JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Effects of Pulsed Electric Fields on the Bioactive Compound Content and Antioxidant Capacity of Tomato Fruit

Anna Vallverdú-Queralt,[†,‡] Gemma Oms-Oliu,[§] Isabel Odriozola-Serrano,[§] Rosa María Lamuela-Raventos,[†,‡] Olga Martín-Belloso,*[,§] and Pedro Elez-Martínez[§]

[†]Nutrition and Food Science Department, XaRTA, INSA, Pharmacy School, University of Barcelona, Av. Joan XXIII s/n, Barcelona 08028, Spain

[‡]CIBER CB06/03 Fisiopatología de la Obesidad y la Nutrición, (CIBEROBN), and RETICS RD06/0045/0003, Instituto de Salud Carlos III, Spain

[§]Department of Food Technology, University of Lleida, Rovira Roure 191, Lleida 25198, Spain

ABSTRACT: The effect of moderate intensity pulsed electric fields (MIPEF) on the bioactive compounds (total polyphenol, lycopene, and vitamin C content) as well as on the antioxidant capacity of tomato fruit was studied. The MIPEF treatment conditions were optimized to obtain tomato fruit with a high content of bioactive compounds. Tomato fruits were subjected to different electric field strengths (from 0.4 to 2.0 kV/cm) and number of pulses (from 5 to 30) and then immediately refrigerated at 4 °C for 24 h. A concentration of bioactive compounds higher than that of untreated tomatoes was obtained in MIPEF-treated tomatoes. A 44% increase in total polyphenol content was achieved under 30 pulses at 1.2 kV/cm. The hydrophilic antioxidant capacity was also enhanced by 44% applying 18 pulses at 1.2 kV/cm, and the lipophilic antioxidant capacity was increased by 37% under 5 pulses at 1.2 kV/cm. The maximum overall level of bioactive compounds and antioxidant capacity in the treated tomatoes was obtained under 16 pulses at 1 kV/cm. Therefore, MIPEF treatments could be considered an effective method to enhance the bioactive compound content and antioxidant potential of tomatoes.

KEYWORDS: *MIPEF treatments, lycopene, total polyphenols, antioxidant capacity, vitamin C, stress reactions*

INTRODUCTION

Diet-related chronic diseases have become a major public health concern due to their increasing prevalence in recent years. In this context, the consumption of tomatoes could be considered as a nutritional indicator of good dietary habits and healthy lifestyle due to the important health protecting role of their bioactive compounds, principally polyphenols, carotenoids, and vitamin C.[1,2] In particular, lycopene has been associated in epidemiological studies with a lower risk of prostate cancer.[3] The high content of polyphenols in tomatoes has also been gaining interest because of their multiple biological effects, including free-radical scavenging, inhibition of cellular proliferation, and modulation of enzymatic activity and signal transduction pathways.[4]

High intensity pulsed electric fields have been proposed as an alternative to conventional techniques of food preservation. Several studies have demonstrated the ability of high intensity pulsed electric fields to obtain shelf-stable liquid foods with high nutritional value by inactivating microorganisms and enzymes.[5] Other applications of pulsed electric fields technology are currently being developed. Moderate intensity pulsed electric fields (MIPEF) may cause lethal damage to cells or induce sublethal stress by permeabilizing tissue structures, thus improving intracellular metabolite extraction[5,6] and enhancing drying efficiency.[7] Recent studies have also suggested the possibility of using MIPEF to stress cells and thus stimulate the biosynthesis of secondary metabolites.[8] MIPEF affect the metabolism of vegetables with the consequent generation of reactive oxygen species (ROS).[9,10] ROS are endogenous signal components required for the synthesis of secondary metabolites such as polyphenols or carotenoids, which are known to be part of the defense response of plants to stress.[11]

Metabolic responses to MIPEF treatments have been studied in potato tissues,[10,12] but as far as we know, no data is available on the effects of MIPEF on other fruit and vegetables. Therefore, the aim of this research was to study the effects of processing parameters of MIPEF, namely, electric field strength and number of pulses, on the bioproduction of polyphenols, lycopene and vitamin C as well as on the antioxidant capacity of tomato fruits. In addition, MIPEF processing parameters were optimized to obtain tomato fruit with enhanced levels of bioactive compounds.

MATERIALS AND METHODS

Reagents. All samples and standards were handled without exposure to light. Folin−Ciocalteau (F−C) reagent, L-ascorbic acid, lycopene, 2,2′azino-bis(3)-ethylbenzothiazoline-6-sulfonic acid (ABTS), Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) 97% ,and manganese dioxide were purchased from Sigma (Madrid, Spain); metaphosphoric acid and DL-1,4-dithiotreitol (DTT) were purchased from Acros Organics (New Jersey, U.S.A.); hydrochloric acid 35% and acetic acid 99.8% were from Panreac (Barcelona, Spain); and anhydrous sodium acetate (2 M) was from

Received: December 19, 2011
Revised: February 15, 2012
Accepted: February 29, 2012
Published: February 29, 2012

Merck (Darmstadt, Germany). Butylated hydroxytoluene (BHT), methanol, hexane, and formic acid were obtained from Scharlau (Barcelona, Spain). Ultrapure water (Milli-Q) was from Millipore Corporation (Bedford, MA, USA).

Tomatoes. Commercially mature tomato fruit (*Licopersicon esculentum* Mill. cv. Daniella) were purchased from a local supermarket (Lleida, Spain). The pH (Crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), soluble solids content (Atago RX-1000 refractometer; Atago Company Ltd., Japan), firmness (Texturometer-XT2 Stable Micro Systems Ltd., Surrey, England), and color (Minolta CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) of the tomato fruit were determined (Table 1).

Table 1. Analytical Characteristics of Tomato Fruits

parameters[a]	tomato fruits
pH	4.45 ± 0.01
firmness (N·s[b])	20.4 ± 2.51
soluble solids (°Brix)	4.42 ± 0.02
L^*	38.5 ± 0.4
a^*	18.1 ± 1.9
b^*	24.6 ± 1.8

[a]Results are the mean ± SD of three measurements. [b]N·s: Newtons·second.

MIPEF Processing. MIPEF treatments were conducted in bath equipment manufactured by Physics International (San Leandro, CA, USA), which can deliver pulses from a capacitor of 0.1 μF with an exponential decaying waveform. A stainless steel parallel plate treatment chamber was used. A batch of tomato fruit was placed in the treatment chamber filled with tap water. Tomato fruits were treated at 0.4−2 kV/cm using 5−30 monopolar pulses of 4 μs at a frequency of 0.1 Hz. Each treatment was repeated twice.

MIPEF-treated tomato fruits were collected and immediately refrigerated at 4 °C for 24 h as previously described by Galindo et al.[10] Untreated tomatoes were stored separately at 4 °C for 24 h. Both untreated and MIPEF-treated tomatoes were lyophilized after 24 h and frozen at −20 °C until analysis.

Extraction and Analyses of Total Polyphenol Content. Samples were treated in triplicate following the procedure of Vallverdú-Queralt et al.[13] with some modifications. Lyophilized tomato fruits (0.2 g) were weighed and homogenized with 80% ethanol in Milli-Q water (4 mL); the homogenate was then sonicated for 5 min and centrifuged (4000 rpm at 4 °C) for 15 min. The supernatant was transferred into a flask, and the extraction was repeated. Both supernatants were combined and evaporated under nitrogen flow. Finally, the residue was reconstituted with Milli-Q water (0.1% of formic acid) up to 2 mL and filtered through a 13 mm, 0.45 μm polytetrafluoroethylene (PTFE) filter from Waters (Milford, USA) into an insert-amber vial.

