelon juice was determined following the method validated by Odriozola-Serrano, Hernández-Jover, and Martín-Belloso (2007). A sample of 25 mL of watermelon juice was mixed with 25 mL of a solution containing 45 g/L of metaphosphoric acid and 7.2 g/L of DTT. The mixture was centrifuged at 22,100*g* for 15 min at 4 °C and the supernatant was vacuum-filtered through Whatman No. 1 paper. The sample was then passed through a millipore 0.45 μm membrane into an opaque vial and kept at −42 °C until required for analysis. An aliquot of 20 μL was injected into a HPLC system fitted with a reverse-phase C18 Spherisorb® ODS2 (5 μm) stainless-steel column (4.6 mm × 250 cm). The mobile phase was a 0.01% sulphuric acid solution adjusted to a pH of 2.6. The flow rate was fixed at 1 mL/min at room temperature. Detection was performed with a 486 Absorbance Detector (Waters, Milford, MA) set at 245 nm. Vitamin C was quantified through a calibration curve built with ascorbic acid pure standards and results were expressed as relative vitamin C concentration compared to the untreated sample.

2.4. Lycopene

Total lycopene content was measured spectrophotometrically following the method proposed by Davis, Fish, and Perkins-Veazie (2003). Approximately 0.6 g of watermelon juice was weighed and added to 5 mL of 0.05% (w/v) butylated hydroxytoluene (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 according to the following equation:

$$\text{lycopene} = \frac{\Delta_{503} \times \text{MW} \times \text{DF} \times 1000}{\varepsilon \times L} \tag{1}$$

where MW is the molecular weight of lycopene (536.9 g/mol), DF is the dilution factor, L is the pathlength in cm and ε is the molar extinction coefficient for lycopene (172,000 L mol/cm). Lycopene was expressed as the percentage of lycopene compared to that of the untreated samples.

2.5. Antioxidant capacity

This assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH), according to the method described by Elez-Martínez and Martín-Belloso (2007). The measurement is based in a decolouration assay, which evaluates the absorbance decrease at 515 nm produced by the addition of the antioxidant to a DPPH solution in methanol. The $DPPH^{\cdot}$ method is recommended to measure the radical-scavenging activity of fruit juices as it is an easy and accurate procedure (Gil, Tomás-Barberán, Hess-Pierce, Holcroft, & Kader, 2000). Watermelon juice was centrifuged at 6000*g* for 15 min at 4 °C (Centrifuge Medigifer; Select, Barcelona, Spain) and 0.01 mL of the supernatant was added to 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. 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. Antioxidant capacity was calculated as percentage of inhibition of the radical DPPH, which is the decrease in absorbance with respect to the control value (DPPH initial absorption value). Results were expressed as antioxidant capacity related to the untreated sample.

2.6. Experimental design

A face-centered central composite response surface analysis was used to determine the effect of electric field strength, frequency, pulse width, treatment time and polarity mode on the antioxidant potential of watermelon juice. The selected responses were lycopene, vitamin C, and antioxidant capacity. The independent variables were electric field strength (from 25 to 35 kV/cm), pulse frequency (from 50 to 250 Hz), pulse width (from 1 to 7 μs), treatment time (from 50 to 2050 μs) and polarity mode (monopolar or bipolar). The levels for each independent parameter were chosen considering sample and equipment limitations. The experimental design along with each experimental condition is shown in Table 1. The experimental design was performed twice, resulting in two blocks of experiments. The order of assays within each block was randomised and performed in triplicate. Experimental data were fitted to a polynomial response surface function. The sec-

Table 1
Central composite response surface design followed to evaluate lycopene, vitamin C and antioxidant capacity retention of HIPEF-treated watermelon juices.

Assay number[a]	Intensity (kV/cm)	Frequency (Hz)	Pulse width (μs)	Treatment time (μs)	Lycopene relative content (%)[b]		Vitamin C retention (%)[b]		AC retention (%)[b]	
					Monopolar	Bipolar	Monopolar	Bipolar	Monopolar	Bipolar
1	30	150	4	1050	97.6 ± 2.4[c]	103.5 ± 3.4[c]	84.9 ± 3.7[c]	80.9 ± 1.8[c]	86.2 ± 2.0[c]	93.0 ± 2.9[c]
2	35	250	1	50	103.2	107.4	86.5	80.3	91.0	96.3
3	30	150	4	50	95.4	100.4	92.9	87.1	84.7	90.7
4	30	250	4	1050	90.6	92.4	82.6	70.9	80.1	83.0
5	25	250	1	2050	92.8	99.8	85.4	80.4	82.2	89.8
6	35	250	7	50	105.3	110.4	73.7	70.2	92.2	98.3
7	25	50	1	50	90.5	95.5	99.9	96.4	80.9	87.0
8	30	50	4	1050	94.9	99.9	94.4	90.2	84.4	90.4
9	25	50	7	2050	93.2	100.9	92.8	88.8	82.9	91.2
10	25	250	7	50	100.4	103.6	83.6	85.2	88.5	93.3
11	35	50	7	50	92.6	97.6	90.9	82.1	82.3	88.1
12	35	250	7	2050	110.3	121.2	45.8	39.8	95.0	106.0
13	25	50	1	2050	89.6	93.9	97.4	92.5	80.0	85.4
14	30	150	4	2050	98.4	104.3	80.3	73.3	86.6	93.3
15	35	50	1	50	89.6	93.6	93.5	90.4	79.9	85.0
16	25	50	7	50	94.6	101.4	96.2	94.2	84.2	91.9
17	25	150	4	1050	95.4	106.5	92.8	86.4	84.7	95.9
18	35	250	1	2050	107.2	110.6	61.3	58.2	93.2	97.9
19	30	150	1	1050	92.1	95.4	90.4	85.4	81.8	86.3
20	25	250	7	2050	102.6	106.9	80.3	76.3	90.2	95.6
21	35	50	1	2050	87.6	96.4	90.4	80.3	78.0	87.0
22	35	150	4	1050	98.4	108.3	75.3	72.2	86.4	96.6
23	30	150	7	1050	100.3	103.6	83.3	74.2	88.4	92.8
24	35	50	7	2050	90.5	95.6	87.3	78.2	80.3	86.2
25	25	250	1	50	96.5	101.1	89.2	86.2	85.5	91.3

