

## Changes in the Polyphenol Profile of Tomato Juices Processed by Pulsed Electric Fields

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**ABSTRACT:** The effect of pulsed electric fields on the polyphenol profile of tomato juices was studied. First, tomatoes were subjected to moderate-intensity pulsed electric fields (MIPEFs) and then were immediately refrigerated at 4 °C for 24 h. Treated and untreated juices were then subjected to high-intensity pulsed electric fields (HIPEFs) or thermal treatment (90 °C for 60 s). In comparison to references, tomatoes subjected to MIPEF treatments led to juices with a higher content of polyphenol compounds. A slight decrease in polyphenol compounds was observed over time in thermal- and HIPEF-treated juices, with the exception of caffeic acid. However, HIPEF-processed tomato juices had a higher content of polyphenol compounds (ferulic acid, caffeic-*O*-glucoside acid, *p*-coumaric acid, chlorogenic acid, rutin, and naringenin) just after processing and through storage than those thermally treated. Therefore, the combination of MIPEFs and HIPEFs could be proposed as a strategy for producing tomato juices with a higher content of phenolic compounds.

**KEYWORDS:** *Tomato juices, HIPEF, MIPEF, thermal treatments, polyphenols, tomato fruit*

### ■ INTRODUCTION

The consumption of raw tomato and tomato-based products, such as tomato juices, is associated with a decrease in chronic degenerative diseases.<sup>1</sup> These beneficial effects could be attributed in part to their content in polyphenols, which have been gaining interest because of their multiple biological effects, such as free-radical scavenging, inhibition of cellular proliferation, and modulation of enzymatic activity and signal transduction pathways.<sup>2</sup> Polyphenols in tomatoes are mainly represented by flavanones (naringenin-glycosylated derivatives) and flavonols (quercetin-, rutin-, and kaempferol-glycosylated derivatives).<sup>3,4</sup>

The application of pulsed electric fields in food processing has been studied as a possible treatment to induce stress reactions in plant systems to enhance or stimulate the production of secondary plant metabolites, such as polyphenols.<sup>5</sup> Vallverdú-Queralt et al.<sup>6</sup> reported that moderate-intensity pulsed electric field (MIPEF) treatments induce stress reactions in tomato fruits after 24 h of refrigeration by stimulating metabolic activity and accumulating secondary metabolites. MIPEFs affect metabolism as a result of stress, with the consequent generation of reactive oxygen species (ROS). ROS are required for the synthesis of polyphenols, which are widely known to be part of the defense response of plants to stress.<sup>6,7</sup>

Thermal processing is the most common method to extend the shelf life of juices, by inactivating microorganisms and enzymes. However, heat treatments reduce the sensory and nutritional qualities of these products. Up to now, studies have suggested that high-intensity pulsed electric field (HIPEF)

treatment is efficient enough to destroy microorganisms in fruit juices at levels equivalent to those achieved by heat pasteurization without greatly affecting their nutritional and sensory properties.<sup>8,9</sup> On the other hand, some studies suggested that HIPEF processing may enhance the antioxidant properties of juices compared to untreated juices.<sup>10,11</sup> However, knowledge about the effects of this emerging technology on the antioxidant potential of juices prepared with fruits treated by MIPEFs is not currently available. Therefore, the aim of this work was to evaluate the influence of the consecutive application of MIPEF and HIPEF treatments on the polyphenol profile of tomato juices.

### ■ MATERIALS AND METHODS

**Standards and Reagents.** All samples and standards were handled without exposure to light. Caffeic, ferulic, *p*-coumaric, and chlorogenic acids, rutin, quercetin, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, 97%), and manganese dioxide were purchased from Sigma (Madrid, Spain). Naringenin, naringenin-7-*O*-glucoside, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Extrasynthèse (Genay, France). Hydrochloric acid (35%) and acetic acid (99.8%) were purchased from Panreac (Barcelona, Spain). Anhydrous sodium acetate (2 M) was purchased from Merck (Darmstadt, Germany). Ethanol, methanol, and formic acid [high-performance liquid chromatography (HPLC) grade] were obtained

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from Scharlau (Barcelona, Spain). Ultrapure water (Milli-Q) was obtained from Millipore (Bedford, MA).

**Tomato Juice.** *Tomatoes.* Tomato fruits (*Lycopersicon esculentum* Mill., cv. Daniella) at commercial maturity were purchased from a local supermarket (Lleida, Spain). pH (Crison 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, U.K.), and color (Minolta CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) of the tomatoes were determined. The physicochemical characteristics of tomatoes were as follows: pH,  $4.45 \pm 0.0$ ; soluble solids,  $3.8 \pm 0.1^\circ$  Brix; firmness,  $20.4 \pm 2.51$  N s; and color, with  $L^*$ ,  $38.5 \pm 0.4$ ;  $a^*$ ,  $18.1 \pm 1.9$ ; and  $b^*$ ,  $24.6 \pm 1.8$ .