Solid phase extraction (SPE) was carried out to eliminate interferences such as ascorbic acid, amino acids, and reducing sugars, which are proved to overestimate values of total polyphenol (TP) content. For this procedure, Oasis MAX cartridges with 30 mg of mixed-mode anion-exchange and reversed-phase solvent from Waters (Milford, USA) were used following the procedure of Vallverdú-Queralt et al.[14] The eluted fractions were evaporated under nitrogen flow, and the residue was reconstituted with up to 500 μL of Milli-Q water containing 0.1% formic acid.

For the TP content assay, each extract was analyzed as follows: 20 μL of the eluted fractions was mixed with 188 μL of Milli-Q water in a thermo microtiter 96-well plate (Nunc, Roskilde, Denmark), and 12 μL of Folin−Ciocalteu (F−C) reagent and 30 μL of sodium carbonate (200 g/L) were added. The mixtures were incubated for 1 h at room temperature in the dark. After the reaction period, 50 μL of Milli-Q water was added, and the absorbance was measured at 765 nm in a UV/vis Thermo Multiskan Spectrum spectrophotometer (Vantaa, Finland). This spectrophotometer allowed the absorbance of a 96-well plate to be read in 10 s. TP content was expressed as mg of gallic acid equivalents (GAE)/100 g dry weight.

Extraction and Analyses of Vitamin C Content. The extraction procedure was carried out in triplicate following a method described by Odriozola-Serrano et al.[15] Five grams of lyophilized tomato fruits were weighed and homogenized with 5 mL of a solution containing 45 g of metaphosphoric acid and 7.5 g of DTT per liter. The mixture was centrifuged at 4000 rpm for 10 min at 4 °C. The supernatant was transferred into a flask, and the extraction was repeated. Both supernatants were combined and filtered through a 0.45 μm PTFE filter into an insert-amber vial for HPLC analysis.

For chromatographic separations an HP 1100 HPLC system (Hewlett-Packard, Waldbronn, Germany) equipped with a diode-array detector and an automatic sample injector was used. The mobile phase was a 0.01% solution of sulphuric acid adjusted to pH 2.6. The separation of ascorbic acid was performed with a Luna C_{18} column 50 × 2.0 mm i.d., 5 μm (Phenomenex, Torrance, CA, USA). The injection volume was 20 μL, and the flow rate was 1 mL/min. All UV spectra were recorded at 245 nm. Identification of the ascorbic acid was carried out comparing the retention time and UV−visible absorption spectrum with those of the standard. Vitamin C content was expressed as mg vitamin C/100 g dry weight.

Extraction and Analyses of Lycopene Content. The extraction of lycopene was carried out in triplicate following the procedure of Odriozola-Serrano et al.[16] This method determines the content of lycopene and other derivates such as hydroxy lycopene and lycopene epoxides. Approximately 0.2 g of lyophilized tomatoes were weighed and added to 2.5 mL of 0.05% (w/v) BHT in acetone, 2.5 mL of 95% USP-grade ethanol, and 5 mL of hexane. The homogenate was centrifuged at 4000 rpm for 20 min at 4 °C. After shaking, 1.5 mL of distilled water was added. The vials were then agitated for 5 min and left at room temperature to allow phase separation. The lycopene content of each sample was measured using the absorbance at 503 nm. Lycopene content was expressed as mg lycopene/100 g dry weight.

Antioxidant Capacity Determination. The tomato extracts prepared to determine the TP and lycopene contents were also used to analyze the hydrophilic antioxidant capacity (HAC) and lipophilic antioxidant capacity (LAC), respectively. The HAC and LAC were measured using an $ABTS^+$ radical decolorization assay and DPPH assay following the procedure of Vallverdú-Queralt et al.[17] with minor modifications.

$ABTS^+$ Assay. An $ABTS^+$ radical cation was prepared by passing a 5 mM aqueous stock solution of ABTS (in methanol) through manganese dioxide powder. Excess manganese dioxide was filtered through a 13 mm 0.45 μm filter PTFE. Before analysis, the solution was diluted in methanol pH 7.4 to give an absorbance at 734 nm of 0.9 ± 0.1 and preincubated in ice. Two hundred forty-five microliters of $ABTS^+$ solution was added to 5 μL of tomato extracts, and solutions were stirred for 30 s. The homogenates were kept in darkness for 1 h, and the absorbance was recorded with a UV/vis Thermo Multiskan Spectrum spectrophotometer at 734 nm against a blank of methanol without $ABTS^+$. The results were expressed as mmol Trolox equivalent (TE)/100 g dry weight.

DPPH Assay. HAC and LAC were also measured by the DPPH assay. Five microliters of tomato extracts or Trolox were mixed with 250 μL of methanolic DPPH (0.025 g/L). The homogenate was shaken vigorously and kept in darkness for 30 min. Absorption of the samples was measured on the spectrophotometer at 515 nm against a blank of methanol without DPPH. The results were expressed as mmol Trolox equivalent (TE)/100 g dry weight.

TP, lycopene, and vitamin C relative contents were defined as the percentage of compound content of MIPEF-treated tomatoes compared to that of the untreated tomatoes. Relative HAC and LAC were defined as the percentage of HAC or LAC of MIPEF-treated tomatoes compared to that of the untreated tomatoes.

Experimental Design. A face-centered central composite response surface design was used to determine the effect of electric field strength and number of pulses on TP, vitamin C, and lycopene content as well as on HAC and LAC. The independent variables were electric field strength (from 0.4 to 2 kV/cm) and number of pulses

Table 2. Central Composite Response Surface Design for Relative TP, Vitamin C, and Lycopene Content and Relative Antioxidant Capacity of Tomato Fruits under Different MIPEF Treatments[a]

