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Kinetic Study of Anthocyanins, Vitamin C, and Antioxidant Capacity in Strawberry Juices Treated by High-Intensity Pulsed Electric Fields

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A kinetic study of anthocyanins, vitamin C, and antioxidant capacity was carried out in strawberry juice treated with high-intensity pulsed electric fields. Samples were subjected to electric field strengths from 20 to 35 kV/cm for up to 2000 μs applying 1 μs bipolar pulses at 232 Hz. The suitability of simple first-order kinetics and an empirical model based on Weibull distribution function to describe changes in experimental data are discussed. In addition, different secondary models relating the antioxidant property retention to the electric field strength and treatment time are given. The Weibull kinetic model was the most accurate ($R^2_{adj} \geq 0.727$) to predict anthocyanins, vitamin C, and antioxidant capacity changes in strawberry juice through the HIPEF treatment time. The combined effect of treatment time and electric field strength on health-related compounds of strawberry juice was successfully predicted ($R^2_{adj} \geq 0.874$) through secondary expressions. The proposed models are useful to predict the variation of the antioxidant potential of strawberry juice with the key parameters involved in HIPEF treatments.

KEYWORDS: Kinetics; high intensity pulsed electric fields; strawberry juice; anthocyanins; vitamin C; antioxidant capacity

INTRODUCTION

Strawberries and strawberry based-products are a good source of vitamin C and anthocyanins, thus having high antioxidant capacities (*1*). It is well-known that anthocyanins are unstable pigments and can be decolorized and degraded by many factors such as temperature, pH, oxygen, enzymes, light, the presence of copigments and metallic ions, ascorbic acid, sulfur dioxide, and sugars (*2, 3*). Vitamin C is a thermolabile vitamin, which is oxidized to nonantioxidant effective substances in the presence of oxygen (*4*). Heat processing is the most common method used to extend the shelf life of juices, but it is one of the most important factors influencing the stability of anthocyanins and vitamin C. According to Nicoli et al. (*5*), the antioxidant capacity of foods may be affected by processing in several ways, including losses of water-soluble antioxidants such as phenolic compounds, alterations in the compounds that improve or reduce the antioxidant capacity of plant constituents, as well as formation of novel compounds by Maillard or other reactions that affect antioxidant activity. Therefore, the market demand for fresh-like juices with high nutritional content has increased the awareness of the food industry for the development of milder preservation technologies to replace the existing pasteurization methods (*6*). HIPEF processing (35 kV/cm for 1700 μs in bipolar 4-μs pulses at 100 Hz) has been shown to effectively inactivate microorganisms in strawberry juice, thus leading to microbial inactivation levels similar to those achieved with heat pasteurization (*7*). In addition, enzymes such as lipoxygenase, which is involved in the formation of undesirable flavor compounds in strawberry juice, can be partially or totally reduced (*8*). Up to now, the studies evaluating the effects of HIPEF processing conditions on juices have demonstrated that electric field strength and treatment time are important variables to be controlled in order to optimize the inactivation of microorganisms (*9, 10*), enzymes (*11, 12*), and health-related compounds (*13, 14*) by HIPEF. Therefore, to evaluate the influence of HIPEF processing on vitamin C, anthocyanins and antioxidant capacity of juices is important for the consumer, particularly with regard to recommended daily servings. This information is also of interest to processors who wish to retain or enhance health-related compound levels in their products.

However, several models have been used to describe the microbial destruction (*9, 15*) and enzymatic inactivation (*12, 16*) as a function of the HIPEF critical parameters. Although retention of health-related compounds can be a limiting factor when defining process conditions, there are few works modeling the changes in concentration of antioxidant compounds as affected by HIPEF treatment parameters (*17−19*). Therefore, the aim of this work was to propose mathematical models that properly relate changes in the content of health-related com-

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pounds, namely, anthocyanins and vitamin C, as well as antioxidant capacity of strawberry juice to electric field strength and HIPEF treatment time.

MATERIALS AND METHODS

Reagents. Metaphosphoric acid, DL-1,4 -dithiotreitol (DTT) were purchased from Acros Organics (NJ, USA); potassium chloride, sodium acetate, ascorbic acid, sulfuric acid, methanol, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Scharlau Chemie, SA (Barcelona, Spain).

Sample Preparation. Strawberry fruits (*Fragaria ananassa* Duch, cultivar Camarosa) were purchased from a local supermarket (Lleida, Spain). The fruits were washed, drained, and chopped. Then, the squeezed strawberry juice was centrifuged at 24,000 × *g* for 15 min, and the supernatant was filtered using a steel sieve with an approximate mesh of 2 mm. Electric conductivity (Testo 240 conductivimeter; Testo GmBh & Co, Lenzkirch, germany), pH (crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), soluble solid content (Atago RX-1000 refractometer; Atago Company Ltd., Japan), and color (Macbeth-Kollmorgen inst. Corp., Newburg, NY) of strawberry juice were determined. Analytical characteristics of fresh strawberry juice were soluble solids 7.8 ± 0.1 °Brix, electric conductivity 0.41 ± 0.21 S/m, pH 3.45 ± 0.22, and color L* = 18.76 ± 0.25, a* = 5.32 ± 0.12, and b* = 2.25 ± 0.1 (results are expressed as the mean ± standard deviation).