AC = antioxidant capacity.
[a] Order the assays was randomised.
[b] Data shown are the mean ± SD of 2 treatment repetitions, each assay was performed in triplicate.
[c] Data shown are the mean of 6 repetitions.

ond-order response function was predicted by the following equation:

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

where Y is the dependent variable, β_0 is the centre point of the system, β_i, β_{ii} and β_{ij} represent the coefficients of the linear (X_i), quadratic (X_i^2) and interactive (X_iX_j) effects of independent variables, respectively.

After carrying out an analysis of variance, the nonsignificant terms were deleted from the second-order polynomial model, and a new ANOVA was performed to obtain the coefficients of the final equation for better accuracy. Design Expert 6.0.1 software (Stat Ease Inc., Minneapolis, MN) was used to generate quadratic models that fit the experimental data, draw the response surface plots and optimise HIPEF treatment. Three-dimensional surface plots and contour plots were drawn to illustrate the interactive effects of two factors on the dependent variable, while keeping constant the other variables. The optimisation was done according to the method proposed by Derringer and Suich (1980). 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 watermelon juice with the highest levels of lycopene, vitamin C and antioxidant capacity.

A second set of experiments was performed to validate the developed predictive equations. Fresh watermelon juice aliquots were randomly treated at 25–35 kV/cm for 50–2050 μs, using monopolar and bipolar pulses of 1–7 μs applied at frequencies of 50–250 Hz. The correlation coefficients between the polynomial model predictions and the experimental data were taken as a measure of the prediction accuracy.

3. Results and discussion

3.1. Effects of HIPEF treatment conditions on vitamin C retention in watermelon juice

The vitamin C content of fresh watermelon juice was 2.6 mg/100 mL. Concentration was low compared with other fruit juices such as orange, tomato or strawberry (Elez-Martínez & Martín-Belloso, 2007; Odriozola-Serrano, Soliva-Fortuny, Gimeno-Añó, & Martín-Belloso, 2008; Odriozola-Serrano, Aguiló-Aguayo, et al., 2007; Odriozola-Serrano et al., 2009). Juices treated at 25 kV/cm for 50 μs at 50 Hz using mono- or bipolar 1-μs pulses exhibited the highest vitamin C retention (96.4–99.9%). On the other hand, vitamin C loss was higher than 50% when HIPEF treatment was set up at 35 kV/cm for 2050 μs at 250 Hz applying mono- or bipolar 7-μs pulses (Table 1, assay 12). Such severe conditions seem to greater affect vitamin C retention in watermelon juice than in other juices such as orange, orange–carrot or strawberry juices, which exhibited retention of vitamin C above 80% (Elez-Martínez & Martín-Belloso, 2007; Odriozola-Serrano et al., 2009; Torregrosa, Esteve, Frígola, & Cortés, 2006). Applying the same HIPEF conditions, differences in vitamin C retention among HIPEF-treated juices could be due to their different pH, since more acidic conditions are known to stabilise vitamin C (Tannenbaum, Archer, & Young, 1985).

A first-order regression model fitted vitamin C retention with accuracy ($p < 0.001$). The determination coefficient (R^2) was 0.92 and the lack of fit was not significant, showing that the model is adequate to predict vitamin C retention. Pulse polarity, electric field strength, pulse frequency, pulse width and treatment time affected vitamin C retention of fruit juices after HIPEF-processing ($p < 0.05$) (Table 2). Since polarity is a categorical variable, two different equations in terms of the studied HIPEF parameters were obtained for both monopolar and bipolar mode. Coefficients of

Table 2
Analysis of variance of the second-order function for vitamin C, lycopene and antioxidant capacity retention of HIPEF-treated watermelon juices.