E (kV/cm)	*n* (number of pulses)	relative TP content (%)[b]	relative vitamin C content (%)[b]	relative lycopene content (%)[b]	relative HAC[c] (DPPH) (%)[b]	relative HAC[c] ($ABTS^+$) (%)[b]	relative LAC[c] (DPPH) (%)[b]	relative LAC[c] ($ABTS^+$) (%)[b]
1.2	30	144.61 ± 2.29 a	98.97 ± 1.11 a	110.13 ± 2.74 a	134.67 ± 1.64 a	129.76 ± 1.30 a	123.34 ± 2.77 a	115.45 ± 2.96 a
1.2	5	121.89 ± 2.92 b	86.05 ± 1.45 b	131.75 ± 2.96 b	120.80 ± 2.38 b	112.69 ± 1.77 b	137.35 ± 2.02 b	130.72 ± 2.23 b
1.2	18	137.19 ± 2.73 c	99.36 ± 0.99 a	117.77 ± 1.37 c	144.20 ± 2.08 c	141.36 ± 1.76 c	126.47 ± 1.33 c	120.42 ± 1.50 c
2	18	90.59 ± 2.88 d	94.51 ± 1.96 c	100.56 ± 2.40 d	122.48 ± 1.87 d	114.69 ± 1.38 b	113.93 ± 1.64 d	105.51 ± 3.57 d
0.4	18	121.21 ± 2.69 b	95.30 ± 1.88 d	112.98 ± 2.41 a	133.80 ± 1.99 a	130.20 ± 2.38 a	122.10 ± 2.31 a	118.76 ± 2.12 c
2	30	98.96 ± 4.65 e	94.94 ± 1.50 c	93.01 ± 1.39 e	109.85 ± 1.67 e	103.06 ± 1.53 d	110.42 ± 2.43 e	99.93 ± 2.38 e
0.4	30	134.49 ± 3.00 c	94.31 ± 0.97 c	103.83 ± 3.00 f	125.81 ± 2.15 f	117.30 ± 1.37 e	116.75 ± 3.08 f	111.16 ± 2.26 f
2	5	81.37 ± 1.95 f	81.00 ± 1.85 e	110.97 ± 3.09 a	101.60 ± 2.45 g	92.61 ± 0.95 f	120.74 ± 2.27 g	114.62 ± 1.84 a
0.4	5	106.64 ± 1.68 g	83.22 ± 1.60 f	126.02 ± 2.82 g	114.15 ± 2.57 h	106.57 ± 1.51 g	130.86 ± 1.99 h	123.47 ± 3.37 g

[a]Different letters in the columns represent statistically significant differences ($P<0.05$). [b]Data shown are the mean ± SD of two PEF treatment repetitions; each assay was performed in triplicate. [c]HAC, hydrophilic antioxidant capacity; LAC, lipophilic antioxidant capacity.

Table 3. Analysis of Variance of the Second-Order Models for Relative TP, Vitamin C, and Lycopene Content and Relative Antioxidant Capacity

source[a]	relative TP content	relative vitamin C content	relative lycopene content	relative HAC[b] (DPPH)	relative HAC[b] ($ABTS^+$)	relative LAC[b] (DPPH)	relative LAC[b] ($ABTS^+$)
quadratic model	200.13[c]	6463.34[c]	178.83[c]	285.53[c]	176.78[c]	118.43[c]	62.54[c]
E	267.08[c]	94.23[c]	171.15[c]	142.18[c]	79.41[c]	99.17[c]	77.34[c]
n	148.49[c]	14777.62[c]	445.27[c]	102.25[c]	60.76[c]	241.80[c]	124.43[c]
E^2	465.53[c]	3279.01[c]	265.51[c]	356.29[c]	215.01[c]	245.60[c]	103.99[c]
n^2	2.80	7656.7[c]	11.69[d]	375.28[c]	245.68[c]	21.43[d]	2.43
E·*n*	5.06	187.89[c]	3.14	1.56	0.01	3.50	0.59
lack of fit	0.38	2.61	0.42	2.89	2.90	2.13	1.47
std. dev.	2.28	1.36	1.20	1.36	2.00	1.01	1.55
mean	121.98	129.55	113.70	129.55	124.13	123.68	117.06
coefficient of variation	1.87	1.05	1.05	1.05	1.61	0.82	1.32
R^2	0.9931	0.9898	0.9922	0.9951	0.9921	0.9883	0.9781
adj R^2	0.9881	0.9896	0.9867	0.9916	0.9865	0.9799	0.9624

[a]*E*) electric field strength, *n*)number of pulses. [b]HAC, hydrophilic antioxidant capacity; LAC, lipophilic antioxidant capacity. [c]Significant at $p < 0.001$. [d]Significant at $p < 0.05$.

(from 5 to 30). The levels for each independent parameter were chosen considering sample and equipment limitations. MIPEF experimental design was performed twice, and the order of assays was randomized. Experimental data were fitted to a polynomial response surface. The second-order response function was predicted by eq 1:

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

where Y is the dependent variable, β_0 is the center point of the system, β_i, β_{ii}, and β_{ij} represent the coefficients of the linear, quadratic, and interactive effect, respectively, and X_i,X_i^2 and X_iX_j represent the linear, quadratic, and interactive effect of the independent variables, respectively. The nonsignificant terms were deleted from the second-order polynomial model after an ANOVA test, and a new ANOVA was performed to obtain the coefficients of the final equation for improved accuracy. Design Expert 7.0.1 software (Stat Ease Inc., Minneapolis, MN) was used to generate quadratic models that fit the experimental data and to draw the response surface plots.

The optimization was done following the method proposed by Derringer et al.[18] 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 closer the desirability value is to the unit, the more suitable is the system. In the present study, desirability functions were developed to obtain tomato fruits with optimum levels of health-related compounds.

A set of 88 experiments, choosing intensities between 0.4 and 2 kV/cm and number of pulses between 5 and 30, was carried out to validate the predictive models on TP, vitamin C, and lycopene content as well as on HAC and LAC. Correlations between predicted and observed retentions were evaluated by Pearson's test.

RESULTS AND DISCUSSION

Effect of MIPEF on TP Content. The TP content of untreated tomatoes was 138.2 mg/100 g dry weight, which was consistent with values reported in the literature.[14,19] Results for relative TP content obtained under the different experimental conditions are shown in Table 2. After 24 h of MIPEF treatments, a higher TP content was observed in MIPEF-treated tomatoes than in untreated samples except at 2 kV/cm. The increases in relative TP content of tomatoes ranged from 6.6% (5 pulses at 0.4 kV/cm) to 44.6% (30 pulses at 1.2 kV/cm). These results are in accordance with Galindo et al.,[10] who reported that 24 h after MIPEF treatments, potato tissue metabolism showed plant stress responses characterized by changes in polyphenols, amino acids, and the hexose pool. Similar responses in plants to other types of stress have also been observed. Matsuda et al.[20] reported a 50% increase of chlorogenic acid in wound-healing potato tuber tissue, which is known to boost tissue protection against oxidative stress.

Increases of TP content are difficult to explain due to the complexity of chemical reactions occurring in natural systems.

Table 4. Significant Regression Coefficients of the Quadratic Model for Relative TP, Vitamin C, and Lycopene Content and Relative Antioxidant Capacity of MIPEF-Treated Tomatoes

source[a]	relative TP content	relative vitamin C content	relative lycopene content	relative HAC[b] (DPPH)	relative HAC[b] ($ABTS^+$)	relative LAC[b] (DPPH)	relative LAC[b] ($ABTS^+$)
intercept value	74.338	69.237	120.413	80.107	66.220	125.996	119.264
E	95.417	14.778	35.962	49.782	57.167	30.562	26.589
n	0.909	1.967	−1.374	4.011	4.742	−1.142	−0.564
E^2	−47.692	−7.087	−18.308	−24.200	−27.615	−14.871	−13.973
n^2		−0.044	0.016	−0.102	−0.121	−0.018	
$E{\cdot}n$		0.090					

[a] E, electric field strength; n, number of pulses. [b] HAC, hydrophilic antioxidant capacity; LAC, lipophilic antioxidant capacity.

