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# Volatile Profile and Sensory Evaluation of Tomato Juices Treated with Pulsed Electric Fields

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**ABSTRACT:** Tomato juices produced from tomatoes subjected to moderate-intensity pulsed electric fields (MIPEF) and from untreated tomatoes were preserved by high-intensity pulsed electric fields (HIPEF) or by thermal treatment (TH) having, in both cases, the fresh juice as a reference. The chemical and sensory changes of tomato juices stored at 4 °C for increasing period of time were analyzed. A quantitative descriptive analysis was developed to characterize the sensory quality of samples. Tomatoes subjected to MIPEF treatments led to tomato juices with a higher content of volatile compounds and better sensory properties than those prepared with untreated tomatoes. An enhancement was observed in hexanal and (E)-2-hexenal just after processing in juices prepared with MIPEF-treated tomatoes. A slight decrease in volatile compounds and a loss of sensory quality was observed over time in TH and HIPEF juices, but HIPEF-processed samples just after processing and through storage maintained higher overall quality.

KEYWORDS: HIPEF, MIPEF, pulsed electric fields, sensory analysis, tomato juices, volatile compounds

# INTRODUCTION

Tomato, either fresh or industrially processed, is a widely consumed vegetable, and its flavor is an important criterion to determine its acceptability.<sup>1</sup> Approximately 400 volatile compounds have been identified in tomatoes, but only some of them are considered to have a high impact on tomato aroma due to their amount and threshold of perception by humans. These compounds are (E)-2-hexenal, (Z)-3-hexenal, hexanal, (Z)-3-hexen-1-ol, hexanol, 2-isobutylthiazole, and 6-methyl-5hepten-2-one.<sup>1,2</sup> Volatile compounds are formed in the intact tomato fruit upon tissue disruption or during ripening and originate from many substrates including carotenoids, terpenoids, amino acids, lipids, and lignin.<sup>3</sup> Tomatoes described as full-flavored are characterized by a low level of titratable acidity, high contents of total sugars and soluble solids, and intermediate contents of hexanal, (Z)-3-hexenal, 2- and 3methyl-1-butanol, (E)-2-hexenal, (Z)-3-hexenol, geranyl acetone,  $\beta$ -ionone, and 1-penten-3-one.<sup>3</sup>

The most common method to extend the shelf life of juices by inactivating microorganisms and enzymes is thermal processing. However, heat treatments reduce the sensory and nutritional qualities of these products.<sup>4</sup> Up to now, studies have suggested that high-intensity pulsed electric fields (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>1,5</sup>

Recently, high HIPEF have been developed as a nonthermal emerging technology for food preservation, whereas moderate-

intensity pulsed electric fields (MIPEF) have been applied as a possible treatment to induce stress reactions in plant systems stimulating the production of secondary plant metabolites.<sup>6</sup> In particular, Vallverdú-Queralt et al. reported that MIPEF treatments induce stress reactions in tomato fruits after 24 h of refrigeration.<sup>7</sup>

The first aim of the present work was to study and compare the profile in volatile compounds of HIPEF and heat-processed tomato juices prepared with MIPEF-treated and untreated tomatoes during the commercial shelf life. The volatile molecules were analyzed by solid phase microextraction coupled with gas chromatographic separation and mass detection (SPME/GC-MS). Another important scope of this work was to develop a quantitative descriptive sensory analysis able to describe the most important attributes perceivable by sight and smell on these tomato juice samples. The conjoint analysis between chemical and sensory data permitted the definition of volatile molecules responsible for positive or negative sensory olfactive attributes. As far as we know, it is the first time that a study has been carried out to characterize in terms of volatile compounds and sensory properties processed tomato juices by the application of new technologies such as moderate-intensity and high-intensity pulsed electric fields.

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#### High intensity pulsed electric fields (HIPEF)

Figure 1. Flowchart of sampling.