Source Model		Vitamin C	Lycopene	Antioxidant capacity
F-value[a]				
Linear	E	153.66[b]	8.45[c]	5.01[c]
	f	225.82[b]	61.51[b]	50.22[b]
	τ	36.68[b]	17.73[b]	15.36[b]
	t	90.41[b]	1.18	0.31
	p	34.56[b]	39.47[b]	75.22[b]
Interactions	$E \times f$	20.62[b]	239.70[b]	17.95[b]
	$E \times \tau$	5.23[b]	7.68	0.87
	$E \times t$	22.54[b]	17.64	0.90
	$E \times p$	1.18	0.18	3.63×10^{-3}
	$f \times \tau$	5.06[c]	4.66	0.25
	$f \times t$	23.53[b]	30.31	1.75
	$f \times p$	0.19	1.41	0.10
	$\tau \times t$	0.31	7.00	0.54
	$\tau \times p$	2.03×10^{-3}	1.38	0.11
	$t \times p$	0.91	5.84	0.42
Quadratic	E^2	1.45	5.44[c]	5.26[c]
	f^2	0.54	7.27[c]	7.23[c]
	τ^2	5.76×10^{-3}	2.67	0.23
	t^2	9.89×10^{-4}	5.54	0.46
Lack of fit		1.41	1.54	1.51
Standard deviation		3.33	3.49	2.93
Mean		82.66	99.41	88.63
Coefficient of variation		4.02	3.51	3.30
R^2		0.9397	0.8046	0.8176
Adjusted R^2		0.9110	0.7118	0.7309

E: electric field strength (kV/cm); f: frequency (Hz); τ: pulse width (μs); t: treatment time (μs); p: polarity.

[a] *F*-value stands for the variance explained by a factor compared with the unexplained variance.

[b] Significant at $p < 0.05$.

[c] Significant at $p < 0.001$.

Table 3
Coefficients of the second-order equation for vitamin C, lycopene and antioxidant capacity of HIPEF-treated watermelon juices.

Source	Vitamin C	Lycopene	Antioxidant capacity
Coefficient estimate[a]			
Intercept			
Monopolar pulses	89.41 ± 2.27	225.59 ± 24.81	193.58 ± 21.11
Bipolar pulses	84.36 ± 2.68	231.25 ± 25.25	200.13 ± 21.48
E	0.37 ± 0.11	−9.38 ± 3.78	−7.78 ± 3.11
f	0.12 ± 0.25	$1.31 \times 10^{-3} \pm 0.26$	$3.45 \times 10^{-3} \pm 0.21$
τ	2.23 ± 0.31	0.81 ± 0.37	0.64 ± 0.31
t	0.02 ± 0.26	–	–
$E \times f$	$-5.34 \times 10^{-3} \pm 0.28$	$5.47 \times 10^{-3} \pm 0.30$	$4.38 \times 10^{-3} \pm 0.24$
$E \times \tau$	−0.09 ± 0.30	–	–
$E \times t$	$-5.58 \times 10^{-4} \pm 0.28$	–	–
$f \times \tau$	$-4.41 \times 10^{-3} \pm 0.28$	–	–
$f \times t$	$-2.85 \times 10^{-5} \pm 0.28$	–	–
$\tau \times t$	–	–	–
E^2	–	0.15 ± 0.67	0.12 ± 0.55
f^2	–	$-3.99 \times 10^{-4} \pm 0.64$	$-3.35 \times 10^{-4} \pm 0.53$
τ^2	–	–	–
t^2	–	–	–

E: electric field strength (kV/cm); f: frequency (Hz); τ: pulse width (μs); t: treatment time (μs).

[a] Values ± confidence intervals ($p = 0.05$).

the fitted equations are shown in Table 3. Vitamin C content was better maintained when HIPEF treatments were performed in monopolar than in bipolar mode (Fig. 1). In agreement with our results, vitamin C retention of tomato juices treated at 35 kV/cm for 1000 μs applying 7-μs pulses at 250 Hz was 5% higher for monopolar than for bipolar treatments (Odriozola-Serrano, Aguiló-Aguayo, et al., 2007). Increased vitamin C retention of HIPEF-treated fruit juices in monopolar mode may be related to inactivation of enzymes that catalyse vitamin C oxidation. In HIPEF-treated orange juices, enzymes such as peroxidase were more inactivated with monopolar pulses than with bipolar pulses (Elez-Martinez, Aguiló-Aguayo, & Martín-Belloso, 2006). Loss of vitamin C in watermelon juice was accelerated when increasing severity of HIPEF treatments. In accordance with our results, the lower the electric field strength, the treatment time, the pulse frequency or the pulse width, the higher the vitamin C retention in orange, tomato and strawberry juices (Elez-Martínez & Martín-Belloso, 2007; Odriozola-Serrano, Aguiló-Aguayo, et al., 2007; Odriozola-Serrano et al., 2008, 2009). Oxidation of ascorbic acid occurs mainly during the processing of juices and depends upon many factors such as oxygen presence, heat and light (Robertson & Samaniego, 1986). Ascorbic acid is an unstable compound, which under less desirable conditions decomposes easily; thus the milder the treatment, the better the vitamin C retention in juices (Odriozola-Serrano et al., 2008).