Polyphenols are formed in plant products via the action of phenylalanine ammonia-lyase (PAL) in the phenylpropanoid metabolism.[21] It could be hypothesized that MIPEF induced stress and increased PAL activity, thus enhancing TP content. This stress response is initiated when the plant recognizes a stimulus at the cellular level, which is initiated by the activity of specific ion channels.[22] Voltage-gated ion channels are a specific type of transmembrane ion channel embedded in a cell membrane, which are activated by changes in the membrane electrical potential. Therefore, MIPEF may influence the voltage-gated ion channels and increase the membrane permeability for Ca^{2+} at the cellular level, followed by a rapid influx of Ca^{2+} through cation channels. Through this process, Ca^{2+}-dependent protein kinase (CDPK) phosphorylates PAL,[23] which regulates the phenylpropanoid metabolism. The CDPK can also increase the ROS,[24] which are endogenous signal components required for the synthesis of secondary metabolites, such as polyphenols, known to be part of the plant defense response to stress.[11]

The effect of the number of pulses and electric field strength on relative TP content was evaluated by a design surface methodology. The statistical analyses showed that the quadratic model proposed for relative TP content was adequate ($P < 0.001$), with a satisfactory determination coefficient ($R^2 = 0.9931$) (Table 3). The model showed no significant ($P > 0.05$) lack-of-fit, indicating a good fit for prediction within the range of assayed conditions. The linear term of electric field strength ($P < 0.001$), the linear term of number of pulses ($P < 0.001$), and the quadratic term of electric field strength ($P < 0.001$) significantly affected the relative TP content of tomato fruits (Table 3). Coefficients of the fitted equations are shown in Table 4. An overall increase in relative TP content was observed as the number of pulses rose (Figure 1). These results provide evidence that a fast metabolic response took place under 25−30 pulses at 0.7−1.1 kV/cm. The application of MIPEF results in an opening of pores in the cell membrane and consequently an efflux and influx of molecules. The influence of different stress factors such as wounding and light on the increased expression of phenol-biosynthetic genes has been reported,[25] and MIPEF may elicit a similar response. As can be seen in Figure 1, tomato fruit treated at the highest intensity field strength (2 kV/cm) and minimum number of pulses (5 pulses) showed the lowest relative TP content. It seems that treatments at 2 kV/cm may cause lethal damage to cells due to irreversible loss of cell membrane permeability properties.[26]

Effect of MIPEF on Vitamin C Content. The vitamin C content of untreated tomato fruits was 98 mg/100 g dry weight, which is consistent with values reported in the literature.[16] The relative vitamin C content of tomatoes 24 h after MIPEF

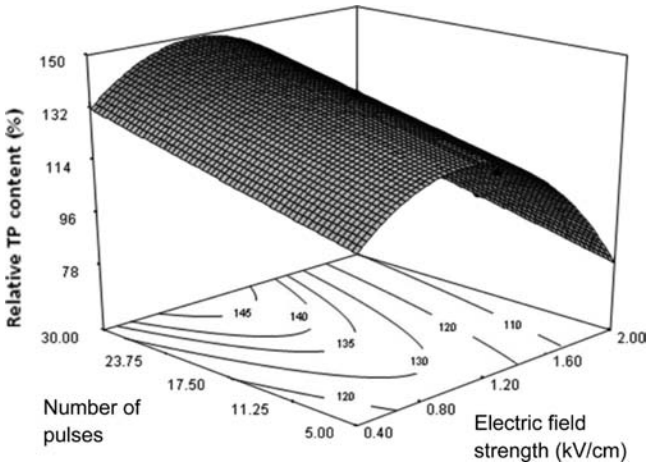

Figure 1. Effect of electric field strength and number of pulses on the relative TP content of tomatoes.

treatments ranged between 81.0% (5 pulses at 2 kV/cm) and 99.4% (18 pulses at 1.2 kV/cm) (Table 2). Ade-Omowaye et al.[6] reported a relative vitamin C content ranging from 89.6% to 96.5% in red bell peppers immediately after the application of MIPEF treatments (50 pulses at 2 kV/cm, pulse duration 400 ms). Therefore, the application of MIPEF does not produce synthesis of vitamin C.

In order to evaluate the effects of number of pulses and electric field strength on relative vitamin C content, a surface design methodology was used. A second-order model showed a good fit with the relative vitamin C content results ($P < 0.001$). The determination coefficient, R^2, was 0.9898, and the lack of fit was not significant ($P > 0.05$). The linear terms of electric field strength ($P < 0.001$) and number of pulses ($P < 0.001$), the quadratic terms of electric field strength ($P < 0.001$) and number of pulses ($P < 0.001$), and the interaction between both parameters ($P < 0.001$) had significant effects on the vitamin C content of the tomato fruits (Table 3). Coefficients of the fitted equations are presented in Table 4. As shown in Figure 2, a lower relative vitamin C content was observed in MIPEF-treated tomatoes than in untreated samples. The highest relative vitamin C content (99.4%) was obtained under 18−25 pulses at 0.8−1.2 kV/cm. Vitamin C is a typically labile nutrient and is also vulnerable to enzyme-catalyzed oxidation (specifically by ascorbate oxidase and peroxidase).[27] Our results demonstrate that 24 h after the application of MIPEF, tomato metabolism did not show an increase in vitamin C. Even more a slight decrease was observed in vitamin C in treated fruits.

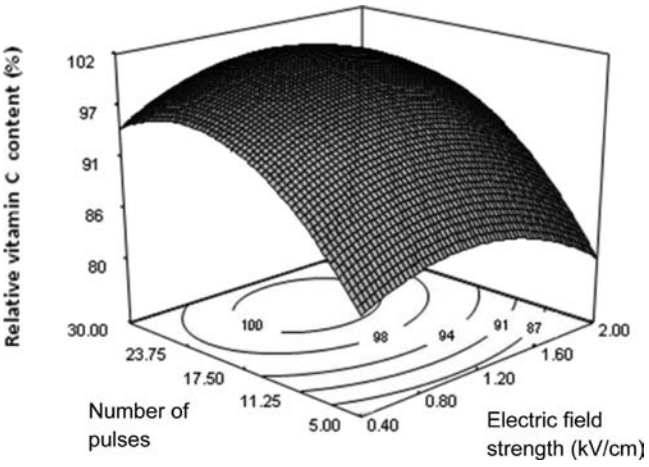

Figure 2. Effect of electric field strength and number of pulses on the relative vitamin C content of tomatoes.

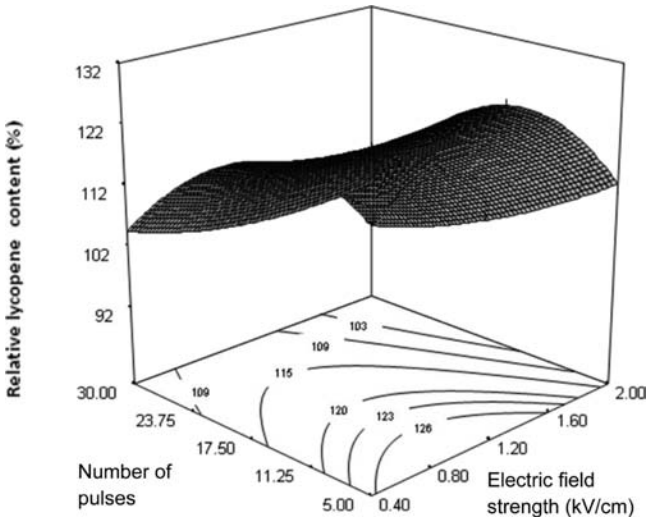

Figure 3. Effect of electric field strength and number of pulses on the relative lycopene content of tomatoes.