### MATERIALS AND METHODS

**Preparation of Tomato Juice.** Tomato fruits (*Lycopersicon esculentum* Mill. cv. Daniella) were purchased from a local supermarket (Lleida, Spain). For each operation of tomato juice production some basic chemical and physical parameters were determined: pH (Crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), soluble solids content (Atago RX-1000 refractometer; Atago Co. Ltd., Tokyo, Japan), firmness (Texturometer-XT2 Stable Micro Systems Ltd., Surrey, UK), and color (Minolta CR-400, Konica Minolta Sensing, Inc., Osaka, Japan), according to the methods of Vallverdú-Queralt et al.<sup>7</sup>

**MIPEF Processing of Tomatoes.** MIPEF treatments were conducted in batch equipment manufactured by Physics International (San Leandro, CA, USA), which can deliver pulses from a capacitor of 0.1  $\mu$ F 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 1 kV cm<sup>-1</sup> using 16 monopolar pulses of 4  $\mu$ s at a frequency of 0.1 Hz according to a previous study.<sup>7</sup> MIPEF-treated tomato and untreated tomato fruits were collected and immediately refrigerated at 4 °C during 24 h (Figure 1).

**Preparation of Tomato Juice.** Both untreated and MIPEFtreated tomatoes were ground and filtered through 2 mm steel to produce tomato juices. The tomato juices obtained from these samples were analyzed at three subsequent intervals (days) during storage (0-MIPEF-FR, 6-MIPEF-FR, 13-MIPEF-FR and 0-NT-FR, 6-NT-FR, 13-NT-FR, respectively; Figure 1).

HIPEF Processing of Tomato Juices. Pulse treatments were carried out using a continuous flow bench scale system (OSU-4F, Ohio State University, Columbus, OH, USA), which provides squared-wave pulses within eight cofield flow chambers in series. Each chamber had a treatment volume of 0.012 cm<sup>3</sup>, 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 mL min<sup>-1</sup> and controlled by a variable-speed pump (model 752210-25, Cole Palmer Instrument Co., Vermon Hills, IL, USA). 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 cm<sup>-1</sup> for 1500  $\mu$ s using bipolar squared-wave pulses of 4  $\mu$ s and a frequency of 100 Hz, as described in the literature.<sup>5</sup> The codes used for tomato samples treated with MIPEF and not treated (NT), and then subjected to HIPEF treatment and

checked at different periods of time during storage were as follows: 0-MIPEF-HIPEF, 0-NT-HIPEF, 6-MIPEF-HIPEF, 6-NT-HIPEF, 13-MIPEF-HIPEF, 13-NT-HIPEF, 28-MIPEF-HIPEF, 28-NT-HIPEF, 42-MIPEF-HIPEF, 42-NT-HIPEF, 56-MIPEF-HIPEF, 56-NT-HIPEF (Figure 1).

**Thermal Treatment of Tomato Juice.** To compare the effect of HIPEF treatment to that of the conventional thermal treatment (TH), tomato juice was subjected to heat processes at 90 °C for 60 s. These conditions were selected on the basis of the literature, where typical heat treatments of juices vary from 95 to 90 °C for 15–60 s.<sup>8</sup> 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. The codes used for tomato treated with MIPEF and not treated (NT) and then subjected to thermal treatment and checked at different periods of time during storage were as follows: 0-MIPEF-TH, 0-NT-TH, 6-MIPEF-TH, 6-NT-TH, 13-MIPEF-TH, 13-NT-TH, 28-MIPEF-TH, 28-NT-TH, 42-MIPEF-TH, 42-NT-TH, 56-MIPEF-TH, 56-NT-TH (Figure 1).

**Packaging and Storage Conditions.** Sterile 100 mL polypropylene 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.

Analysis of Volatile Compounds by SPME/GC-MS. The tomato juice (1.5 g), weighed into a 10 mL vial fitted with a silicone septum, was spiked with 0.15 g of 4-methyl-2-pentanone (internal standard dissolved in water) to a concentration of 2.5 mg kg<sup>-1</sup>. Tomato juice was thermostated at 40 °C and maintained under magnetic stirring. A divinylbenzene/carboxen/polydimethylsiloxane fiber (DVB/CAR/PDMS, 50/30 mm, 2 cm long from Supelco Ltd., Bellefonte, PA, USA) was conditioned for 2 min, exposed to the sample headspace for 30 min, and immediately desorbed for 3 min at 250 °C in an Agilent 6890N Network gas chromatograph (Agilent Technologies, Palo Alto, CA, USA). Volatile compounds were identified and quantified by GC coupled with an Agilent 5973 Network quadrupolar mass selective spectrometry (Agilent Technologies, Palo Alto, CA, USA). Analytes were separated on a ZB-WAX column, 30 m × 0.25 mm i.d., 1.00 mm film thickness (Phenomenex, Torrance, CA, USA). Column temperature was held at 40 °C for 10 min and increased to 200 °C at 3 °C min<sup>-1</sup>. The FID temperature was set at 250 °C, and the ion source and the transfer line were at 180 and 230 °C, respectively. Electron impact mass spectra were recorded at 70