3.2. Effects of HIPEF treatment conditions on lycopene retention in watermelon juice

Fresh watermelon juice had a lycopene content of 6.2 mg/100 mL. Lycopene retention in HIPEF-processed watermelon juice ranged from 87.6% to 121.2%. The content achieved with HIPEF treatments set up at 35 kV/cm with pulses of 250 Hz was slightly higher than that of untreated samples (Table 1, assays 2, 6, 12 and 18). Cortés, Esteve, Rodrigo, Torregrosa, and Frígola (2006) observed that the carotenoid concentration in orange juice rose slightly after applying intense HIPEF treatments of 35 and 40 kV/cm for 30–240 μs. A significant increase in the lycopene content was attained in tomato juice subjected to 35 kV/cm for 1000 μs at frequencies from 50 to 250 Hz (Odriozola-Serrano, Aguiló-Aguayo, et al., 2007). However, as can be seen in Fig. 2, the application of 35 kV/cm at low frequency led to a decrease in the lycopene content of treated watermelon juice of up to 10–12% compared to the fresh fruit juice. Maximal lycopene content of 114% in watermelon juice was achieved with 7-μs bipolar pulses for 1050 μs at 35 kV/cm and frequencies ranging from 200 to 250 Hz (Fig. 2). These same treatment conditions have been reported by other authors to reach lycopene relative content of 146.2% in tomato juice (Odriozola-Serrano, Aguiló-Aguayo, et al., 2007). These authors related the increase in lycopene to the conversion of some carotenoids to lycopene as a result of an intense

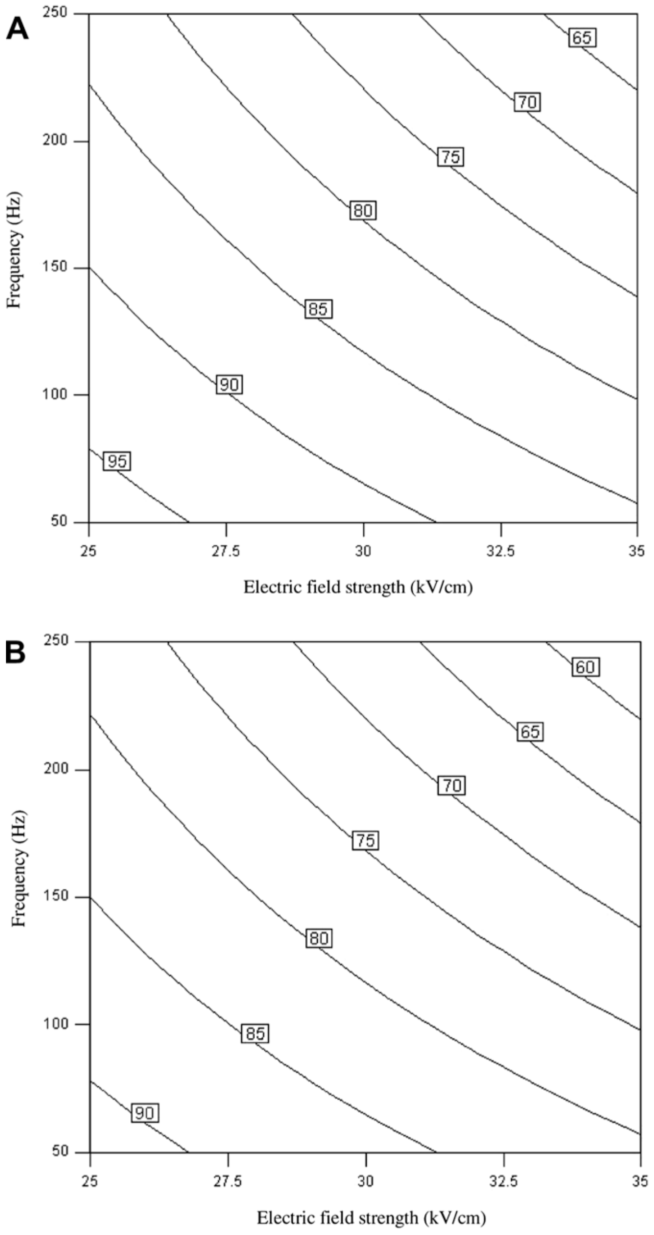

Fig. 1. Effect of pulse frequency and electric field strength on vitamin C relative content (%) of watermelon juice treated with high-intensity pulse electric fields (HIPEF) applying 7-μs monopolar (A) or bipolar (B) pulses for 1050 μs.