Effect of MIPEF on Lycopene Content. In untreated tomatoes, the lycopene content was 65 mg/100 g dry weight, which is consistent with values reported in the literature.[16] A higher relative lycopene content was achieved in tomato fruits 24 h after the application of MIPEF, except under 30 pulses at 2 kV/cm (Table 2). The increase in the relative lycopene content of tomato fruit ranged from 0.6% (18 pulses at 2 kV/cm,) to 31.8% (5 pulses at 1.2 kV/cm), indicating an influence of MIPEF on the carotenoid pattern. The maturation of tomato fruit is characteristically accompanied by a burst of ethylene.[28] Ethylene production, which leads to lycopene biosynthesis in tomatoes, is also known to be rapidly increased by stress, e.g., a pathogenic attack or elevated salinity.[28,29] Thus, it is possible that MIPEF promoted ethylene production and activated enzymes involved in lycopene biosynthesis, such as carotenoid isomerase or ζ-carotene desaturase.[30]

A second-order model showed an adequate fit with the relative lycopene content ($P < 0.001$) with a satisfactory determination coefficient ($R^2 = 0.9922$) and insignificant lack of fit ($P > 0.05$) (Table 3). Coefficients of the fitted equations are shown in Table 4. The relative lycopene content was significantly affected by the linear terms of electric field strength ($P < 0.001$) and number of pulses ($P < 0.001$), the quadratic terms of electric field strength ($P < 0.001$), and number of pulses ($P < 0.05$). A fast metabolic response was observed when electric field strength increased between 0.4 and 1.2 kV/cm, showing a maximum bioproduction between 0.8 and 1.2 kV/cm, although the relative lycopene content decreases when electric field strength increases above 1.2 kV/cm. Moreover, treatments carried out at 5 pulses resulted in the highest lycopene content of tomato fruits. At 0.4 kV/cm, an enhancement of about 22% was observed with 5 pulses compared to 30 pulses (Figure 3). Similarly, increases of 22% and 18% were obtained with 5 pulses at 1.2 kV/cm and 30 pulses at 2 kV/cm, respectively. The metabolic response is initiated when the plant recognizes a stimulus at the cellular level, including changes in membrane electric potential. Therefore, MIPEF may influence the voltage-gated ion channels and increase the membrane permeability for Ca^{2+} at the cellular level, followed by a rapid influx of Ca^{2+} through cation channels[23] and an acceleration of the biosynthesis of carotenoids in tomatoes.

Effect of MIPEF on Antioxidant Capacity. *Hydrophilic Antioxidant Capacity (HAC).* Table 2 shows the relative HAC of MIPEF-processed tomato fruits treated under the studied experimental conditions using DPPH and $ABTS^+$ methods. In untreated tomatoes, the HAC (DPPH) was 5.30 mmol TE/100 g dry weight, and HAC ($ABTS^+$) was 4.85 mmol TE/100 g dry weight, being consistent with values reported in the literature.[14] The increases in relative HAC of tomatoes 24 h after MIPEF treatments ranged from 1.6% (5 pulses at 2 kV/cm) to 44.2% (18 pulses at 1.2 kV/cm) for the DPPH assay, and from 3.1% (30 pulses at 2 kV/cm) to 41.4% (18 pulses at 1.2 kV/cm) for the $ABTS^+$ assay. Thus, MIPEF treatment significantly affected the metabolome after 24 h in promoting an increase in hydrophilic compounds. These results are in agreement with those reported by Galindo et al.,[10] who strongly suggested that MIPEF treatments significantly affect the tissue metabolome only in the long term (time scale of hours).

The effects of electric field strength and number of pulses on the relative HAC were studied. A second-order model fitted well ($P < 0.001$) with the relative HAC results in both assays ($ABTS^+$ and DPPH). The determination coefficients, R^2, were 0.9951 (DPPH) and 0.9921 ($ABTS^+$), and the lack of fit was not significant ($P > 0.05$). The linear terms of electric field strength ($P < 0.001$) and number of pulses ($P < 0.001$), and the quadratic terms of electric field strength ($P < 0.001$) and number of pulses ($P < 0.001$) had significant effects on the relative HAC of tomato fruits in both DPPH and $ABTS^+$ assays (Table 3). Thus, the HAC was represented by polynomial quadratic equations in terms of the studied PEF parameters (Table 4).

As can be seen in Figure 4a and b, the analyzed tomato fruits showed the same tendencies in both DPPH and $ABTS^+$ assays. A rise in relative HAC was observed when the electric field strength and number of pulses were increased between 0.4 and 1.2 kV/cm and 5−18, respectively, although it seemed to decrease when these parameters were increased further (Figure 4a and b). The maximum relative HAC was achieved with 0.6−1 kV/cm and 15−18 pulses (Figure 4a and b).

Antioxidant capacity is related to the amount and composition of bioactive compounds present in food.[31] It is widely known that ascorbic acid together with polyphenols may be important in conferring antioxidative-related health benefits in tomatoes.[14] A moderate relationship was found between relative HAC and TP content, $R^2 = 0.6794$ (DPPH) and $R^2 =$

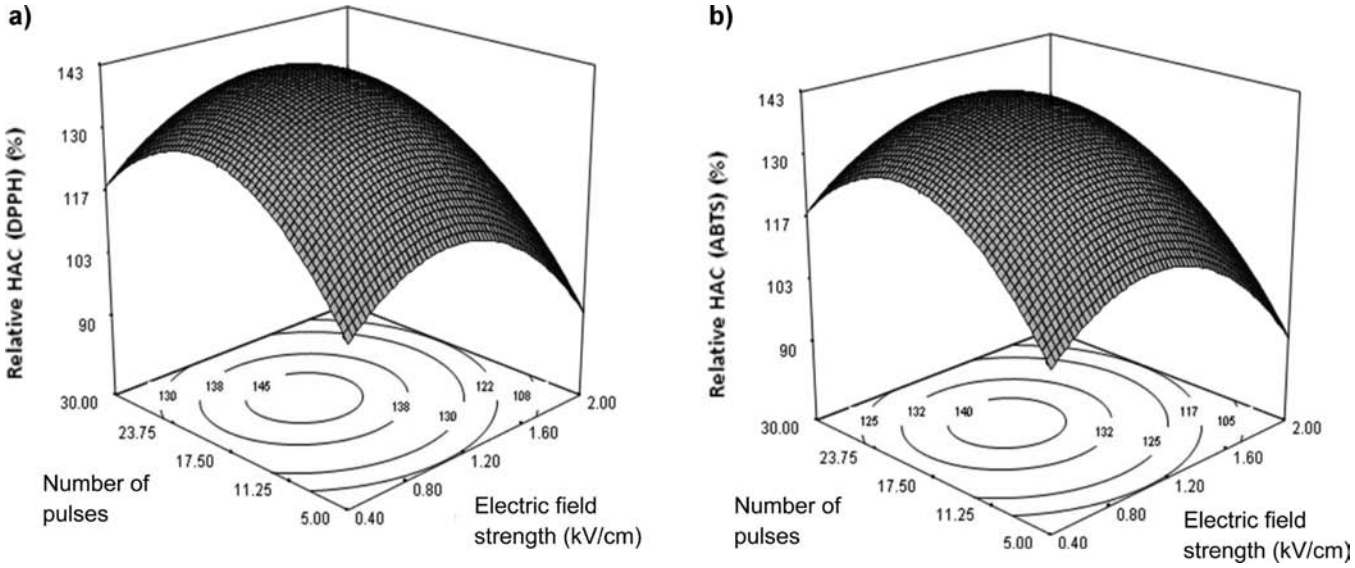

Figure 4. Effect of electric field strength and number of pulses on the relative hydrophilic antioxidant capacity of tomatoes. (a) DPPH. (b) ABTS$^+$.