volatile compound	Т0	Т6	T13	T28	T42	T56	KI	threshold values in water
1-penten-3-one	10.00-13.08	9.88-13.07	9.80-12.90	9.70-12.70	9.45-12.55	9.31-12.31	1047	
hexanal	3.40-18.11	1.88-6.80	1.75-7.81	1.61-8.23	1.51-8.59	1.39-8.77	1111	$4.5 \times 10^{-3},$ $5.8 \times 10^{-3}$
(E)-2-hexenal	2.32-14.70	1.98-7.96	1.76-5.50	1.51-4.29	1.21-4.10	1.01-4.01	1248	$17 \times 10^{-3}$
6-methyl-5-hepten- 2-one	2.56-7.01	0.82-3.06	0.61-2.94	0.61-2.59	0.51-2.20	0.21-1.99	1368	$0.05 \times 10^{-3}$
hexanol	1.51-5.75	1.55-4.53	0.88-3.88	0.88-3.50	0.91-3.59	0.99-3.85	1381	
(Z)-3-hexenol	7.50-10.03	7.31-9.88	7.10-9.50	7.05-9.33	6.70-9.10	5.50-8.54	1414	
2-isobutylthiazole	1.01-3.81	0.88-3.52	0.80-3.61	0.80-3.60	0.78-3.41	0.79-3.40	1437	$0.0035 \times 10^{-3}$
(E,E)-2,4-decadienal	0.08-0.09	0.11-0.72	0.22-0.99	0.44-0.65	0.70-0.90	1.01-1.45	1839	
geranyl acetone	1.53-3.31	0.90-2.88	0.56-2.69	0.31-2.44	0.10-2.31	0.00-2.11	1889	$0.06 \times 10^{-3}$
<sup>a</sup> Threshold values in water are expressed as mg L <sup>-1</sup> . T0, day 0; T6, day 6; T13, day 13; T28, day 28; T42, day 42; T56, day 56. KI, Kovats index.								

Table 1. Main Volatile Compounds Selected as Markers of Analyzed Tomato Juices Expressed as Milligrams of 4-Methyl-2pentanone per Kilogram of Tomato Juice<sup>a</sup>

eV ionization energy in the 20–250 amu mass range, 2 scan s<sup>-1,9</sup> The identification of the volatile compounds was carried out by a comparison of their mass spectral data with information from the National Institute of Standards and Technology (NIST) library (2005 version) and confirmed matching reference standards (hexanal, (E)-2-hexenal, (Z)-3-hexenol, hexanol, 1-penten-3-one, 6-methyl-5-hepten-2-one, geranyl acetone, and 2-isobutylthiazole). Relative amounts of volatile compounds were expressed with respect to internal standard as milligrams per kilogram of tomato juice. Analysis were carried out in triplicate. Moreover, the Kovats indices were calculated using an appropriate mixture of *n*-alkanes.