Pulsed Electric Field Equipment. HIPEF treatments were carried out in a continuous flow bench scale system (OSU-4F, Ohio State University, Columbus, OH, USA). The treatment system consists of eight collinear chambers in series, each one with two stainless steel electrodes separated by a gap of 0.29 cm, thus defining a treatment volume of 0.012 cm^3. The flow rate of the process was adjusted to 60 mL/min and controlled with a variable speed pump (model 752210−25, Cole Palmer Instrument Company, Vermon Hills, IL, USA). A cooling coil was connected between each pair of chambers and submerged in an ice−water shaking bath. Product outlet temperatures never exceeded 40 °C. Samples of strawberry juice were subjected to field strengths of 20, 25, 30, and 35 kV/cm during 100, 300, 600, 1000, 1500, and 2000 μs, using 1 μs square-wave bipolar pulses at 232 Hz. Treatment conditions were selected according to a previous study (*20*). Each processing condition was assayed in duplicate, and two replicate analyses were carried out in order to obtain the mean value.

Anthocyanins. The anthocyanin content of strawberry juice was determined with a modified pH differential method described by Meyers et al. (*21*), using two buffer systems: 0.025 M potassium chloride at pH 1, and 0.4 M sodium acetate at pH 4.5. Briefly, 5 mL of strawberry juice was transferred to a 50 mL volumetric flask and made up with each buffer. The absorbance of the mixtures at pH 1 and 4.5 was then measured with a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) at 510 and 700 nm against a blank of distilled water. The anthocyanin content was calculated according to eq 1 and expressed as pelargonidin-3-glucoside per liter:

$$TA = \frac{[(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}] \cdot MW \cdot DF \cdot 1000}{\varepsilon \cdot L} \quad (1)$$

where *MW* is the molecular weight of pelargonidin-3-glucoside (433.0 g/mol), *DF* is the dilution factor, *L* is the path length in cm, and ε is the molar extinction coefficient for pelargonidin-3-glucoside (22400 L/mol·cm). Results were expressed as pelargonidin-3- glucoside content (plg-3-glu) compared to the untreated sample.

Vitamin C. Vitamin C content of strawberry juice was analyzed by HPLC. The extraction procedure was based on a method validated by Odriozola-Serrano et al. (*22*). A sample of 25 mL of juice was mixed with 25 mL of a solution containing 45 g/L metaphosphoric acid and 7.2 g/L of DTT. The homogenate was centrifuged at 22,100 × *g* for 15 min at 4 °C (Centrifuge Avanti J-25, Beckman Instruments Inc., Fullerton, CA, USA). The supernatant was vacuum-filtered through Whatman No. 1 paper. Then, the samples were filtered with a Millipore 0.45 μm membrane. An aliquot of 20 μL was injected into the HPLC system consisting of a reverse-phase C18 Spherisorb ODS2 (5 μm) stainless steel column (4.6 mm × 250 cm) and a 486 Absorbance Detector (Waters, Milford, MA). A 0.01% solution of sulfuric acid adjusted to pH 2.6 was used as eluent. The flow was isocratic at a rate of 1 mL/min at room temperature. Detection was performed at 245 nm. Identification of the ascorbic acid was carried out comparing the retention time and UV−visible absorption spectrum of the juice samples with those of the standards. Results were expressed as vitamin C retention related to the untreated sample.

Antioxidant Capacity. The antioxidant capacity of strawberry juice was studied through the evaluation of free radical-scavenging effect on 1,1-diphenyl-2-picrylhydrazyl radical, according to the method described by Odriozola-Serrano et al. (*20*). Samples of strawberry juice were centrifuged at 6000 × *g* for 15 min at 4 °C (Centrifuge Medigifer; Select, Barcelona, Spain), and aliquots of 0.01 mL of the supernatant were mixed with 3.9 mL of methanolic DPPH (0.025 g/L) and 0.090 mL of distilled water. The homogenate was shaken vigorously and kept in darkness for 60 min. Absorption of the samples was measured with a spectrophotometer at 515 nm against a blank of methanol without DPPH. Results were expressed as antioxidant capacity retention related to the untreated sample.

Kinetic Models. The fitting of several kinetic models to experimental data, namely, the simple first-order model (eq 2) and Weibull distribution function (eq 3), was evaluated in order to properly relate health-related compounds and antioxidant capacity retention in strawberry juices to HIPEF processing parameters.