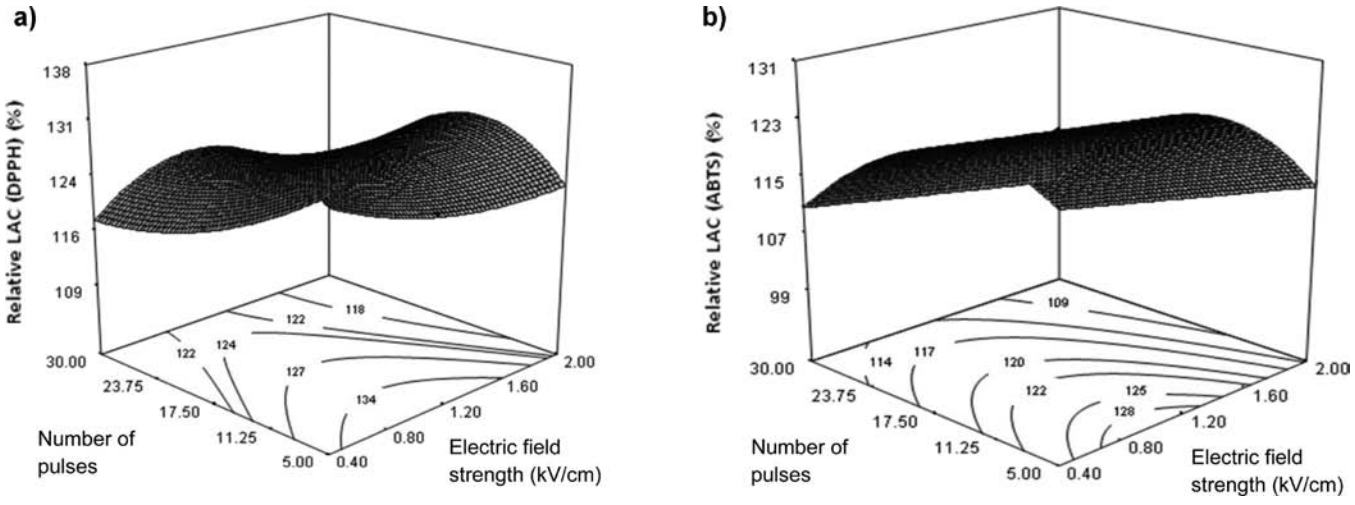

Figure 5. Effect of electric field strength and number of pulses on the relative lipophilic antioxidant capacity of tomatoes. (a) DPPH. (b) ABTS$^+$.

0.6515 (ABTS$^+$), whereas a stronger relationship was observed between relative HAC and vitamin C content, R^2 = 0.7272 (DPPH) and R^2 = 0.7319 (ABTS$^+$). As can be seen by comparing Figure 1 with Figure 4a and b, relative HAC and relative TP content did not follow the same pattern, the former reaching a peak at 15−22 pulses and the latter at 30 pulses. Thus, the levels of vitamin C and other antioxidant compounds in the tomato fruits seem to have exerted an important effect on the HAC, which increased 24 h after the MIPEF treatments.

Lipophilic Antioxidant Capacity (LAC). The LAC of tomato fruits was measured on the basis of the DPPH and ABTS$^+$ assays. The LAC (DPPH) of untreated tomatoes was 4.73 mmol TE/100 g dry weight, and LAC (ABTS$^+$) was 4.51 mmol TE/100 g dry weight. These results are in the range of those reported by Cano et al.[32] MIPEF-processed tomato fruits showed an increase in relative LAC between 10.4% (30 pulses at 2 kV/cm) and 37.4% (5 pulses at 1.2 kV/cm,) in the DPPH assay and between 5.5% (18 pulses at 2 kV/cm) and 30.7% (5 pulses at 1.2 kV/cm) in the ABTS$^+$ assay 24 h after MIPEF treatments (Table 2).

The effects of the number of pulses and electric field strength on relative LAC were also evaluated. A second-order model showed a good fit with the relative LAC data (Table 3). The determination coefficient, R^2, was 0.9883 (DDPH) and 0.9781 (ABTS$^+$), and the lack-of-fit was not significant ($P > 0.05$). The linear terms of electric field strength ($P < 0.001$) and number of pulses ($P < 0.001$), and the quadratic term of electric field strength ($P < 0.001$) had significant effects on the relative LAC of tomato fruits in both DPPH and ABTS$^+$ assays (Table 3), while the quadratic term of number of pulses ($P < 0.05$) significantly affected the relative LAC of tomato fruits in the DPPH assay (Table 3). Coefficients of the fitted equations are shown in Table 4.

A rise in relative LAC was observed when electric field strength was increased between 0.4 and 1 kV/cm, beyond which it decreased (Figure 5a and 5b). The number of pulses also had a significant effect ($P < 0.001$) on the relative LAC of tomatoes. A maximum content of tomato lipophilic antioxidants was achieved by combining an electric field strength of 0.8−1.3 kV/cm with 5−8 pulses (Figure 5a and b), when a fast metabolic response was observed.

Lipophilic compounds such as carotenoids are responsible for the LAC of tomato fruits.[14] A close relationship was found between the relative LAC and lycopene content: R^2 = 0.9708