**MIPEF Processing of Tomatoes.** MIPEF treatments were conducted in a batch equipment manufactured by Physics International (San Leandro, CA), which deliver pulses from a capacitor of 0.1  $\mu$ F. 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 fruit were treated at 1 kV  $\text{cm}^{-1}$  using 16 monopolar pulses of 4  $\mu$ s at a frequency of 0.1 Hz according to a previous study.<sup>6</sup> MIPEF-treated tomato fruit were collected and immediately refrigerated at 4 °C for 24 h, as previously described by Galindo et al.<sup>12</sup> Untreated tomatoes were stored separately at 4 °C for 24 h.

**Preparation of Tomato Juice.** Both untreated and MIPEF-treated tomatoes were ground and then filtered through 2 mm steel sieves. The physicochemical characteristics of untreated tomato juices were as follows: electrical conductivity,  $0.73 \pm 0.02$  S  $\text{m}^{-1}$ ; pH,  $4.24 \pm 0.19$ ; soluble solids,  $3.8 \pm 0.1^\circ$  Brix; and color, with  $L^*$ ,  $22.8 \pm 0.8$ ; and  $h^*$ ,  $35.6 \pm 1.8$ . The parameters of MIPEF-treated tomato juices were as follows: electrical conductivity,  $0.76 \pm 0.01$  S  $\text{m}^{-1}$ ; pH,  $4.41 \pm 0.17$ ; soluble solids,  $3.9 \pm 0.1^\circ$  Brix; and color, with  $L^*$ ,  $24.8 \pm 0.9$ ; and  $h^*$ ,  $38.6 \pm 1.4$ .

**HIPEF Processing of Tomato Juices.** Pulse treatments were carried out using a continuous flow bench-scale system (OSU-4F, The Ohio State University, Columbus, OH) that provides squared-wave pulses within eight co-field flow chambers in series. Each chamber had a treatment volume of 0.012  $\text{cm}^3$ , delimited by two stainless-steel electrodes and separated by a gap of 0.29 cm. The flow rate of the process was adjusted to 60  $\text{mL min}^{-1}$  and controlled by a variable-speed pump (model 752210-2S, Cole Palmer Instrument Company, Vernon Hills, IL). The treatment temperature was kept below 40 °C using a cooling coil, which was connected before and after each pair of chambers and submerged in an ice–water shaking bath. HIPEF treatment was set up at 35 kV  $\text{cm}^{-1}$  for 1500  $\mu$ s using bipolar squared-wave pulses of 4  $\mu$ s and a frequency of 100 Hz.<sup>8</sup>

**Thermal Treatment of Tomato Juice.** To compare the effect of HIPEF treatment to that of conventional thermal treatment, tomato juice was subjected to heat processes at 90 °C for 60 s. Tomato juice was thermally processed in a tubular stainless-steel heat-exchange coil immersed in a hot-water shaking bath (University of Lleida, Lleida, Spain). A gear pump was used to maintain the desirable juice flow rate. After thermal processing, the juice was immediately cooled in a heat-exchange coil immersed in an ice–water bath.

**Packaging and Storage Conditions.** Polypropylene, sterile, 100 mL bottles were filled directly from the outlet of the treatment systems, leaving as little headspace as possible. Afterward, the container was tightly closed and stored at  $4 \pm 1$  °C for 56 days.

**Extraction and Analysis of Polyphenols.** *Extraction of Polyphenols.* Samples were treated in a dark room with a red safety light to avoid oxidation of the analytes. Tomato juices (0.5 g) were weighed and homogenized with 80% ethanol in Milli-Q water (4 mL); the homogenates were sonicated for 5 min and centrifugated (4000 rpm at 4 °C) for 15 min. The supernatant was transferred into a flask, and extraction was repeated. The supernatants were combined and evaporated under nitrogen flow. Finally, the residue was redissolved with up to 2 mL of Milli-Q water containing 0.1% formic acid and filtered through a 13 mm, 0.45  $\mu$ m polytetrafluoroethylene (PTFE) filter from Waters (Mildford, MA) into an insert-amber vial.<sup>13</sup>

Solid-phase extraction (SPE) was carried out to eliminate interferences, such as ascorbic acid, amino acids, and reducing sugars.