Simple First-Order Model. Simple first-order kinetics have been commonly used to fit thermal degradation of anthocyanins (*23*−*25*) and vitamin C (*26, 27*) in juices and nectars as a function of treatment time. In addition, Zhang et al. (*19*) observed that the degradation of cyanidin-3-glu in methanolic solution exposed to PEF treatments at 1.2−3 kV/cm ($T^a \leq 47$ °C) for 20−140 μs was well fitted by simple first-order reactions. In this way, Bendicho et al. (*17*) proposed a first-order model to describe the vitamin C changes in milk as affected by HIPEF treatment time.

$$RA = RA_0 \cdot \exp(-k_1 \cdot t) \quad (2)$$

where *RA* (%) is the relative antioxidant property, RA_0 (%) is the intercept of the curve, k_1 is the first-order kinetic constant (μs^{-1}), and *t* is the treatment time (μs).

Weibull Model. Weibull distribution (eq 3) has been used to describe the destruction of microorganisms (*15*) and enzyme inactivation (*16, 28, 29*) under HIPEF. The use of Weibull distribution function to describe the retention of tomato health-related compounds and antioxidant capacity has been reported by Odriozola-Serrano et al. (*30*)

$$RA = RA_0 \exp\left[-\left(\frac{t}{\alpha}\right)^{\gamma}\right] \quad (3)$$

where *RA* (%) is the relative antioxidant property, RA_0 (%) is the intercept of the curve, *t* is the treatment time (μs), α is the scale factor (μs), and γ is the shape parameter that indicates concavity (tail-forming) or convexity (shoulder-forming) of the curve when it takes values below and above 1, respectively. Derived from the Weibull distribution function parameters (α, γ), t_m is defined as the mean processing time to achieve complete destruction/inactivation of the health-related compound or antioxidant capacity and can be used as a measurement of the resistance of these compounds to HIPEF treatments (eq 4):

$$t_m = \alpha \cdot \Gamma\left(1 + \frac{1}{\gamma}\right) \quad (4)$$

where α and β are the parameters of the Weibull distribution, and Γ is the gamma function.

Secondary Models. Additionally, kinetic rate constants obtained for each model were related, when possible, to the applied electric field strength through mathematical expressions. The combined effect of electric field strength and treatment time on strawberry juice antioxidant properties was described by rearranging these mathematical expressions into the best kinetic model for each compound.

Statistical Analysis. Each processing condition was assayed in duplicate, and two replicate analyses were carried out in order to obtain the mean value. The analysis of variance was carried out with

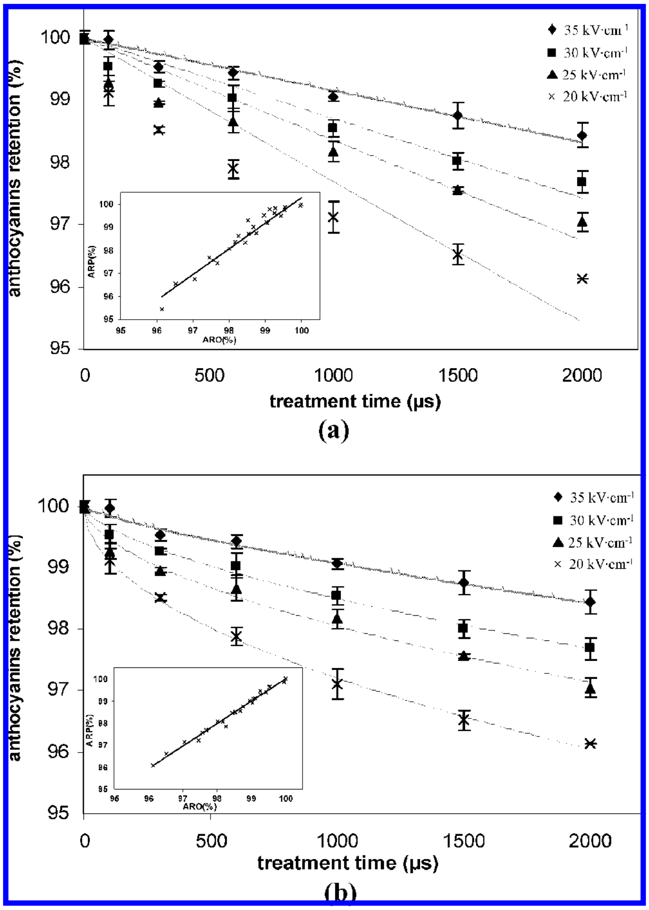

Figure 1. Effect of treatment time and electric field strength on the anthocyanin retention of strawberry juice (mean ± SD) as modeled by the simple first-order model (**a**) and Weibull approach (**b**). Treatments were performed at 232 Hz and square bipolar pulses of 1-μs. ARO, anthocyanin retention (observed); ARP, anthocyanin retention (predicted).