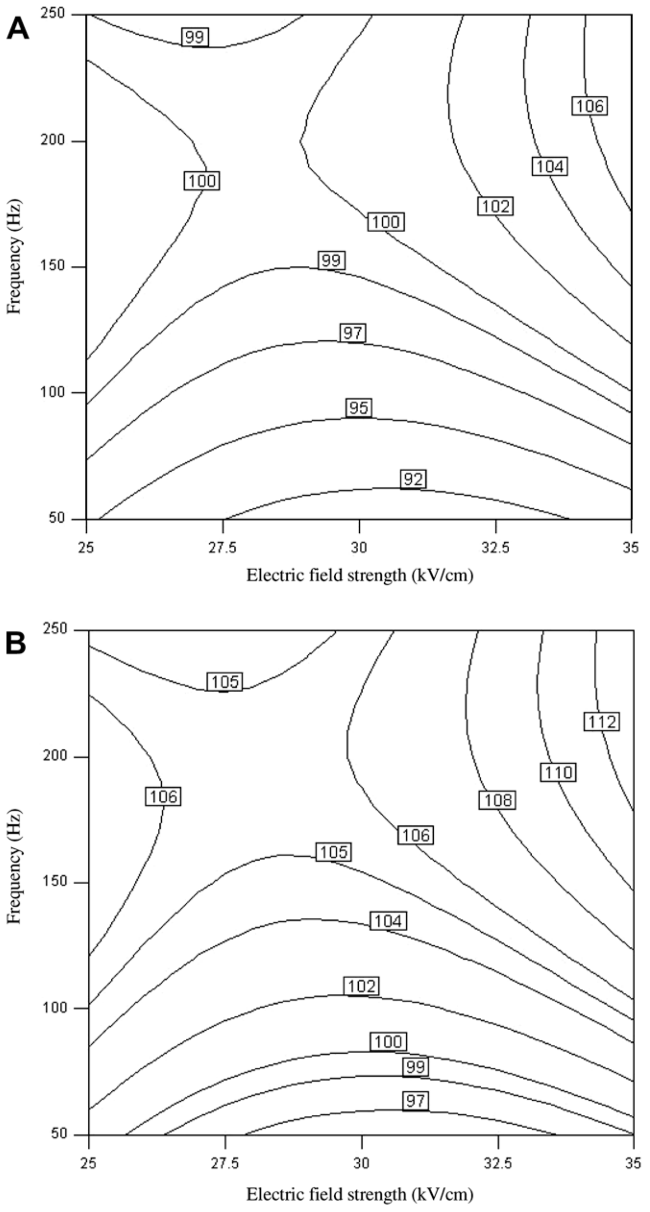

Fig. 2. Effect of pulse frequency and electric field strength on lycopene relative content (%) of watermelon juice treated with high-intensity pulse electric fields (HIPEF) applying 7-μs monopolar (A) or bipolar (B) pulses for 1050 μs.

HIPEF treatment at temperatures lower than 40 °C. The temperature attained during processing may accelerate lycopene synthesis in watermelon, which has a carotenoid pathway similar to that of tomato (Tadmor et al., 2005), involving the conversion of geranyl–geranyl diphosphate (GGPP) to phytoene by phytoene synthase and the conversion of phytoene to phytofluene, ζ-carotene, and lycopene by phytoene desaturase (Fraser, Truesdale, Bird, Schuch, & Bramley, 1994).

The statistical analysis indicates that the proposed quadratic model for lycopene was adequate ($p < 0.0001$) with satisfactory determination coefficients ($R^2 = 0.71$) (Table 2). No significant lack of fit of the model was found, showing that the model was sufficiently accurate for predicting the response within the range of assayed conditions. Electric field strength, frequency, pulse width and polarity affected the lycopene content (Table 2). Lycopene relative content in watermelon juice was fitted through two different equations for both monopolar and bipolar pulses. Bipolar treatments resulted in higher lycopene concentrations in watermelon juice than monopolar treatments ($p < 0.05$, Fig. 2). Moreover, relative lycopene retention increased at high pulse width or frequencies, whereas it decreased at low electric field strength. However, linear effects of electric field strength and frequency were masked by the quadratic effects and interaction of both parameters. Thus, lycopene retention did not decrease as electric field strength rose beyond 30 kV/cm, nor did it increase at frequencies higher than 200 Hz. Moreover, the effects of increasing electric field strength on lycopene retention depended on the applied frequencies. For 7-μs bipolar pulses, the effect of an increase in electric field strength from 25 kV/cm to 35 kV/cm on lycopene retention led to a 2% decrease at 50 Hz but to a 9% increase at 250 Hz (Fig. 2).

3.3. Effects of HIPEF treatment conditions on antioxidant capacity of watermelon juice

The antioxidant capacity of watermelon juice was measured as free radical-scavenging capacity on DPPH radical. Antioxidant capacity of untreated watermelon juice was 4.92% of DPPH inhibition. Antioxidant capacity retention of HIPEF-treated watermelon juice ranged from 78% to 106% (Table 1). These results differ from those reported for tomato and strawberry juices, that exhibited an antioxidant capacity retention from 50.7% to 100% after a HIPEF treatment at 35 kV/cm for 1000 μs using 1–7 μs monopolar or bipolar pulses at frequencies from 50 to 250 Hz (Odriozola-Serrano, Aguiló-Aguayo, et al., 2007; Odriozola-Serrano et al., 2009). Table 1 (assay 12) shows that a HIPEF treatment set up at 35 kV/cm for 2050 μs with 7-μs bipolar pulses at 250 Hz did not affect the antioxidant capacity of watermelon juice. On the other hand, watermelon juice treated at 35 kV/cm for 2050 μs with 1-μs monopolar pulses at 50 Hz exhibited the lowest antioxidant capacity retention (78%) (Table 1, assay 21). In contrast with our results, nonsignificant differences were detected between HIPEF-treated and fresh orange juices, within a range of processing conditions similar to the assayed in the present study (Elez-Martínez & Martín-Belloso, 2007; Sánchez-Moreno et al., 2005).