For this procedure, Oasis MAX cartridges with 30 mg of mixed-mode anion-exchange and reversed-phase sorbent from Waters were used.<sup>14</sup> The eluted fractions were evaporated under nitrogen flow, and the residue was redissolved with up to 500  $\mu$ L of Milli-Q water containing 0.1% formic acid and filtered through a 13 mm, 0.45  $\mu$ m PTFE filter into an insert-amber vial for HPLC analysis.

**Analysis of Polyphenols.** HPLC–electrospray ionization (ESI)–tandem mass spectrometry (MS/MS) was used to evaluate the content of flavonols, flavanones, and hydroxycinnamic acids.<sup>15</sup> The liquid chromatography equipment was an Agilent series 1100 HPLC instrument (Agilent, Waldbronn, Germany) equipped with a quaternary pump, an autosampler, and a column oven set to 30 °C. The mobile phase consisted of Milli-Q water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). The separation of phenolic compounds was performed with a Luna C<sub>18</sub> column, 50  $\times$  2.0 mm inner diameter, 5  $\mu$ m (Phenomenex, Torrance, CA). The injection volume was 20  $\mu$ L, and the flow rate was 0.4  $\text{mL min}^{-1}$ . Separation was carried out in 20 min under the following conditions: 0 min, 5% B; 16 min, 40% B; 17 min, 95% B; 19 min, 95% B; and 19.5 min, 5% B. The column was equilibrated for 5 min prior to each analysis.

An API 3000 (PE Sciex, Concord, Ontario, Canada) triple quadrupole mass spectrometer equipped with a Turbo Ionspray source in negative-ion mode was used to obtain MS/MS data.<sup>15</sup> Quantification of polyphenols was performed by the internal standard method. Polyphenols were quantified in relation to their corresponding standard, and results were expressed as  $\mu\text{g g}^{-1}$  of dry weight. When standards were not available, as in the case of caffeic-*O*-glucoside and ferulic-*O*-glucoside acids, they were quantified with respect to the corresponding hydroxycinnamic acid (caffeic and ferulic acids).

The total polyphenol (TP) content was obtained by the sum of each individual polyphenol.

**Antioxidant Capacity of the Hydrophilic Fraction.** The tomato juice extracts, which were prepared for the analysis of the polyphenols, were used to analyze the hydrophilic antioxidant capacity (HAC). The HAC was measured using an ABTS<sup>+</sup> radical decolorization assay and a DPPH assay.<sup>16</sup>

**ABTS<sup>+</sup> Assay.** A total of 1 mM Trolox (antioxidant standard) was prepared in methanol. Working standards were carried out by diluting 1 mM Trolox with methanol. Solutions of known Trolox were used for calibration.

An ABTS<sup>+</sup> 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  $\mu$ m PTFE filter (Waters). Then, 245  $\mu$ L of ABTS<sup>+</sup> solution was added to 5  $\mu$ L of Trolox or tomato juice extracts, and the solutions were stirred for 30 s. The homogenate was shaken vigorously and kept in the dark for 1 h. Absorption of the samples was measured on a UV–vis Thermo Multiskan Spectrum spectrophotometer at 734 nm, and methanol blanks were run in each assay. Results were expressed as millimoles of Trolox equivalent (TE) 100  $\text{g}^{-1}$  of fresh weight (FW).

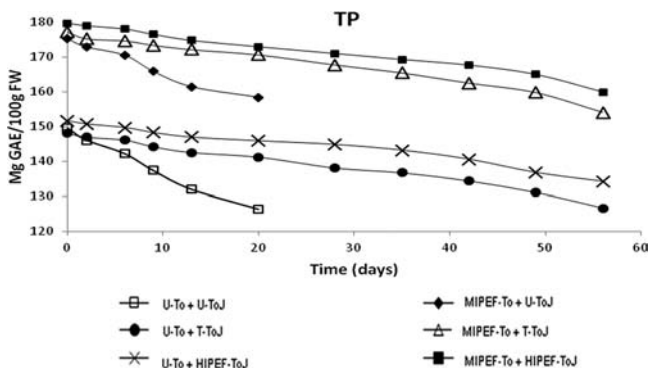
**DPPH Assay.** The antioxidant capacity was also studied through the evaluation of the free-radical-scavenging effect on the DPPH radical. Solutions of known Trolox were used for calibration. A total of 5  $\mu$ L of tomato juice extracts or Trolox were mixed with 250  $\mu$ L of methanolic DPPH (0.025  $\text{g L}^{-1}$ ). The homogenate was shaken vigorously and kept in the dark for 30 min. Absorption of the samples was measured on the spectrophotometer at 515 nm. Results were expressed as millimoles of TE 100  $\text{g}^{-1}$  of FW.