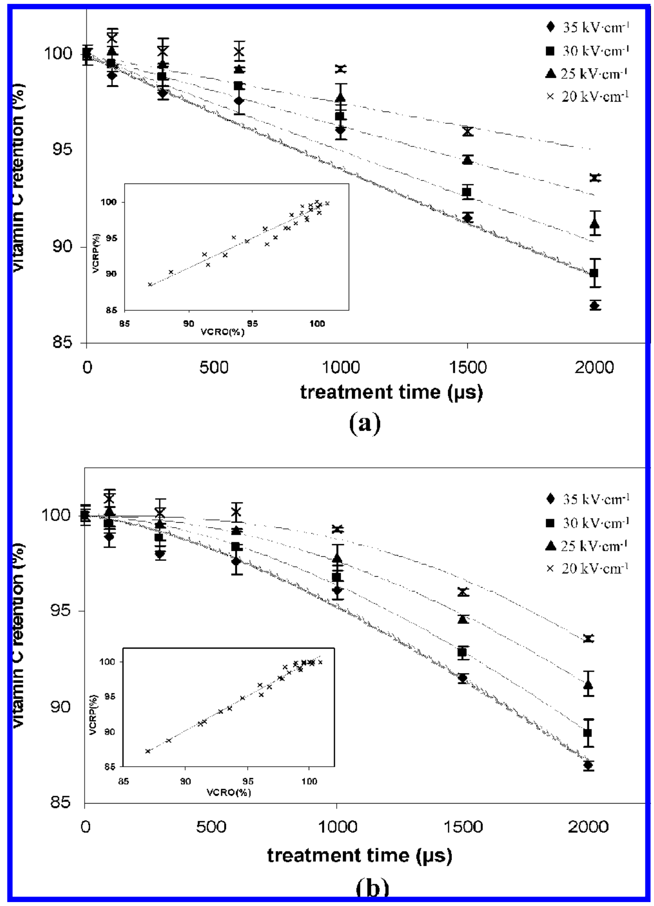

Figure 2. Effect of treatment time and electric field strength on the vitamin C retention of strawberry juice (mean ± SD) as modeled by the simple first-order model (**a**) and Weibull approach (**b**). Treatments were performed at 232 Hz and square bipolar pulses of 1-μs. VCRO, vitamin C retention (observed); VCRP, vitamin C retention (predicted).

Statgraphics Plus v.5.1 Windows package (Statistical Graphics Co., Rockville, MD). The least significant difference test was used to determine differences between treatments at a 5% significance level. The models were fitted to experimental data by nonlinear regression procedures, using the Statgraphics Plus v.5.1 Windows package. Estimated parameters are given of the estimates by the Student's-*t* adjusted at the corresponding degree of freedom. The adjusted regression coefficients (R^2_{adj}) and the statistical parameters, root-mean-square error (RMSE), reduced chi-square (χ^2) and mean bias error (MBE) were calculated to evaluate the fitting of a model to experimental data (*31*). The higher the values of R^2_{adj} and the lower values of RMSE, χ^2 and MBE, the better the models fit the experimental data.

RESULTS AND DISCUSSION

Anthocyanins. The total concentration of anthocyanins, expressed as pelargonidin-3-glucoside, in fresh strawberry juice was 27.3 ± 0.3 mg/100 mL. As can be seen in **Figure 1**, the total anthocyanin retention ranged between 96.1% and 100.5% in HIPEF-processed juice treated under the studied experimental conditions. Lower retentions (80%−94%) of cyanidin-3-glucoside (cyn-3-glu) in a methanolic solution were obtained after carrying out less intense HIPEF treatment conditions (1.2, 2.2 and 3.0 kV/cm, 300 numbers of pulses, $T^a \leq 47$ °C) than those reported in the present study (*19*). Differences in anthocyanins due to HIPEF treatments can be attributed not only to factors intrinsic to the food product but also to other factors such as HIPEF system, treatment chambers, pulse characteristics, and electrical conditions. Anthocyanin content significantly depended on the HIPEF treatment time and electric field strength applied during HIPEF-processing of the samples. The lower the treatment time and the higher the electric field strength, the greater the anthocyanin retention (**Figure 1**). Contrarily, Zhang et al. (*19*) reported that the degradation of cy-3-glu in a methanolic solution increased as the electric field strength rose. Not all anthocyanins appear to react equally regarding their resistance to the degrading effects of various agents (*32*). In addition, intensive HIPEF treatments might stimulate the degradation of proanthocyanins to anthocynins. It has been reported that proanthocyanins are converted into anthocyanins after treatments at high temperature in acidic water-free conditions (*33*−*35*). Although the maximum temperature reached in the sample during HIPEF treatments at 35 kV/cm was 37 °C, the stress produced by HIPEF-processing at high electric field strength might explain this transformation. Further investigations are still needed to explain the mechanisms that mediate this HIPEF-induced conversion of proanthocyanins to anthocynins.