Sensory Evaluation. The procedures for selection, training, and monitoring of the assessors, the choice of optimal descriptors and appropriate measure scale, and the evaluation of results were developed according to ISO 13299:2010.10 A total of 30 samples with 2 replicates were evaluated by the panelists. A total of eight trained panelists (four female and four male panelists aged 20-50 years old) participated in the sessions. The panelists were recruited on the basis of their previous experience in descriptive sensory analysis (staff and Ph.D. students at the Campus of Food Science, University of Bologna, Cesena, Italy). The panel worked in a panel room, and each assessor carried out the sensory analysis in a single booth. Data acquisition and data treatment were conducted with the Fizz software (Biosystemes, Dijon, France). The panelists were trained and samples were evaluated using a quantitative descriptive method. During the training phase, each panelist received tomato juice samples and found perceivable product attributes, by identification of appearance and odor attributes to be used in describing the tomato juice samples. The panel decided if descriptors were redundant and should be removed from the list or if there were terms that should be added. The final list of terms was written, and the panel defined each attribute. Panelists also identified possible reference standards on which the rating of the generated attributes will be based. The identified references were presented to each panelist, and specific training sessions were carried out. During the training sessions reference tomato juice samples were presented to assessors. The panel leader entered the assessment data and checked if the robust coefficient of variation of the single attributes was  $\leq 20\%$ . When the panel leader found anomalous values, the analysis was repeated. After the calibration session, all samples were presented to the panelists for evaluation. The panelists rated the samples, indicating the intensities of each attribute on a graduated scale of 10 cm with well-defined anchor points from 0 (not perceivable) on the left to 10 (perceivable at the level of saturation) on the right. A small white plastic cup, usually used for coffee, was employed for the appearance and odor evaluation. Around 10 g of tomato juice was flowed into the plastic cup. Panelists were advised to spit out the tomato juice after the analysis, and between one analysis and the following, panelists were required to wash the mouth using sparkling or natural water.

**Statistical Treatment of Data.** The software XLSTAT 7.5.2 version (Addinsoft, New York, NY) was used to analyze both the

sensory and chemical results by analysis of variance (ANOVA) and by principal components analysis (PCA).

#### RESULTS AND DISCUSSION

Physical–Chemical Characteristics of Processed Tomatoes. MIPEF-treated and untreated tomatoes were analyzed to determined some basic physical–chemical characteristics. The pH as well as the soluble solids showed proximally the same values for fresh tomatoes and MIPEF-treated and untreated tomatoes (pH, 4.45  $\pm$  0.01, 4.24  $\pm$  0.19, and 4.41  $\pm$  0.17, respectively; soluble solids, 3.8  $\pm$  0.1, 3.8  $\pm$  0.1, and 3.9  $\pm$  0.1 °Brix, respectively).

For MIPEF-treated and untreated tomato juices the electrical conductivity was also determined and no significant difference was evidenced (0.73  $\pm$  0.02 and 0.76  $\pm$  0.01 S m<sup>-1</sup>, respectively). A slight difference was found for the same samples concerning the  $L^*$  and  $h^*$  parameters ( $L^* = 24.8 \pm 0.9$  and 22.8  $\pm$  0.8 and  $h^* = 38.6 \pm 1.4$  and 35.6  $\pm 1.8$ , respectively).

**Volatile Compounds.** The effects of processing and storage time on the flavor compounds of tomato juice were investigated by using the SPME/GC-MS technique and by quantifying the variation within the main volatile compounds. The study confirmed the presence, as major markers, of hexanal, (E)-2-hexenal, (E,E)-2,4-decadienal, (Z)-3-hexenol, hexanol, 1-penten-3-one, 6-methyl-5-hepten-2-one, geranyl acetone, and 2-isobutylthiazole (Table 1), which have previously been identified as the major contributors to tomato aroma.<sup>11,12</sup> The trend of main volatile compounds during commercial shelf life was analyzed and, as examples, the decrease of (E)-2-hexenal and the increase of (E,E)-2,4-decadienal are depicted in Figure 2.

With the aldehydes taken into account, tomato juices made with untreated tomatoes contained less hexanal (from 15.01 to 1.95 mg L<sup>-1</sup> for 0-NT-FR and 13-NT-FR, respectively) and (*E*)-2-hexenal (from 11.01 to 3.88 mg L<sup>-1</sup> for 0-NT-FR and 13-NT-FR, respectively) than juices prepared with MIPEF-treated tomatoes. In these juices the concentrations varied between 18.11 and 2.51 mg L<sup>-1</sup> for hexanal and between 14.70 and 5.50 mg L<sup>-1</sup> for (*E*)-2-hexenal. As suggest by some authors,<sup>13</sup> MIPEF affects the metabolism of vegetables with the consequent generation of reactive oxygen species (ROS). ROS are endogenous signal components required for the synthesis of secondary metabolites, which are known to be part of the defense response of plants to stress.<sup>7</sup>



**Figure 2.** Decreasing trend of (E)-2-hexenal and increasing trend of (E,E)-2,4-decadienal during commercial shelf life.