**Statistical Analysis.** Treatments of tomato juice were carried out in duplicate, and each replicate was analyzed 3 times. Significance of the results and statistical differences were analyzed using Statgraphics Plus, version 5.1, software (Manugistics, Inc., Rockville, MA). Data were analyzed by multifactor analysis of variance, and a Duncan multiple range test was applied to determine differences among means, with a significance level of  $p = 0.05$ .

## RESULTS AND DISCUSSION

### Effect of Processing and Storage on Polyphenol Compounds.

The initial TP content of tomato juices, obtained by the sum of each individual polyphenol, ranged from 148 to 151  $\mu\text{g g}^{-1}$  of FW for those prepared with untreated tomatoes and from 175 to 180  $\mu\text{g g}^{-1}$  of FW for juices made of MIPEF-treated tomatoes (Figure 1). The results

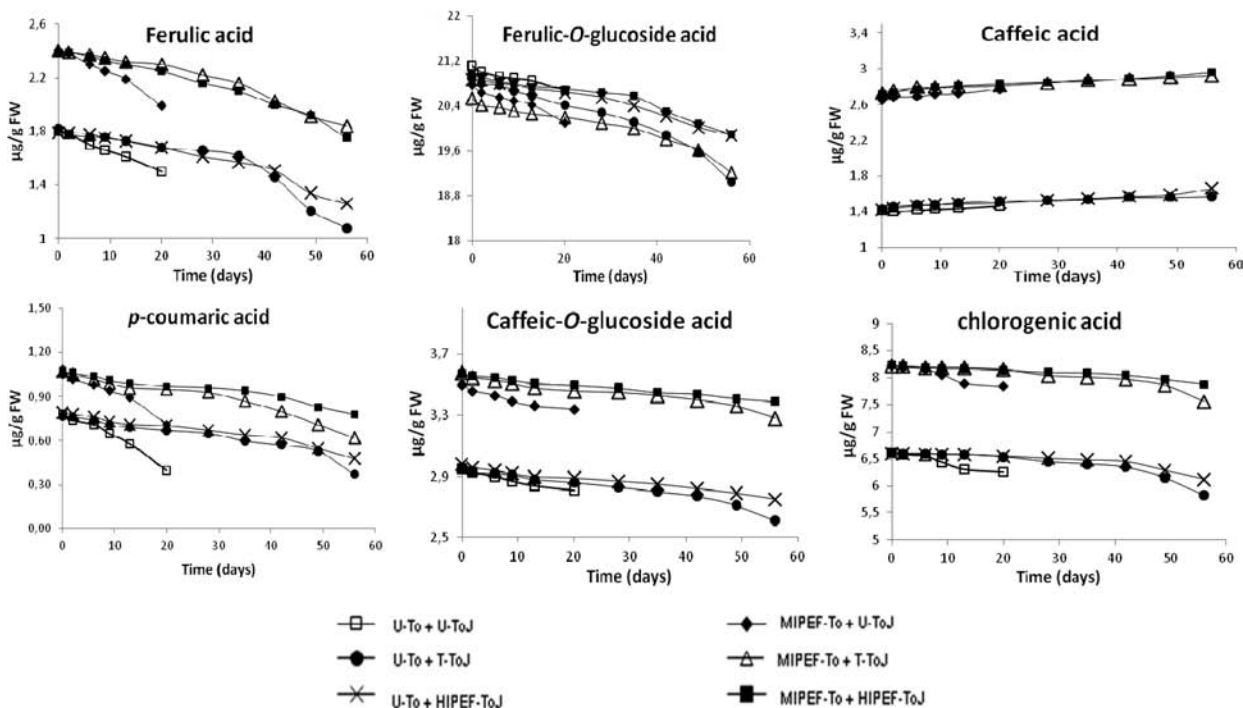


**Figure 1.** Effects of HIPEFs and heat treatments on TP of tomato juices made of untreated and MIPEF-treated tomatoes through storage at 4 °C. U-To + U-ToJ, untreated tomato + untreated tomato juice; U-To + HIPEF-ToJ, untreated tomato + HIPEF-treated tomato juice; U-To + T-ToJ, untreated tomato + thermal-treated tomato juice; MIPEF-To + U-ToJ, MIPEF-treated tomato + untreated tomato juice; MIPEF-To + HIPEF-ToJ, MIPEF-treated tomato + HIPEF-treated tomato juice; and MIPEF-To + T-ToJ, MIPEF-treated tomato + thermal-treated tomato juice. Data shown are the mean  $\pm$  standard deviation.

were significantly higher ( $p < 0.05$ ) in juices made of MIPEF-treated tomatoes in comparison to those made of untreated tomatoes. This fact could be attributed to the defense response of plants to MIPEFs.<sup>6</sup> MIPEF treatment provides potential to induce stress reactions in tomato fruits after 24 h of refrigeration by enhancing metabolic activity and accumulating secondary metabolites but also an increased permeability of the cellular membrane because of MIPEF processing, which could potentially make the extraction of the bioactive constituent more efficient.