The kinetic constants estimated by the simple first-order and Weibull models as well as the adjusted determination coefficients, RMSE, χ^2, and MBE, of the fitted models at different electric field strengths are shown in **Table 1**. On the basis of these coefficients, it can be observed that the two models predicted well the relationship between anthocyanin retention and HIPEF treatment time irrespective of electric field strength. However, the Weibull approach exhibits the highest R^2_{adj} and the lowest RMSE, χ^2, and MBE. Furthermore, the deviations in the values estimated by a first-order model in relation to the observed values are higher than

Table 1. Results of the Statistical Analyses on the Kinetic Changes of Anthocyanins in Strawberry Juices Subjected to High Intensity Pulsed Electric Fields

		statistical parameters					
model	E (kV/cm)	constants[a]		R^2_{adj}	RMSE	χ^2	MBE
first-order	20	$k_1 = 2.33 \times 10^{-5}\ \mu s^{-1}$		0.771	0.443	0.141	0.097
	25	$k_1 = 1.66 \times 10^{-5}\ \mu s^{-1}$		0.817	0.373	0.134	0.094
	30	$k_1 = 1.30 \times 10^{-5}\ \mu s^{-1}$		0.846	0.310	0.054	0.060
	35	$k_1 = 8.39 \times 10^{-6}\ \mu s^{-1}$		0.731	0.196	0.004	0.017
Weibull	20	$\alpha = 11.33 \times 10^5\ \mu s$	$\gamma = 5.07 \times 10^{-1}$	0.959	0.248	0.040	0.051
	25	$\alpha = 10.91 \times 10^5\ \mu s$	$\gamma = 5.62 \times 10^{-1}$	0.930	0.205	0.0005	0.006
	30	$\alpha = 7.56 \times 10^5\ \mu s$	$\gamma = 6.32 \times 10^{-1}$	0.912	0.161	0.0002	0.004
	35	$\alpha = 4.12 \times 10^5\ \mu s$	$\gamma = 7.76 \times 10^{-1}$	0.727	0.147	0.0001	0.003

[a] Mean values. k_1, first-order rate constant; α, scale factor; γ, shape factor; R^2_{adj}, adjusted determination coefficient; RMSE, root mean square error; χ^2, reduced chi-square; MBE, mean bias error.

Table 2. Results of the Statistical Analyses on the Kinetic Changes of Vitamin C in Strawberry Juices Subjected to High Intensity Pulsed Electric Fields

		statistical parameters					
model	E (kV/cm)	constants[a]		R^2_{adj}	RMSE	χ^2	MBE
first-order	20	$k_1 = 2.55 \times 10^{-5}\ \mu s^{-1}$		0.752	0.717	1.102	−0.270
	25	$k_1 = 3.78 \times 10^{-5}\ \mu s^{-1}$		0.869	0.647	0.650	−0.207
	30	$k_1 = 5.12 \times 10^{-5}\ \mu s^{-1}$		0.909	0.625	0.374	−0.157
	35	$k_1 = 6.08 \times 10^{-5}\ \mu s^{-1}$		0.920	0.635	0.106	−0.083
Weibull	20	$\alpha = 7.49 \times 10^3\ \mu s$	$\gamma = 1.50$	0.955	0.461	0.196	−0.114
	25	$\alpha = 6.94 \times 10^3\ \mu s$	$\gamma = 1.70$	0.974	0.282	0.003	−0.014
	30	$\alpha = 6.81 \times 10^3\ \mu s$	$\gamma = 1.94$	0.969	0.344	0.056	0.061
	35	$\alpha = 5.83 \times 10^3\ \mu s$	$\gamma = 2.50$	0.934	0.493	0.218	0.120

[a] Mean values. k_1, first-order rate constant; α, scale factor; γ,: shape factor. R^2_{adj}, adjusted determination coefficient; RMSE, root mean square error; χ^2, reduced chi-square; MBE: mean bias error.

those estimated by the Weibull model (**Figure 1**). A simple first-order model was used by Zhang et al. (*19*) to describe degradation of cy-3-glu in a methanolic solution. The authors obtained k-values ranging from 4.2×10^{-7} to $1.1 \times 10^{-6}\ \mu s^{-1}$ for cy-3-glu using electric field strengths between 1.2 and 3.0 kV/cm and HIPEF treatment times up to 140 μs. In our study, kinetic rates defined by the simple first-order model ranged from 8.39×10^{-6} to $2.33 \times 10^{-5}\ \mu s^{-1}$, which suggests that the resistance of anthocyanins to HIPEF processing is quite similar when treating strawberry juice or a methanolic solution.

The scale parameter (α) and the shape parameter (γ) of the Weibull model were obtained by fitting eq 3 to the experimental data. The γ parameter took values of 5.07×10^{-1} to 7.76×10^{-1}, and the electric field strength dependency was found to be exponential ($R^2_{adj} = 0.972$) in the range of the applied conditions (eq 5). Values of γ below 1 could be regarded as evidence that a great amount of anthocyanins were degraded at a relatively fast rate leaving behind anthocyanins with higher resistance to HIPEF treatment. This trend is in accordance with the fact that anthocyanin stability is influenced by the composition of the food matrix, as certain compounds such as flavonoids and phenolic acids can react with them enhancing their resistance to different stresses (*36*). However, estimated α values decreased when the electric field strength increased, following a trend that could be described with good accuracy ($R^2_{adj} = 0.873$) using a simple first-order model (eq 6). According to our results, the studies performed with enzymes showed that the plots of the shape and scale factor obtained from the Weibull model adjustment versus the applied electric field strength matched an exponential trend with good agreement (*29*). Mean time (t_m) can be defined as the mean processing time to completely destroy the anthocyanin content of strawberry juice. The values of t_m were calculated from eq 4 and varied from 959 to 3368 ms. Therefore, it may be assumed that total anthocyanin degradation by HIPEF treatments in the range of the studied electric field strength will not be reached since these prolonged treatments are not used in HIPEF processing.