The proposed second-order model is adequate to fit the experimental data ($p < 0.001$), showing no significant lack of fit and satisfactory values of R^2 (0.76). Electric field strength, frequency, pulse width and polarity significantly ($p < 0.05$) affected antioxidant capacity retention, similarly to what has been described for lycopene retention (Table 2). The higher the pulse width, the greater the antioxidant capacity retention of HIPEF-treated watermelon. In agreement with other studies carried out in tomato and strawberry juices (Odriozola-Serrano, Aguiló-Aguayo, et al., 2007; Odriozola-Serrano et al., 2009), bipolar treatments were substantially more effective than monopolar treatments in maintaining antioxidant capacity of watermelon juice (Fig. 3). Antioxidant capacity retention rose when both the frequency and pulse width were increased from 50 to 200 Hz and from 1 to 7 μs, respectively. In addition, antioxidant capacity retention was the lowest at 30 kV/cm and the highest at about 35 kV/cm. Thus, antioxidant capacity retention was best maintained after HIPEF treatment at 200 Hz and 35 kV/cm applying 7 μs bipolar pulses for 1050 μs (Fig. 3). The antioxidant capacity is related to the amount and composition of bioactive compounds present in food (Sánchez-Moreno et al., 2005). Watermelon contains small amounts of vitamin C and phenolic compounds, whereas it has been reported to be an excellent source of carotenoids. In fact, a 75% of the total carotenoid content in some watermelon cultivars was lycopene (Gil et al., 2006). In this study, the antioxidant capacity of HIPEF-treated watermelon juice could be mainly attributed to lycopene content, as antioxidant capacity retention was highly correlated with lycopene relative content ($R^2 = 0.964$).

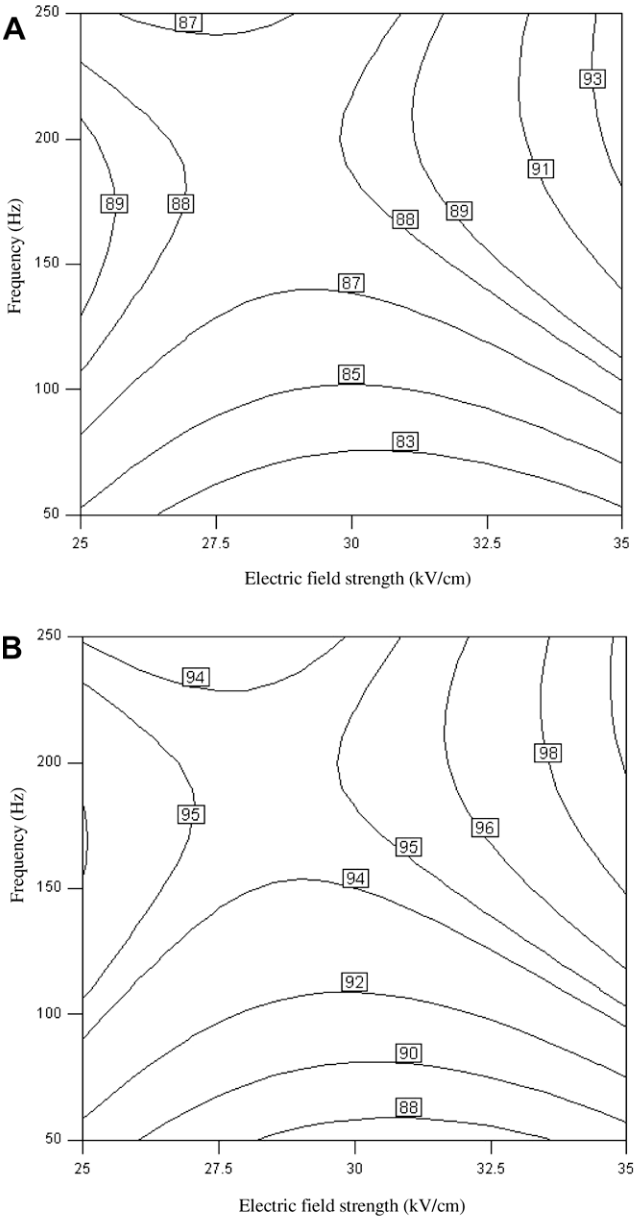

Fig. 3. Effect of pulse frequency and electric field strength on antioxidant capacity relative content (%) of watermelon juice treated with high-intensity pulse electric fields (HIPEF) applying 7-μs monopolar (A) or bipolar (B) pulses for 1050 μs.

Table 4
Optimal high-intensity pulsed electric field conditions to achieve maximal lycopene, vitamin C and antioxidant capacity in HIPEF-treated watermelon juice.

HIPEF parameters					Variables			Desirability
E (kV/cm)	f (Hz)	τ (μs)	t (μs)	p	Lycopene retention (%)	Vitamin C retention (%)	Antioxidant capacity retention (%)	
35	200	7	50	Bipolar	113	72	100	0.679
35	200	4	50	Bipolar	111	77	98	0.674
25	175	7	50	Bipolar	112	73	99	0.662
25	150	7	215	Bipolar	107	87	96	0.661

E: electric field strength; f: frequency; τ: pulse width; t: treatment time.