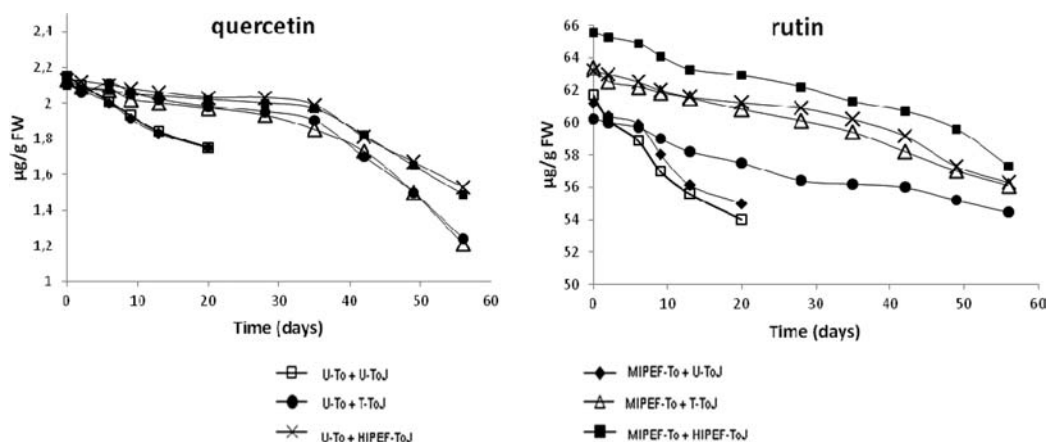
HIPEF processing better maintained the content of polyphenols in tomato juices than thermal treatments. Lower processing temperatures reached through HIPEF processing ( $T < 40$  °C) would explain the higher retention of polyphenols in HIPEF-treated tomato juices compared to the thermally processed samples.

HIPEF-processed tomato juices from untreated and MIPEF-treated tomatoes presented significantly ( $p < 0.05$ ) higher TP content (134.2–160.0  $\mu\text{g g}^{-1}$  of FW) throughout the storage period than those treated at 90 °C for 60 s. Juices treated by heat showed levels of TP content ranging from 127.5 to 155.2  $\mu\text{g g}^{-1}$  of FW (Figure 1) at 56 days of cold storage. The TP concentration was kept stable during the first 10 days of storage in fresh and treated juices. Afterward, a significant decrease ( $p > 0.05$ ) was observed in fresh juices, whereas for HIPEF- and thermal-treated juices, the TP content significantly depleted ( $p > 0.05$ ) after day 30 of cold storage. This trend is in accordance with other studies that determined the effects of heat and HIPEF treatments over storage on tomato juices made of untreated tomatoes.<sup>17</sup> Peroxidase and oxidase are involved in the oxidative degradation of phenolic compounds. Thus, the degradation of phenolic compounds during storage might be



**Figure 2.** Effects of HIPEF and heat treatments on hydroxycinnamic acids of tomato juices made of untreated and MIPEF-treated tomatoes through storage at 4 °C. U-To + U-ToJ, untreated tomato + untreated tomato juice; U-To + HIPEF-ToJ, untreated tomato + HIPEF-treated tomato juice; U-To + T-ToJ, untreated tomato + thermal-treated tomato juice; MIPEF-To + U-ToJ, MIPEF-treated tomato + untreated tomato juice; MIPEF-To + HIPEF-ToJ, MIPEF-treated tomato + HIPEF-treated tomato juice; and MIPEF-To + T-ToJ, MIPEF-treated tomato + thermal-treated tomato juice. Data shown are the mean  $\pm$  standard deviation.





**Figure 3.** Effects of HIPEF and heat treatments on flavonols of tomato juices made of untreated and MIPEF-treated tomatoes through storage at 4 °C. U-To + U-ToJ, untreated tomato + untreated tomato juice; U-To + HIPEF-ToJ, untreated tomato + HIPEF-treated tomato juice; U-To + T-ToJ, untreated tomato + thermal-treated tomato juice; MIPEF-To + U-ToJ, MIPEF-treated tomato + untreated tomato juice; MIPEF-To + HIPEF-ToJ, MIPEF-treated tomato + HIPEF-treated tomato juice; and MIPEF-To + T-ToJ, MIPEF-treated tomato + thermal-treated tomato juice. Data shown are the mean  $\pm$  standard deviation.

associated with the residual activity of phenol oxidase or phenol peroxidase. It has been demonstrated that both thermal and HIPEF treatments could partially inhibit peroxidase in tomato juices.<sup>18</sup> However, a residual peroxidase activity of 10 and 5% was obtained after applying thermal and HIPEF treatments, respectively. Therefore, it could be the reason for the high content of polyphenols in juices treated with HIPEF treatments.