$$\alpha = [3569110 \cdot \exp(-0.0536 \cdot E)] \quad (5)$$

$$\gamma = [0.2738 \cdot \exp(0.0292 \cdot E)] \quad (6)$$

The combined effect of treatment time and electric field strength on total anthocyanins of strawberry juice (AR) is described by eq 7, where α and γ in the Weibull model have been replaced by eqs 5 and 6, respectively.

$$AR(\%) = 100\left[-\left(\frac{t}{[3569110 \cdot \exp(-0.0536 \cdot E)]}\right)^{[0.2738 \cdot \exp(0.0292 \cdot E)]}\right] \quad (7)$$

The good fitting of the model is confirmed by the statistical parameters (RMSE = 0.389; $\chi^2 = 0.024$; MBE = 0.389) and the high adjusted determination coefficient ($R^2_{adj} = 0.937$).

Vitamin C. The vitamin C content of the untreated strawberry juice was 48.6 ± 0.4 mg/100 mL. The effect of HIPEF processing parameters on the concentration of vitamin C in strawberry juice is shown in **Figure 2**. High vitamin C retention (≥87%) with respect to the fresh juice was observed after the applied HIPEF-treatments. Sanchez-Moreno et al. (*37*) reported a vitamin C retention of 93% after processing orange juice at 35 kV/cm during 750 μs with bipolar pulses of 4-μs and 800 Hz. In this way, Evrendilek et al. (*38*) did not observe vitamin C degradation in apple juice subjected to a HIPEF treatment at 35 kV/cm for 94 μs with 1.92 μs monopolar pulses at 952 Hz. Vitamin C content significantly depended on the HIPEF treatment time and electric field strength. The lower the treatment time and electric field strength, the greater the vitamin C retention (**Figure 2**). Consistently, some authors (*13, 18*) reported an increase of vitamin C degradation when electric field strengths and treatment time rise in HIPEF-treated orange and orange-carrot juice, respectively. Oxidation of ascorbic acid occurs mainly during the processing of juices and depends upon many factors such as oxygen presence, heat, and light (*39*). 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 (*40*). The calculated first-order rate, Weibull parameters, and regression coefficients at different electric field strength are shown in **Table 2**. Up to now, several authors have studied the kinetics of vitamin C thermal degradation in several juices under pasteurization conditions and have stated that this vitamin depletion can be approached by simple first-order models (*26, 41*). However, in this study, the fitting performance of the simple first-order kinetic model was high for treatments with an electric field strength between 25 and 35 kV/cm (R^2_{adj} = 0.869−0.920) but dramatically decreased for treatments conducted at 20 kV/cm (R^2_{adj} = 0.752). The ascorbic acid degradation rates defined by the simple first-order model were statistically influenced by the electric field strength and took values in the range of 2.55×10^{-5} to 6.08×10^{-5} μs^{-1} for field strength between 20 and 35 kV/cm. Torregrosa et al. (*18*) obtained first-order rate constants from 9×10^{-3} to 2.2×10^{-2} μs^{-1} when modeling vitamin C changes in orange−carrot juice after HIPEF treatments of up to 340 μs and electric field strengths from 25 to 40 kV/cm. Thus, HIPEF treatments were found to achieve lower rates of vitamin C destruction in strawberry juice than in orange−carrot juice exposed to less intense conditions than those reported in the present study. As can be seen in **Table 2**, Weibull distribution seemed to be most suitable model to predict kinetic degradation of vitamin C regardless of treatment intensity. The high determination coefficients (0.934−0.974) and the low RMSE, χ^2 and MBE indicate that the Weibull model can be useful to relate vitamin C retention to HIPEF treatment time (**Table 2**). This good accuracy of the Weibull approach is confirmed by the small deviation in the values estimated by the model and the experimental data to the line of equivalence (**Figure 2**). The scale parameter (α) and the shape parameter (γ) of the Weibull distribution were obtained by fitting eq 3 to the experimental data. The γ parameter ranged from 1.5 to 2.5, suggesting that this vitamin became increasingly destroyed overtime. This behavior is in accordance with that observed by Odriozola-Serrano et al. (*30*) who obtained γ-values higher than 1 modeling vitamin C degradation in tomato juice affected by HIPEF processing at conditions similar to those reported in the present work. In addition, the γ parameter increased with electric field strength following a trend that fitted well an exponential equation (R^2_{adj} = 0. 952) in the range of the applied conditions (eq 8). However, α was statistically dependent on the electric field strength and took values from 5834 to 7489 μs. A simple first-order model was found to describe the electric field strength dependency of α (R^2_{adj} = 0.852) (eq 9).