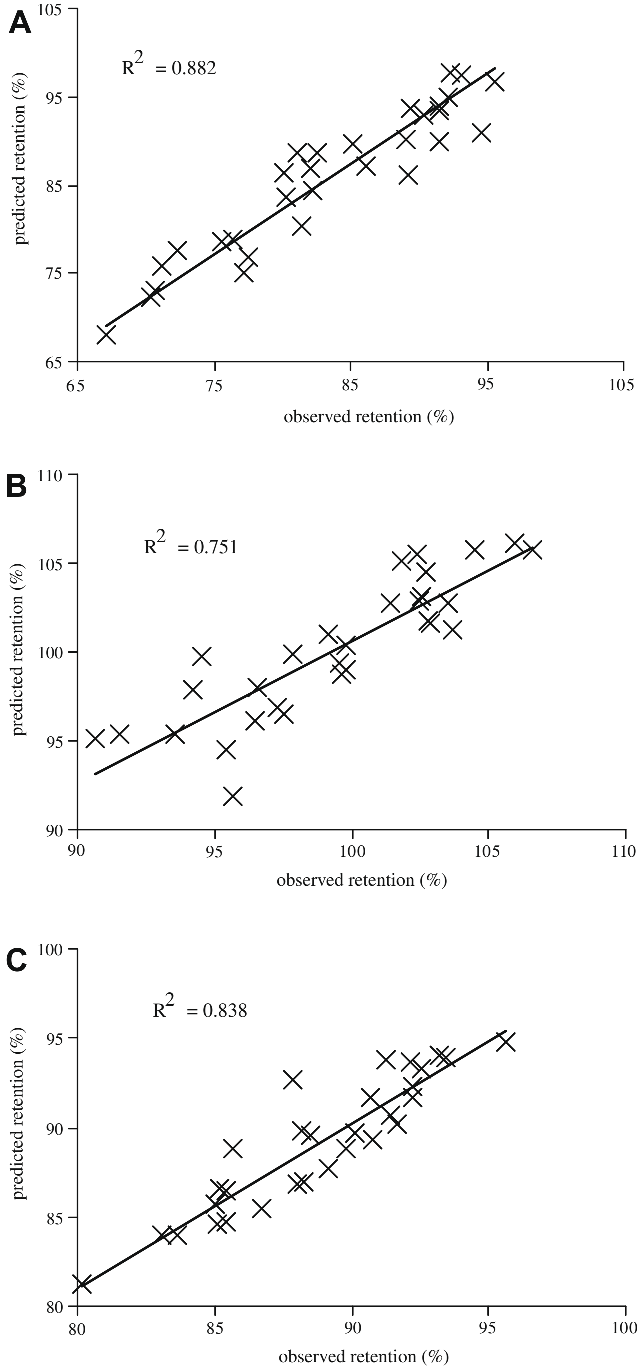

Fig. 4. Scatter plots of the observed and the predicted values for vitamin C (A), lycopene (B) and antioxidant capacity (C) retention in HIPEF-treated watermelon juice.

3.4. Optimal HIPEF treatments for watermelon juice and model validation

Among the studied health-related parameters, vitamin C seems to be more affected by intense HIPEF treatments than lycopene and antioxidant capacity. The combination of HIPEF critical parameters that lead to watermelon juices with the highest health-related compound content was determined. The same priority was assigned to each dependent variable in order to obtain watermelon juices with maximal retention of lycopene, vitamin C and antioxidant capacity. Maximal lycopene (113%), vitamin C (72%) and antioxidant capacity (100%) were obtained when applying 35 kV/cm for 50 μs using 7-μs squared wave bipolar pulses at 200 Hz. Nevertheless, high desirability was also obtained combining high frequencies and pulse width at 25 or 35 kV/cm using bipolar mode (Table 4).

To complete the study, a set of 30 experiments were carried out to validate the prognostics of the developed predictive models. Fig. 4 shows that the proposed predictive models fit with enough accuracy of the experimental results. The correlation coefficients between observed and predicted values were 0.882, 0.751 and 0.838 for vitamin C, lycopene and antioxidant capacity retention, respectively.

4. Conclusions

Electric field strength, pulse frequency, pulse width and polarity significantly affected lycopene, vitamin C and antioxidant capacity of watermelon juice. Moreover, vitamin C retention seemed to be also affected by treatment time. Although the use of bipolar pulses enhanced lycopene and antioxidant capacity of watermelon juice, their application decreased vitamin C retention in comparison with the application of monopolar pulses. The higher the severity of HIPEF-processing parameters, the lower the retention of vitamin C in watermelon juice. On the other hand, the maximum values of lycopene and antioxidant capacity were obtaining at high electric field strengths (25 and 35 kV/cm) and frequencies around 200 Hz, applying bipolar 4–7 μs pulses. As watermelon juice has been shown to be an excellent source of lycopene and antioxidant capacity is highly related to its content, both parameters could be used during storage to assure a juice with high antioxidant potential.

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References

Aguiló-Aguayo, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2008). Effects of thermal and high-intensity pulsed electric field treatments on quality related enzymes of watermelon juices. In *IFT annual meeting*, New Orleans, USA, Paper 135-03.

Cortés, C., Esteve, M. J., Rodrigo, D., 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.

Davey, M. W., Van Montagu, M., Inze, D., Sanmartin, M., Kanellis, A., Smirnoff, N., et al. (2000). Plant L-ascorbic: Chemistry, function, metabolism, bioavailable and effects of processing. *Journal of the Science of Food and Agriculture, 80*, 825–860.