**Phenolic Acids.** The main hydroxycinnamic acid derivative in tomato juices was chlorogenic acid. The content of chlorogenic acid was 25% higher in juices prepared with MIPEF-treated tomatoes than in juices made of untreated tomatoes (Figure 2).

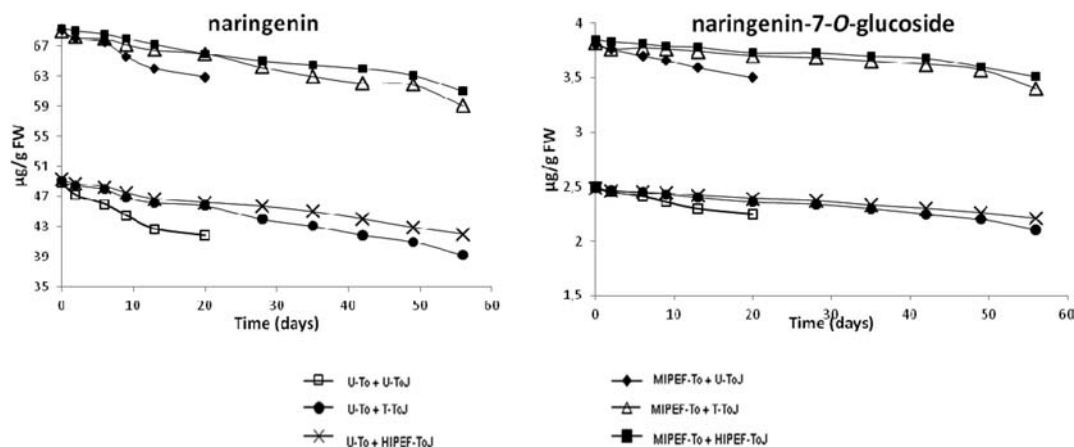
Chlorogenic acid was also found to be in significantly ( $p < 0.05$ ) higher concentrations just after processing and over time in HIPEF-treated tomato juices than in juices processed by heat, irrespective of the tomatoes used. Tomato juices underwent a substantial loss ( $p < 0.05$ ) of chlorogenic acid from 6.6 to 5.8–6.1  $\mu\text{g g}^{-1}$  of FW in juices made of untreated tomatoes and from 8.2 to 7.7–7.9  $\mu\text{g g}^{-1}$  of FW in juices prepared with MIPEF-treated tomatoes at 56 days of storage at 4 °C (Figure 2). These results were similar to those reported by Odriozola-Serrano et al.,<sup>8</sup> in which chlorogenic acid was also found to be in greater concentrations over the time in juices processed by HIPEFs than in those thermally treated, when tomato juice was prepared with untreated tomatoes. Tomato juices have been found to be a rich source of caffeic acid (1.4–2.7  $\mu\text{g g}^{-1}$  of FW), ferulic acid (1.8–2.4  $\mu\text{g g}^{-1}$  of FW), and *p*-coumaric acid (0.8–1.0  $\mu\text{g g}^{-1}$  of FW) and their glycosylated forms. Significantly ( $p < 0.05$ ) lower concentrations of caffeic acid (87%), *p*-coumaric acid (34%), and ferulic acid (33%) were obtained in juices prepared with untreated tomatoes compared to those made of MIPEF-processed tomatoes (Figure 2). Ferulic-*O*-glucoside acid was not affected by MIPEF treatments ( $p > 0.05$ ), whereas caffeic-*O*-glucoside acid was enhanced from 3 to 3.5  $\mu\text{g g}^{-1}$  of FW in juices made of MIPEF-treated tomatoes.