The low temperatures (below 35 °C) during HIPEF processing may also promote higher retention of flavor compounds.<sup>14</sup> It is possible to note that during storage, HIPEF reduced the loss of aldehydes in comparison to heat treatments (see the trend of (*E*)-2-hexenal in Figure 2). Aguiló-Aguayo et al. also observed that the concentration of hexanal and (*E*)-2-hexenal was higher in HIPEF-treated juices than in heat-treated tomato juices.<sup>1</sup> Moreover, Min and Zhang reported an increase in (*E*)-2-hexenal in juices treated with HIPEF treatments at 40 kV cm<sup>-1</sup> for 57  $\mu$ s.<sup>14</sup>

There were no differences in the content of (E,E)-2,4decadienal in juices prepared with untreated or MIPEF-treated tomatoes; this compound, when present in concentration higher than its threshold value, can be responsible for fatty and rancid notes in the juice.<sup>15</sup> The production of (E,E)-2,4decadienal increased over time; the most marked increment was observed in juices treated by thermal treatments from day 42 to day 56 regardless of the tomatoes used for their production (MIPEF-treated or untreated).

With regard to (Z)-3-hexenol and hexanol, these compounds were increased between 18 and 25% and from 42 to 62% in juices prepared with MIPEF-treated tomatoes, respectively, in comparison to juices prepared with untreated tomatoes. These changes could be attributed to a defense response of plants to stress.<sup>7</sup> Moreover, (Z)-3-hexenol and hexanol were better retained in HIPEF-processed than in heat-treated samples beyond the storage period. For (Z)-3-hexenol, the content started to increase progressively from day 28 to day 56, whereas for hexanol, the content decreased significantly during this period. These alcohols can be originated by reductase conversions of the corresponding aldehydes formed from the metabolism of fatty acids.<sup>15</sup> The changes observed in (Z)-3hexenol during storage could also be associated with changes in hydroperoxide lyase (HPL) activity. A lower HPL activity during the storage of treated juice can lead to a lesser synthesis of aldehydes by enzymatic oxidation of unsaturated fatty acids,<sup>1</sup> because the production of lipid-derived alcohol volatiles depends on the availability of substrates in the sample.<sup>16</sup>

Moreover, tomato juices prepared with MIPEF-treated tomatoes contained between 10 and 20% higher content of 1-penten-3-one than tomato juices made with untreated tomatoes; this compound is one of the products from fatty acid oxidation by lipoxygenase pathway. During storage, a slight decrease in the concentration of 1-penten-3-one was observed after HIPEF and heat treatments. However, the initial level of  $10.2-12.2 \text{ mg L}^{-1}$  found in HIPEF-processed tomato juice, regardless of the tomatoes used for their production, remained approximately constant until the end of storage.

On the other hand, 6-methyl-5-hepten-2-one and geranyl acetone have been reported to be carotenoid-related volatile compounds that are characteristic of tomato aroma.<sup>17</sup> Both of them were higher in juices made of MIPEF-treated tomatoes than in juices prepared with untreated tomatoes, and during storage their content decreased slightly until the end of the storage period. For both carotenoid-related volatile compounds, HIPEF-processed tomato juices exhibited the highest levels of these compounds throughout storage. Aguilo-Aguayo et al. reported similar results in tomato juices treated with HIPEF and thermal treatments. They found that treated juices prepared with untreated tomatoes underwent a substantial loss of 6-methyl-5-hepten-2-one and geranyl acetone during 56 days of storage.<sup>1</sup>

Finally, 2-isobutylthiazole is the only alkylthiazole often found in tomatoes providing a grassy and sweet fruity odor.<sup>18</sup> Yilmaz<sup>19</sup> suggested that alcoholic flavors become dominant

Table	2. Attribute	s Used in	the Profile	Sheet 7	To Descri	be the	Perceived	Sensations	of	Conventional	and	Organic	Tomato
Juices,	Anchor Po	ints, and	References	Used d	uring the	Trainir	ng Phase c	of Panelists					

descriptor	definition	references	anchor points
red intensity dimension of the particles	intensity of the red color from light to dark absence or presence of particles	measured using a color scaling ruler measured with a glass rotation	
tomato sauce freshness vegetal notes	odor reminiscent of tomato sauce odor reminiscent of raw tomato intensity of the odor reminiscent of vegetal notes	tomato sauce in a glass fresh tomato carrot celery hasil oregano thyme	strong (10) strong (10) strong (10)
green acidulous	intensity of the odor reminiscent of regeta noces intensity of the odor reminiscent of grass/tomato leaves acidulous sensation generated by heat treatments; remininscent of concentrated tomato	grass/tomato leaves concentrated tomato paste in a glass	strong (10) strong (10)
anomalous odors	anomalous and unpleasant odors; reminiscent of cheese, smoked or rancid	cheese, smoked salami, rancid oil	strong (10)