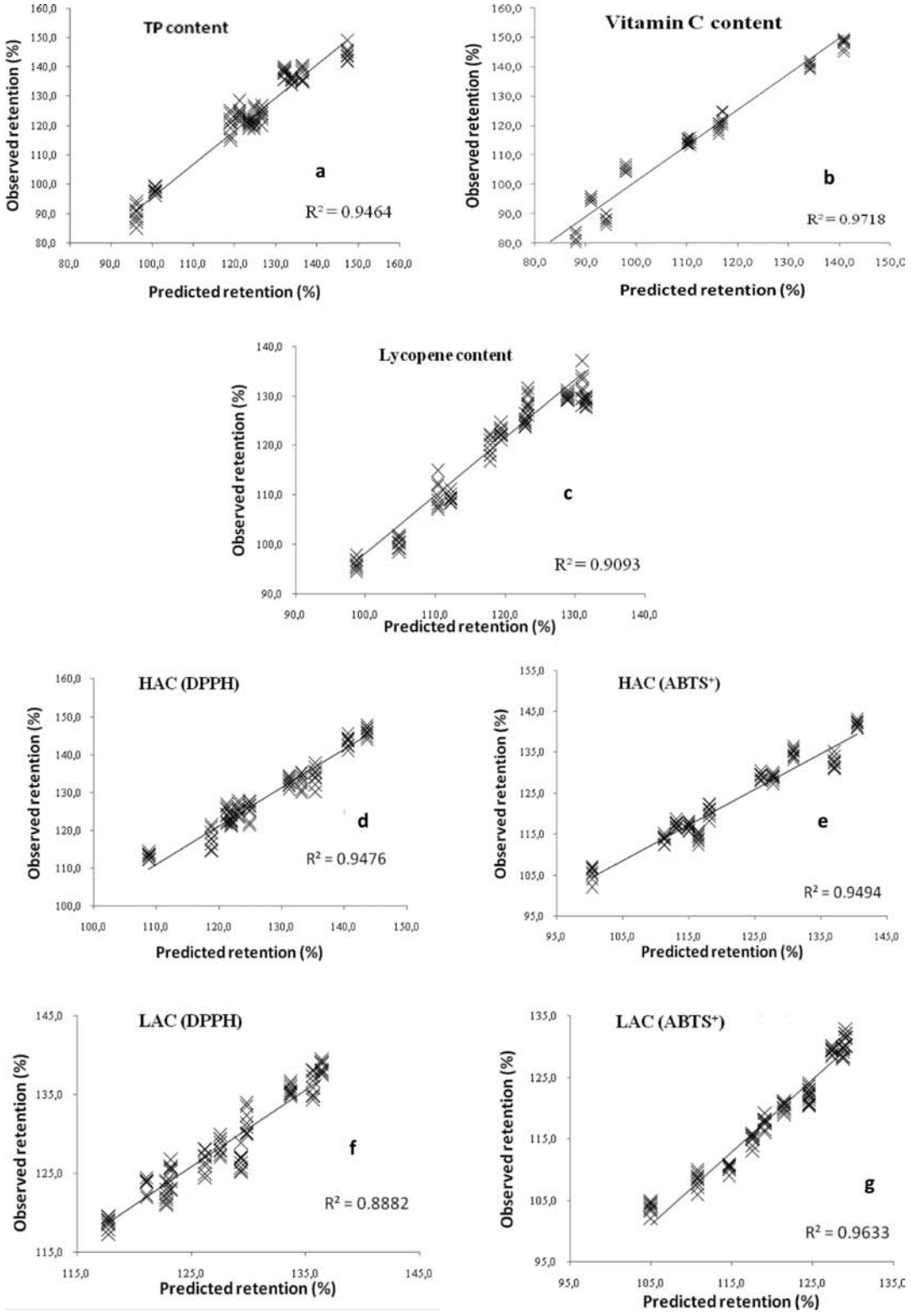

Figure 6. Scatter plots of the observed and predicted data of tomato fruit TP content (a), vitamin C content (b), lycopene content (c), HAC ($ABTS^+$) (d), HAC (DPPH) (e), LAC ($ABTS^+$) (f), and LAC (DPPH) (g). Retention of the validated trials. The straight line indicates the correlation between both groups of data.

(DPPH) and R^2 = 0.9592 ($ABTS^+$). As can be seen when comparing Figure 3 with Figure 5a and b, the relative LAC and lycopene content show a similar tendency, indicating that the increases in LAC observed 24 h after MIPEF treatment could be due to the higher total lycopene content. These results are in agreement with those of Cano et al.[32] who described lycopene as the most important carotenoid in the lipophilic fraction of tomatoes.

Optimization and Validation of the MIPEF Processing Conditions. The MIPEF critical parameters that provided

dx.doi.org/10.1021/jf205216m | J. Agric. Food Chem. 2012, 60, 3126–3134

tomato fruits with the highest nutritional quality were determined. The same priority was assigned to each dependent variable in order to obtain tomatoes with maximal content of TP, vitamin C, lycopene, and antioxidant capacity. 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 closer the desirability value to the unit, the more adequate the system. A desirability value of 0.822 was obtained when MIPEF treatment was conducted with 16 pulses at 1 kV/cm. Under these conditions, the predicted relative contents were TP (136.58%), vitamin C (98.33%), lycopene (120.10%), HAC-DPPH (143.83%), HAC-ABTS$^+$ (140.70%), LAC-DPPH (128.03%), and LAC-ABTS$^+$ (122.84%).

To validate the predictive models, a set of 88 experiments was carried out. The data comparison (Figure 6) showed that the proposed predicted expressions (Table 4) were accurate enough to fit experimental results. There were strong correlations between the observed and predicted retention data: TP (0.9464), vitamin C (0.9718), lycopene (0.9093), HAC-DPPH (0.9476), HAC-ABTS$^+$ (0.9494), LAC-DPPH (0.8882), and LAC-ABTS$^+$ (0.9633).

Conclusions. Increases in TP and lycopene contents as well as in the antioxidant capacity of MIPEF-treated tomato fruit were observed 24 h after treatments, depending on the electric field strength (0.4−2 kV/cm) and number of pulses (5−30). Maximum increases in TP (36.58%) and lycopene (20.10%) contents were obtained by combining 1 kV/cm and 16 pulses, contributing to an increase in the antioxidant capacity of tomato fruit by more than 20%.

Our results confirm an increase in bioactive compounds, which could be attributed to a MIPEF-induced stress response but also to an increased permeability of the cellular membrane due to MIPEF processing, which could potentially make the extraction of bioactive constituent more efficient. MIPEF treatments may induce stress reactions in tomato fruits after 24 h of refrigeration by stimulating metabolic activity and accumulating secondary metabolites. However, further investigations should be carried out to study in depth the MIPEF-induced stresses in plants.

AUTHOR INFORMATION

Corresponding Author

*Tel: 34-973-702593. Fax: 34-973-702596). E-mail: omartin@tecal.udl.cat.

Funding

We express our gratitude to CICYT's (AGL2010-22319-C03), RETICS RD06/0045/0003 from the Spanish Ministry of Science and Innovation (MICINN) for financial support. The CIBERobn CB06/03 is an initiative from the Instituto de Salud Carlos III, Spain. We also acknowledge the financial support of the Spanish Institute of Agricultural and Food Research and Technology (INIA) through the project RTA2010-00079-C02-02. ICREA Academia Award is also acknowledged by O. M-B. A.V-Q received support from MEC for the training of researchers.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Grieb, S. M. D.; Theis, R. P.; Burr, D.; Benardot, D.; Siddiqui, T.; Asal, N. R. Food groups and renal cell carcinoma: results from a case-control study. *J. Am. Diet. Assoc.* **2009**, *109*, 656−667.

(2) Willcox, J. K.; Catignani, G. L.; Lazarus, S. Tomatoes and cardiovascular health. *Crit Rev. Food Sci. Nutr.* **2003**, *43* (1), 1−18.

(3) Giovannucci, E.; Rimm, E. B.; Liu, Y.; Stampfer, M. J.; Willett, W. C. A prospective study of tomato products, lycopene, and prostate cancer risk. *J. Nat. Cancer I.* **2002**, *94*, 391−398.

(4) Crozier, A.; Jaganath, I. B.; Clifford, M. N. Dietary phenolics: chemistry, bioavailability and effects on health. *Nat. Prod. Rep.* **2009**, *26*, 1001−1043.

(5) Soliva-Fortuny, R.; Balasa, A.; Knorr, D.; Martín-Belloso, O. Effects of pulsed electric fields on bioactive compounds in foods: a review. *Trends Food Sci. Technol.* **2009**, *20*, 544−556.

(6) Ade-Omowaye, B. I; Taiwo, K.; Eshtiaghi, N.; Angersbach, A.; Knorr, D. Comparative evaluation of the effects of pulsed electric field and freezing on cell membrane permeabilisation and mass transfer during dehydration of red bell peppers. *Innov. Food Sci. Emerg.* **2003**, *4*, 177−188.