$$\alpha = [4479 \cdot \exp(0.0149 \cdot E)] \quad (8)$$

$$\gamma = [4.879 \cdot \exp(-0.0348 \cdot E)] \quad (9)$$

Moreover, substitution of α and γ by into the Weibull distribution (eq 3), transforms the equation into a function dependent on both the electric field strength (E) and treatment time (t) (eq 10). The model performed well under the whole range of applied conditions, with high determination coefficients (R^2 = 0.948) and good accuracy (RMSE = 0.635; χ^2 = 0.0005; MBE = −0.0037).

$$VCR(\%) = 100 \cdot \exp\left[-\left(\frac{t}{[4479 \cdot \exp(0.0149 \cdot E)]}\right)^{[4.879 \cdot \exp(-0.0348 \cdot E)]}\right] \quad (10)$$

It has been reported that vitamin C is a typical heat sensitive nutrient so that its retention is often considered as a significant marker of overall nutrient recovery (*42*). To establish HIPEF processing time to maximal destruction of vitamin C, t_m was calculated from eq 4 for the different electric field strengths. Values of t_m ranged from 8175 and 12481 μs, decreasing with electric field strength. These values are similar to those obtained by Odriozola-Serrano (*30*), who reported t_m-values varying from 7939 to 11252 μs for vitamin C in tomato juice treated by applying HIPEF conditions similar to those of the present work. These small differences in vitamin C stability between HIPEF-treated juices may be due to the lower pH of strawberry juice in comparison to that of tomato juice, as more acidic conditions are known to stabilize vitamin C (*43*).

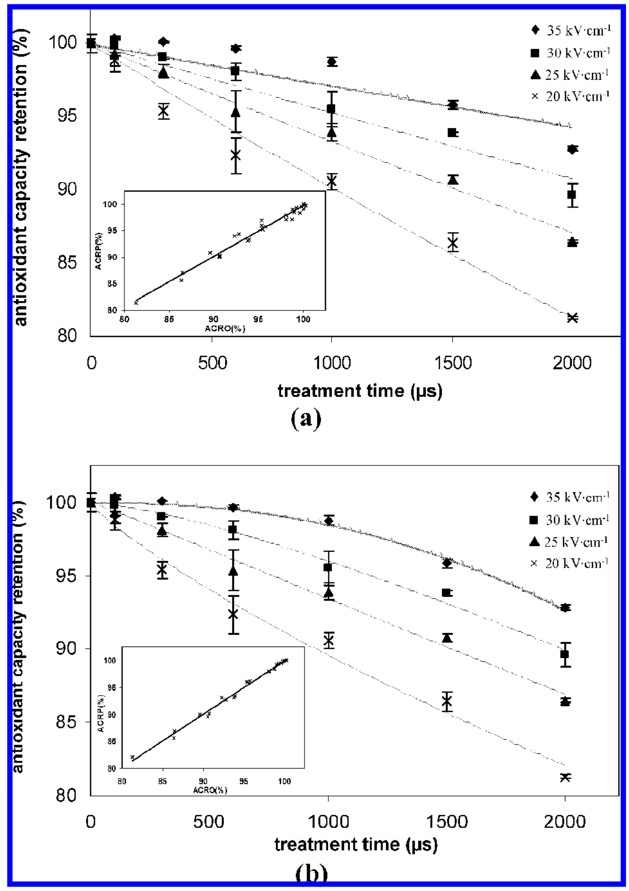

Figure 3. Effect of treatment time and electric field strength on the antioxidant capacity retention of strawberry juice (mean ± SD) as modeled by the simple first-order model (**a**) and Weibull approach (**b**). Treatments were performed at 232 Hz and square bipolar pulses of 1-μs. ACRO, antioxidant capacity retention (observed); ACRP, antioxidant capacity retention (predicted).

Antioxidant Capacity. The DPPH method has been proposed as an easy and accurate method for measuring the antioxidant activity of juice samples (*44*). In addition, the DPPH assay is not specific to any particular antioxidant component, thus determining the overall antioxidant capacity of the sample. The effect of HIPEF treatment conducted at 232 Hz with 1 μs bipolar pulses on the antioxidant capacity of strawberry juice at the assayed electric field strength and treatment time is shown in **Figure 3**. Antioxidant capacity of fresh strawberry juice was 39.3 ± 0.6% of DPPH inhibition. Antioxidant capacity of strawberry juice decreased significantly as electric field strength and treatment time increased (**Figure 3**). HIPEF treatment carried out at 20 kV/cm and 2000 μs led to the maximum antioxidant capacity degradation (18.7%). The results of the antioxidant capacity retention after HIPEF processing are in the range of those reported in the literature working with similar operation conditions but for different fruit juices (*13, 30*). The antioxidant capacity is related to the amount and composition