Article



Figure 3. continued

Article



**Figure 3.** (a) Biplot graph that shows the distribution on the PC1/PC2 surface of tomato juices produced by MIPEF-treated tomato (0-MIPEF-FR) and not-treated tomato fruits (0-NT-FR) during storage (6-MIPEF-FR, 13-MIPEF-FR and 6-NT-FR, 13-NT-FR, respectively). (b) Biplot graph that shows the distribution on the PC1/PC2 surface of tomato juices thermal treated and stored from 0 to 56 days. (c) Biplot graph that shows the distribution on the PC1/PC2 surface of tomato juices produced by not-treated tomatoes and then subjected to HIPEF. (d) Biplot graph that shows the distribution on the PC1/PC2 surface of tomato juices obtained from tomatoes subjected to MIPEF and then treated with HIPEF.

when the effect of 2-isobutylthiazole diminishes. Tomato juices prepared with MIPEF-treated tomatoes contained higher content of 2-isobutylthiazole  $(2.02-3.81 \text{ mg L}^{-1})$  than juices made of untreated tomatoes  $(1.01-2.41 \text{ mg L}^{-1})$ . During the storage period, the content of 2-isobutylthiazole remained nearly constant for both thermally treated and HIPEF-treated juices, regardless of the tomatoes used for its production. However, the contents of 2-isobutylthiazole at the end of storage period were higher in HIPEF-treated tomato juices than in thermally treated juices.

**Sensory Evaluation.** An important task was to select an appropriate vocabulary to explain and share the perceived sensations: for this aim, in the first phase of the focus group assessors worked together to freely describe the attributes of the samples. As a consequence of this training session, red intensity and dimension of the particles were selected as appearance attributes. For odor, the panel distinguished five different attributes perceived by orthonasal routes during the smelling phase. Assessors considered as typical odor notes tomato sauce, acidulous, freshness, green, and vegetable notes. Finally, the panel added another attribute: anomalous odors. The anomalous odors descriptor included cheese, smoked, and rancid unpleasant notes. Positive attributes were considered red intensity, freshness, green, and vegetable notes; negative ones were anomalous odors and acidulous.

Special references (Table 2) of known flavors were selected to have standards for the calibration of the panel and to make possible an unambiguous assignment of sensations and attributes. Several sessions were systematically organized to obtain a satisfactory level of training of the assessors.

Conjoint Analysis of Sensory and Volatile Profiles. Results of sensory and volatile profiles were analyzed by PCA. In general, a PCA picture shows comparison of several samples (products), projected on a two-dimensional space (surface), described by two principal components (PC): PC1 and PC2. Percentages indicate which is the fraction of evaluated product variability related to each PC. To improve the explained variance among samples, the less-significant terms (lower Fvalues and higher *p* values) were deleted from the model after an ANOVA test (among all vectors the red intensity and tomato sauce descriptors were not considered in the PCA model). The first two components were responsible for 78.85% of the variance (64.26% for PC1 and 14.56% for PC2). As shown in Figure 3, it is possible to highlight that PC1 was associated, in the positive direction, to green, freshness, and vegetable notes among sensory descriptors and to (E)-2hexenal, (Z)-3-hexenol, 6-methyl-5-hepten-2-one, geranyl acetone, hexanal, hexanol, 1-penten-3-one, and 2-isobuthylthiazole among volatile molecules. On the other hand, in the negative direction, PC1 was related to dimension of the particles, acidulous, anomalous odors, and  $(E_{,E})$ -2,4-decadienal. With regard to the location of products on PC1/PC2 surface (Figure 3), if close to each other, those products are similar (taking into account the combination of all evaluated vectors); if far away from each other, they differ strongly. The approximate position of the product near certain attribute/ chemical parameter vector(s) allows us to conclude that the product has this attribute/chemical parameter particularly expressed. To better evidence differences among tomato juices otherwise subjected to technological treatments, the analysis of principal components was performed by selecting four groups of samples, and the results are shown in Figure 3. In Figure 3a, it is possible to observe the distribution of tomato juices