Higher concentrations in ferulic, *p*-coumaric, and caffeic acids were obtained just after processing in HIPEF-treated tomato juices than in heat-treated juices, regardless of the tomatoes used, related to the higher processing temperatures reached in thermally processed samples. Changes in ferulic, *p*-coumaric,

and caffeic acids during storage are shown in Figure 2. The caffeic acid concentration was slightly enhanced ( $p < 0.05$ ) during the storage time, reaching maximal values of 1.6–1.7  $\mu\text{g g}^{-1}$  of FW at 56 days in juices prepared with untreated tomatoes and 2.9–3.0  $\mu\text{g g}^{-1}$  of FW in juices made of MIPEF-treated tomatoes. Tomato juices underwent a substantial depletion ( $p < 0.05$ ) of *p*-coumaric acid over time, leading to values of 0.4–0.8  $\mu\text{g g}^{-1}$  of FW at 56 days of cold storage, which may be a consequence of its conversion to caffeic acid.<sup>19</sup> Therefore, the increase of caffeic acid in tomato juices during 56 days of storage may be directly associated with residual hydroxylase activities, which convert *p*-coumaric acid in caffeic acid. Ferulic-acid- and caffeic-acid-glycosylated forms decreased ( $p < 0.05$ ) during storage. The content of ferulic-*O*-glucoside acid was maintained during the first 35 days, whereas the caffeic-*O*-glucoside acid concentration was kept during 49 days of cold storage, irrespective of the processing treatment applied. In caffeic-*O*-glucoside and ferulic-*O*-glucoside acids, the 3-hydroxy function at the C ring of the flavonoid is blocked by a sugar moiety. Thus, the blockage of the 3-hydroxyl group is perhaps one of the reasons for the greater stability of glycosidic forms toward their aglycones.<sup>20</sup>

**Flavonols.** The content of quercetin and rutin was not enhanced in juices prepared with tomatoes processed by MIPEFs. The initial content of rutin in tomato juices was 60.3–63.2  $\mu\text{g g}^{-1}$  of FW in juices made of untreated tomatoes and 61.2–65.6  $\mu\text{g g}^{-1}$  of FW in juices prepared with MIPEF-treated tomatoes. However, quercetin was found at concentrations of 2.1  $\mu\text{g g}^{-1}$  of FW in just processed juices, irrespective of the tomatoes used. Stewart et al.<sup>3</sup> reported levels of quercetin in tomato juices ranging from 2.8 to 3.7 mg 100 mL<sup>-1</sup> of FW. Vallverdú-Queralt et al.<sup>15</sup> measured concentrations of quercetin between 0.4 and 0.8  $\mu\text{g g}^{-1}$  of FW for different commercial tomato cultivars. The quercetin concentration of tomatoes varies according to fruit cultivar, country of origin, harvesting seasons, and growing condition.

The quercetin content depleted significantly ( $p < 0.05$ ) throughout the storage of tomato juices, reaching values of 1.2–1.5  $\mu\text{g g}^{-1}$  of FW (Figure 3) at 56 days, whereas rutin slightly decreased during storage. This fact could be attributed to the bonded 3-hydroxyl group in the case of rutin, which may be one of the reasons for the greater stability of rutin forms



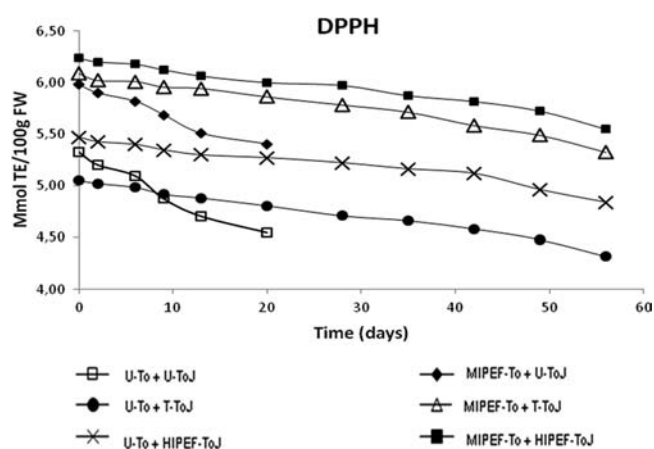
**Figure 4.** Effects of HIPEF and heat treatments on flavanones of tomato juices made of untreated and MIPEF-treated tomatoes through storage at 4 °C. U-To + U-ToJ, untreated tomato + untreated tomato juice; U-To + HIPEF-ToJ, untreated tomato + HIPEF-treated tomato juice; U-To + T-ToJ, untreated tomato + thermal-treated tomato juice; MIPEF-To + U-ToJ, MIPEF-treated tomato + untreated tomato juice; MIPEF-To + HIPEF-ToJ, MIPEF-treated tomato + HIPEF-treated tomato juice; and MIPEF-To + T-ToJ, MIPEF-treated tomato + thermal-treated tomato juice. Data shown are the mean  $\pm$  standard deviation.

toward quercetin.<sup>20</sup> However, HIPEF-treated juices showed significantly ( $p < 0.05$ ) greater quercetin and rutin contents than juices processed by heat just after processing and during storage for 56 days at 4 °C, irrespective of the tomatoes used. These results are in agreement with those reported by Odriozola-Serrano et al.,<sup>8</sup> who studied the effects of thermal and HIPEF treatments on tomato juices made with untreated tomatoes.