Table 3. Results of the Statistical Analyses on the Kinetic Changes of Antioxidant Capacity in Strawberry Juices Subjected to High Intensity Pulsed Electric Fields

		statistical parameters					
model	E (kV/cm)	constants[a]		R^2_{adj}	RMSE	χ^2	MBE
first-order	20	$k_1 = 1.04 \times 10^{-4}\ \mu s^{-1}$		0.973	0.572	0.351	0.153
	25	$k_1 = 6.91 \times 10^{-5}\ \mu s^{-1}$		0.990	0.428	0.001	−0.010
	30	$k_1 = 4.84 \times 10^{-5}\ \mu s^{-1}$		0.948	0.534	0.206	−0.117
	35	$k_1 = 2.91 \times 10^{-5}\ \mu s^{-1}$		0.814	0.667	0.765	−0.225
Weibull	20	$\alpha = 1.36 \times 10^4\ \mu s$	$\gamma = 8.46 \times 10^{-1}$	0.981	0.562	0.002	0.010
	25	$\alpha = 1.30 \times 10^4\ \mu s$	$\gamma = 1.05$	0.989	0.412	0.003	0.013
	30	$\alpha = 1.02 \times 10^4\ \mu s$	$\gamma = 1.38$	0.973	0.362	0.002	0.012
	35	$\alpha = 6.06 \times 10^3\ \mu s$	$\gamma = 2.34$	0.972	0.314	0.034	0.048

[a] Mean values. k_1, first-order rate constant; α, scale factor; γ, shape factor; R^2_{adj}, adjusted determination coefficient; RMSE, root mean square error; χ^2, reduced chi-square; MBE, mean bias error.

of bioactive compounds present in food (*37*). Vitamin C and anthocyanins are reported to be the major antioxidant components in strawberry juice (*1*). The magnitude of the changes in antioxidant capacity may be associated with health-related compound variation due to HIPEF processing. Strawberry juices with the highest antioxidant capacity had the greatest anthocyanin contents and low levels of vitamin C (**Figure 3**). HIPEF treatments applying high electric field strength led to strawberry juices with the greatest antioxidant capacity, although this HIPEF-treated juice had the lowest vitamin C contents. Therefore, these results seem to indicate that changes in antioxidant capacity might be mainly attributed to anthocyanin content rather than to vitamin C concentration.

The kinetic parameters of simple first-order and Weibull models at different electric field strengths are shown in **Table 3**. The adjusted regression coefficients depending upon the electric field strength were greater than 0.814; thus, there is a good correlation between antioxidant capacity degradation and HIPEF treatment time. However, the Weibull model exhibited lower RMSE, χ^2, and MBE values than the first-order model (**Table 3**). Furthermore, the deviations in the values estimated by the simple first-order model in relation to the observed values are higher than those obtained by the Weibull model (**Figure 3**). Therefore, from a quantitative point of view, data were best described by the Weibull function. The antioxidant capacity degradation rate was inversely influenced by the electric field strength, taking values between 2.91×10^{-5} and 1.04×10^{-4} μs^{-1}. In this way, both α and γ resulted in being significantly dependent on electric field strength. The higher the electric field strength, the greater the shape factor and the lower the scale factor. Values of mean time to complete loss of antioxidant capacity (t_m) were calculated according to eq 4 and varied from 8666 to 29662 μs. As no references have been found reporting the use of the Weibull equation to describe antioxidant capacity retention in HIPEF-treated products, Weibull parameters were compared with those reported in other studies for microorganisms (*45*) and enzymes (*12*) to have an idea of the resistance of the antioxidant properties to HIPEF treatments. Comparing t_m-values and according to the Weibull model, strawberry juice antioxidant capacity is shown to be less sensitive to HIPEF treatments than microorganisms, but more than enzymes.

The electric field strength dependency of α and γ estimated by the Weibull approach can be described by simple first-order equations. This model adequately fitted α (eq 11) and γ (eq 12) explaining 76.41% and 90.25% of the variability, respectively.

$$\alpha = [35052 \cdot \exp(-0.0441 \cdot E)] \quad (11)$$

$$\gamma = [0.158 \cdot \exp(0.0758 \cdot E)] \quad (12)$$

To estimate the antioxidant capacity retention (ACR) after HIPEF treatments of a given electric field strength (E) and treatment time (t) in the range of the applied conditions, a secondary model was developed by introducing eqs 11 and 12 in eq 3 (eq 13). The good accuracy of the secondary model was confirmed by the high adjusted determination coefficients ($R^2_{adj} = 0.874$) and the statistical parameters (RMSE = −0.428; $\chi^2 = 6.25$; MBE = 1.01).

$$ACR(\%) = 100 \cdot \exp\left[-\left(\frac{t}{[35052 \cdot \exp(-0.044 \cdot E)]}\right)^{[0.158 \cdot \exp(0.0758 \cdot E)]}\right] \quad (13)$$

In conclusion, the proposed mathematical models can be applied to engineering design to evaluate and optimize HIPEF processing conditions to produce strawberry juices with a high retention of bioactive compounds. The variation of the antioxidant potential of strawberry juice as affected by key parameters involved in HIPEF treatments can be described with good accuracy by secondary models.

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