(7) Taiwo, K. A.; Angersbach, A.; Knorr, D. Influence of high intensity electric field pulses and osmotic dehydration on the rehydration characteristics of apple slices at different temperatures. *J. Food Eng.* **2002**, *52*, 185−192.

(8) Toepfl, S.; Mathys, A.; Heinz, V.; Knorr, D. Review: Potential of high hydrostatic pressure and pulsed electric fields for energy efficient and environmentally friendly food processing. *Food Rev. Int.* **2006**, *22*, 405−423.

(9) Galindo, F. G.; Sjoholm, I.; Rasmusson, A. G.; Widell, S.; Kaack, K. Plant stress physiology: opportunities and challenges for the food industry. *Crit. Rev. Food Sci.* **2007**, *47*, 749−763.

(10) Galindo, F.; Dejmek, P.; Lundgren, K.; Rasmusson, A.; Vicente, A.; Moritz, T. Metabolomic evaluation of pulsed electric field-induced stress on potato tissue. *Planta* **2009**, *230*, 469−479.

(11) Shohael, A. M.; Ali, M. B.; Yu, K. W.; Hahn, E. J.; Islam, R.; Paek, K. Y. Effect of light on oxidative stress, secondary metabolites and induction of antioxidant enzymes in *Eleutherococcus senticosus* somatic embryos in bioreactor. *Process Biochem.* **2006**, *41*, 1179−1185.

(12) Gómez Galindo, F.; Wadso, L.; Vicente, A.; Dejmek, P. Exploring metabolic responses of potato tissue induced by electric pulses. *Food Biophys.* **2008**, *3*, 352−360.

(13) Vallverdú-Queralt, A.; Jáuregui, O.; Medina-Remón, A.; Andrés-Lacueva, C.; Lamuela-Raventós, R. M. Improved characterization of tomato polyphenols using liquid chromatography/electrospray ionization linear ion trap quadrupole Orbitrap mass spectrometry and liquid chromatography/electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2010**, *24*, 2986−2992.

(14) Vallverdú-Queralt, A.; Medina-Remón, A.; Martínez-Huelamo, M.; Jáuregui, O.; Andrés-Lacueva, C.; Lamuela-Raventós, R. M. Phenolic profile and hydrophilic antioxidant capacity as chemotaxonomic markers of tomato varieties. *J. Agric. Food Chem.* **2011**, *59*, 3994−4001.

(15) Odriozola-Serrano, I.; Soliva-Fortuny, R.; Martín-Belloso, O. Impact of high-intensity pulsed electric fields variables on vitamin C, anthocyanins and antioxidant capacity of strawberry juice. *LWT - Food Sci. Technol.* **2009**, *42* (1), 93−100.

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

(17) Vallverdú-Queralt, A.; Medina-Remón, A.; Andrés-Lacueva, C.; Lamuela-Raventós, R. M. Changes in phenolic profile and antioxidant activity during production of diced tomatoes. *Food Chem.* **2011**, *126*, 1700−1707.

(18) Derringer, G.; Suich, R. Simultaneous optimization of several response variables. *J. Qual. Technol.* **1980**, *12*, 214−219.

(19) Bahorun, T.; Luximon-Ramma, A.; Crozier, A.; Aruoma, O. I. Total phenol, flavonoid, proanthocyanidin and vitamin C levels and

antioxidant activities of Mauritian vegetables. *J. Sci. Food Agric.* **2004**, *84*, 1553−1561.
(20) Matsuda, F.; Morino, K.; Miyashita, M.; Miyagawa, H. Metabolic flux analysis of the phenylpropanoid pathway in wound-healing potato tuber tissue using stable isotope-labeled tracer and LC-MS spectroscopy. *Plant Cell Physiol.* **2003**, *44*, 510−517.
(21) Odriozola-Serrano, I.; Oms-Oliu, G.; Soliva-Fortuny, R.; Martín-Belloso, O. Effect of high-oxygen atmospheres on the antioxidant potential of fresh-cut tomatoes. *J. Agric. Food Chem.* **2009**, *57*, 6603−6610.
(22) Gaspar, T.; Franck, T.; Bisbis, B.; Kevers, C.; Jouve, L.; Hausman, J. F.; Dommes, J. Concepts in plant stress physiology. Application to plant tissue cultures. *Plant Growth Regul.* **2002**, *37*, 263−285.
(23) Sanders, D.; Pelloux, J.; Brownlee, C.; Harper, J. F. Calcium at the crossroads of signaling. *Plant Cell* **2002**, *14*, S401−S417.
(24) Xing, T.; Wang, X. J.; Malik, K.; Miki, B. L. Ectopic expression of an arabidopsis calmodulin-like domain protein kinase-enhanced NADPH oxidase activity and oxidative burst in tomato protoplasts. *Mol. Plant Microbe* **2001**, *14*, 1261−1264.
(25) Piironen, V.; Lindsay, D. G.; Miettinen, T. A.; Toivo, J.; Lampi, A. M. Plant sterols: biosynthesis, biological function and their importance to human nutrition. *J. Sci. Food Agric.* **2000**, *80*, 939−966.
(26) Aronsson, K.; Lindgren, M.; Johansson, B. R.; Rönner, U. Inactivation of microorganisms using pulsed electric fields: the influence of process parameters on *Escherichia coli*, *Listeria innocua*, *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae*. *Innov. Food Sci. Emerg.* **2001**, *2* (1), 41−54.
(27) Davey, M. W.; Montagu, M. V.; Inzé, D.; Sanmartin, M.; Kanellis, A.; Smirnoff, N.; Benzie, I. J.; Strain, J. J.; Favell, D.; Fletcher, J. Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. *J. Sci. Food Agric.* **2000**, *80*, 825−860.
(28) Dannehl, D.; Huyskens-keil, S.; Eichholz, I.; Ulrichs, C.; Schmidt, U. Effects of direct-electric-current on secondary plant compounds and antioxidant activity in harvested tomato fruits (*Solanum lycopersicon L.*). *Food Chem.* **2011**, *126*, 157−165.
(29) Shibli, R.; Kushad, M.; Yousef, G.; Lila, M. Physiological and biochemical responses of tomato microshoots to induced salinity stress with associated ethylene accumulation. *Plant Growth Regul.* **2007**, *51*, 159−169.
(30) DellaPenna, D.; Pogson, B. J. Vitamin synthesis in plants: Tocopherols and carotenoids. *Annu. Rev. Plant Biol.* **2006**, *57* (1), 711−738.
(31) Sánchez-Moreno, C.; Pilar Cano, M.; de Ancos, B.; Plaza, L.; Olmedilla, B.; Granado, F.; Elez-Martínez, P.; Martín-Belloso, O.; Martín, A. Intake of Mediterranean vegetable soup treated by pulsed electric fields affects plasma vitamin C and antioxidant biomarkers in humans. *J. Food Sci. Nutr.* **2005**, *56*, 115−124.
(32) Cano, A.; Acosta, M.; Arnao, M. B. Hydrophilic and lipophilic antioxidant activity changes during on-vine ripening of tomatoes (*Lycopersicon esculentum Mill.*). *Postharvest Biol. Technol.* **2003**, *28* (1), 59−65.