Davis, A. R., Fish, W. W., & Perkins-Veazie, P. (2003). A rapid spectrophotometric method for analyzing lycopene content in tomato and tomato products. *Postharvest Biology and Technology, 28*(3), 425–430.

Derringer, G., & Suich, R. (1980). Simultaneous optimization of several response variables. *Journal of Quality Technology, 12*, 214–219.

Elez-Martinez, P., Aguiló-Aguayo, I., & Martín-Belloso, O. (2006). Inactivation of orange juice peroxidase by high-intensity pulsed electric fields as influenced by process parameters. *Journal of the Science of Food and Agriculture, 86*, 71–81.

Elez-Martínez, P., & Martín-Belloso, O. (2007). 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 Chemistry, 102*, 201–209.

Fraser, P. D., & Bramley, P. M. (2004). The biosynthesis and nutritional uses of carotenoids. *Progress in Lipid Research, 43*, 228–265.

Fraser, P. D., Truesdale, M. R., Bird, C. R., Schuch, W., & Bramley, P. M. (1994). Carotenoid biosynthesis during tomato fruit development (evidence for tissue-specific gene expression). *Plant Physiology, 105*, 405–413.

Gardner, P. T., White, T. A. C., McPhail, D. B., & Duthie, G. G. (2000). The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chemistry, 68*, 471–474.

Gil, M. I., Aguayo, E., & Kaer, A. A. (2006). Quality changes and nutrient retention in fresh-cut versus whole fruits during storage. *Journal of Agricultural and Food Chemistry, 54*, 4284–4296.

Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Holcroft, M., & Kader, A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food Chemistry, 48*, 4581–4589.

Giovannucci, E. (2002). A review of epidemiologic studies of tomatoes, lycopene, and prostate cancer. *Experimental Biology and Medicine, 227*, 852–859.

Mosqueda-Melgar, J., Raybaudi-Massilia, R. M., & Martín-Belloso, O. (2007). Influence of treatment time and pulse frequency on *Salmonella enteritidis*, *Escherichia coli* and *Listeria monocytogenes* populations inoculated in melon and watermelon juices treated by pulsed electric fields. *International Journal of Food Microbiology, 117*(2), 192–200.

Nicoli, M. C., Anese, M., & Parpinel, M. (1999). Influence of processing on the antioxidant properties of fruit and vegetables. *Trends in Food Science and Technology, 10*(3), 94–100.

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., Hernández-Jover, T., & Martín-Belloso, O. (2007). Comparative evaluation of UV–HPLC methods and reducing agents to determine vitamin C in fruits. *Food Chemistry, 105*, 1151–1158.

Odriozola-Serrano, I., Soliva-Fortuny, R. C., Gimeno-Añó, V., & Martín-Belloso, O. (2008). Kinetic study of anthocyanins, vitamin C, and antioxidant capacity in strawberry juices treated by high-intensity pulsed electric fields. *Journal of Agricultural and Food Chemistry, 56*, 8387–8393.

Odriozola-Serrano, I., Soliva-Fortuny, R. C., & Martín-Belloso, O. (2009). Impact of high-intensity pulsed electric fields variables on vitamin C, anthocyanins and antioxidant capacity of strawberry juice. *LWT – Food Science and Technology, 42*, 93–100.

Perkins-Veazie, P., Collins, J. K., Pair, S. D., & Roberts, W. (2001). Lycopene content differs among red-fleshed watermelon cultivars. *Journal of the Science of Food and Agriculture, 81*, 983–987.

Robertson, G. L., & Samaniego, C. M. L. (1986). Effect of initial dissolved oxygen levels on the degradation of ascorbic acid and the browning of lemon juice during storage. *Journal of Food Science, 514*, 184–187.

Sánchez-Moreno, C., Plaza, L., De Ancos, B., & Cano, P. (2003). Quantitative bioactive compounds assessment and their relative contribution to the antioxidant capacity of commercial orange juice. *Journal of the Science of Food and Agriculture, 83*, 430–439.

Sánchez-Moreno, C., Plaza, L., Elez-Martínez, P., De Ancos, B., Martín-Belloso, O., & Cano, M. P. (2005). Impact of high pressure and pulsed electric fields on bioactive compounds and antioxidant activity of orange juice in comparison with traditional thermal processing. *Journal of Agricultural and Food Chemistry, 53*, 4403–4409.

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.

Tadmor, Y., King, S., Levi, A., Davis, A., Meir, A., Wasserman, B., et al. (2005). Comparative fruit coloration in watermelon and tomato. *Food Research International, 38*, 837–841.

Tannenbaum, S. R., Archer, M. C., & Young, V. R. (1985). Vitamins and minerals. In O. R. Fennema (Ed.), *Food chemistry* (pp. 488–493). New York: Marcel Dekker.

Tibble, D. L. (1998). Further evidence of the cardiovascular benefits of diets enriched in carotenoids. *American Journal of Clinical Nutrition, 68*, 521–522.

Torregrosa, F., Esteve, M. J., Frígola, A., & Cortés, C. (2006). Ascorbic acid stability during refrigerated storage of orange–carrot juice treated by high pulsed electric field and comparison with pasteurized juice. *Journal of Food Engineering, 73*, 339–345.

Williamson, G. (1999). Protective effects of fruits and vegetables in the diet. *Nutrition and Food Science, 1*, 6–10.