produced by MIPEF-treated tomato (0-MIPEF-FR) and nottreated tomato fruits (0-NT-FR) during storage (6-MIPEF-FR, 13-MIPEF-FR and 6-NT-FR, 13-NT-FR, respectively). Samples just produced (time 0) appeared richer in positive characteristics concerning both volatiles and sensory attributes than those stored for 6 days and then for 13 days. The best sample in terms of the volatiles profile and sensory attributes can be considered to be 0-MIPEF-FR. Figure 3b shows the distribution of tomato juices thermally treated and stored for 0 to 56 days: in this case it is possible to note a trend of the samples stored for a longer time versus the third and fourth quadrants, characterized by negative sensory attributes as acidulous and anomalous odors and by the higher content of (E,E)-2,4-decadienal. For each time of storage was found a tendency to a larger size of particles of the juices obtained from untreated tomatoes with respect to those produced with MIPEF technology. The effect of the HIPEF treatment is shown in Figure 3c,d: these two PCAs show respectively the group of samples produced by not-treated tomatoes and then subjected to HIPEF and juices obtained from tomatoes subjected to MIPEF and then treated with HIPEF.

Juices produced by MIPEF-treated tomatoes and processed by HIPEF were clearly characterized by a higher content of (E)-2-hexenal, freshness, green, and vegetable notes and less affected by the presence of anomalous odors and (E,E)-2,4decadienal with respect to the group of samples obtained from untreated tomatoes and processed by HIPEF, obviously with a lowering of quality properties as a function of the increase of storage time. In fact, during the storage period, (E)-2-hexenal, freshness, vegetable notes decreased while the content of (E,E)-2,4-decadienal, anomalous odors, and acidulous increased. These results are in agreement with Aguiló-Aguayo et al., who reported that the production of (E,E)-2,4-decadienal increased over time. The most marked increment was observed in thermally processed tomato juice from days 28 and 56 of storage.<sup>1</sup> Moreover, they reported that concentrations of (E)-2hexenal, (Z)-3-hexenol, and 1-penten-3-one decreased during the storage period.

Galindo et al. also described that 24 h after MIPEF treatments, potato tissue metabolism showed plant stress responses characterized by changes in metabolites.<sup>13</sup> It is known that polyphenols increase after MIPEF treatments.<sup>7</sup> Therefore, volatile compounds could also increase in MIPEFtreated tomatoes. 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.<sup>20</sup> Voltage-gated ion channels are a specific type of transmembrane ion channel embedded in a cell membrane and 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<sup>2+</sup> at the cellular level, followed by a rapid influx of Ca<sup>2+</sup> through cation channels. Through this process, Ca<sup>2+</sup>-dependent protein kinase (CDPK) can increase the ROS,<sup>21</sup> which are endogenous signal components required for the synthesis of secondary metabolites known to be part of the plant defense response to stress.<sup>22</sup> This could explain the better performance of the MIPEF-treated samples with respect to those not treated when the HIPEF technology was applied. Moreover, comparison of the sample distributions shown in Figure 3c,d with respect to Figure 3b reveals that HIPEFtreated tomato juices preserved better the content of volatile compounds in comparison to thermally treated tomato juices

regardless of the tomatoes used for their production (MIPEFtreated or untreated).

It is possible to summarize the main results obtained in this work in the following points:

(i) The amounts of volatile compounds underwent a substantial loss during storage of tomato juices with the exception of (E,E)-2,4-decadienal content, which increased over time. However, an enhancement of volatile compounds with the exception of (E,E)-2,4-decadienal was observed in juices made of tomatoes processed by MIPEF.

(ii) HIPEF-processed tomato juices better maintained the individual volatiles just after processing and during storage than thermally treated juices.

(iii) MIPEF could be proposed as a method for obtaining tomato juices containing high levels of volatiles. Moreover, HIPEF processing can produce tomato juice with higher sensory quality than those produced by conventional thermal processing.

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#### Notes

The authors declare no competing financial interest.

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