**Flavanones.** The content of naringenin was 40% higher in juices made of MIPEF-treated tomatoes in comparison to juices prepared with untreated tomatoes. The same tendency was observed for the naringenin glycosidic form. The content of naringenin-7-*O*-glucoside in juices prepared with MIPEF-treated tomatoes was 52% higher than in juices made of untreated tomatoes. After MIPEF treatments, the resealing process takes place in a time scale of seconds or minutes. The higher content of naringenin and its glycosylated form in juices made of MIPEF-treated tomatoes could be attributed to the induction of flavanone synthase when MIPEF treatments are applied.<sup>6</sup>

Thermal and HIPEF processing did not modify the initial content of naringenin and naringenin-7-*O*-glucoside in tomato juices. The naringenin concentration depleted significantly ( $p < 0.05$ ) throughout the storage, leading to values of 39.2–42.0  $\mu\text{g g}^{-1}$  of FW for juices made of untreated tomatoes and 59.1–62.9  $\mu\text{g g}^{-1}$  of FW for juices prepared with MIPEF-treated tomatoes (Figure 4), whereas glycosylated naringenin slightly changed during storage.<sup>21</sup> HIPEF-processed tomato juices better maintained the flavanones content during the storage period than thermally treated and untreated juices (Figure 4).

**Effect of Processing and Storage on HAC.** Figure 5 shows changes in the antioxidant capacity of tomato juices measured through the DPPH and ABTS assays. According to both methods, tomato juices made of MIPEF-treated tomatoes had significantly ( $p < 0.05$ ) higher HAC (4.28–6.24 mmol of TE 100  $\text{g}^{-1}$  of FW) than tomato juices prepared with untreated tomatoes (3.07–5.47 mmol of TE 100  $\text{g}^{-1}$  of FW). HIPEF-processed tomato juices obtained from untreated and MIPEF-treated tomatoes showed significantly ( $p < 0.05$ ) higher HAC (3.3–5.6 mmol of TE 100  $\text{g}^{-1}$  of FW) just after processing and throughout the storage period than those treated at 90 °C for 60 s. Juices treated by heat showed levels of HAC ranging from



**Figure 5.** Effects of HIPEF and heat treatments on antioxidant capacities of tomato juices made of untreated and MIPEF-treated tomatoes through storage at 4 °C. U-To + U-ToJ, untreated tomato + untreated tomato juice; U-To + HIPEF-ToJ, untreated tomato + HIPEF-treated tomato juice; U-To + T-ToJ, untreated tomato + thermal-treated tomato juice; MIPEF-To + U-ToJ, MIPEF-treated tomato + untreated tomato juice; MIPEF-To + HIPEF-ToJ, MIPEF-treated tomato + HIPEF-treated tomato juice; and MIPEF-To + T-ToJ, MIPEF-treated tomato + thermal-treated tomato juice. Data shown are the mean  $\pm$  standard deviation.

2.6 to 5.3 mmol of TE 100  $\text{g}^{-1}$  of FW at 56 days of storage (Figure 5). The changes in the antioxidant capacity over time might be associated with the variations of the individual phenolics. The antioxidant capacity of fruits and vegetables is known to depend upon a wide number of compounds. A high relationship was found between relative HAC and TP contents, with  $R^2 = 0.9972$  (DPPH) and  $R^2 = 0.9834$  (ABTS<sup>+</sup>). The magnitude of changes in values of DPPH and ABTS inhibition were highly correlated to the presence of flavonones ( $R^2 = 0.9749$ – $0.9864$ ) and phenolic acids ( $R^2 = 0.9571$ – $0.9986$ ) rather than flavonol content ( $R^2 = 0.2398$ – $0.3554$ ) in tomato juices.

An enhancement of polyphenolic compounds (phenolic acids and flavanones) and HAC were observed in juices made of tomatoes processed by MIPEFs. Increases of chlorogenic acid (25%) and naringenin-7-*O*-glucoside (52%) were obtained

in juices made of MIPEF-treated tomatoes compared to those prepared with untreated tomatoes. However, the content of flavonols was not enhanced after MIPEF treatments. The amounts of individual polyphenol compounds underwent a substantial loss during storage in both juices prepared with MIPEF-treated and untreated tomatoes, with the exception of the caffeic acid content. However, HIPEF-processed tomato juices better maintained the individual polyphenols just after processing and during the storage period than thermally treated and fresh juices. Consecutive application of MIPEF and HIPEF treatments could be proposed as a strategy for producing tomato juices with high antioxidant properties.

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### Notes

The authors declare no competing financial interest.

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