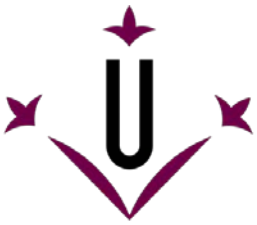

Quality changes in mango juice treated by high-intensity pulsed electric fields throughout the storage

B. Salinas-Roca [1], P. Elez-Martínez[1], J. Welti-Chanes[2], O. Martín-Belloso[1, 2*]

[1] *University of Lleida, Department of Food Technology, Agrotecnio Center, Rovira Roure 191, 25198 Lleida, Spain*

[2] *Tecnológico de Monterrey, Escuela de Ingeniería y Ciencias, Centro de Biotecnología FEMSA, Av. Eugenio Garza Sada 2501 Sur, Col. Tecnológico, 64849 Monterrey, México*

*E-mail: omartin@tecal.udl.es

[*]Author to whom correspondence should be addressed.

ABSTRACT

The effect of high-intensity pulsed electric fields (HIPEF) processes on *Listeria innocua* inhibition, physicochemical parameters and activity of oxidative enzymes of mango juice was evaluated to set the optimal HIPEF treatment time. Quality parameters, microbial population and bioactive compounds of HIPEF-treated (35 kV/cm, 1800 µs) and thermally-treated (TT) (90 °C, 60 s) mango juices were studied and compared with those non-treated during 75 days of storage at 4 °C. HIPEF treatment for 800 µs ensured 5 log reductions of *L. innocua.* Polyphenoloxidase (PPO), lipoxygenase (LOX) and peroxidase (POD) residual activities were significantly reduced to 70, 53 and 44%, respectively, at treatment times of 1800 µs. Similar sensory properties compared with fresh mango juice was attained at product treated at 1800 µs. Moreover, fresh mango juice colour (L*= 38.79, h°= 106.57) was preserved after HIPEF treatment throughout storage. Moulds and yeasts and psychrophilic bacteria counts in HIPEF-treated (1800 µs) mango juice remained below 6 log cycles CFU/mL up to 2 months of refrigerated storage. The content of total phenolic compounds in those HIPEF-treated increased from 333 to 683 µg of GAE/mL from day 0 to the end of storage. Hence, the application of HIPEF may be a feasible treatment in order to ensure microbiological stability, high bioactive compounds content and fresh-like characteristics of mango juice.

Key words: mango juice, *L. innocua*, high intensity pulsed electric fields, thermal treatment, quality attributes

1. INTRODUCTION

Mango (*Mangifera indica L.)*, one of the most harvested tropical fruits, is widely used to produce juices due to its well-appreciated sensorial attributes (FAO 2003, 2012; Nanjundaswamy 1998). Furthermore, this fruit is a rich source of bioactive compounds such as phenolics and carotenoids, hence mango consumption could have health benefits in preventing degenerative diseases (Rawson et al. 2011; Schieber et al. 2000).

Mango juice can undergo quality-degrading reactions triggered by microbial growth population and quality-degrading enzymes, among others. Therefore, preservation treatments are required to ensure its safety and quality stability. On one hand, thermal treatment is commonly used in the juice industry because of its well-known effectiveness in the inactivation of microorganisms and quality-degrading enzymes (Mercadante and Rodriguez-Amaya 1998; Soliva-Fortuny et al. 2009). However, undesired chemical, physical and sensorial changes as well as reduction of bioactive compounds content have been observed in thermally-treated juices (Sánchez-Moreno et al. 2005; Wibowo et al. 2015). On the other hand, non-thermal treatments allow to obtain microbiologically stable fruit juices but also a better preservation of sensorial and nutritional characteristics than conventional treatments (Chen et al. 2013). Hence, high-intensity pulsed electric fields (HIPEF) technology has been considered as a feasible non-thermal technique for the preservation of liquid foods. The electric field strength and treatment time are reported as the main parameters of HIPEF treatment to induce an electric potential across cell membrane conducting the cell damage (Morales-de la Peña et al. 2010).

Several studies have proved the efficiency of HIPEF on the inactivation of microorganisms such as *Listeria innocua*, which is one of the main foodborne microorganisms in fruit juices (Huang et al. 2012; Mosqueda-Melgar et al. 2007;

65 Timmermans et al. 2014). Nevertheless, published data evidenced that the degree of
66 microbial inactivation is strongly dependent on the HIPEF conditions (Jiménez-Sánchez
67 et al. 2017). With regard to enzyme activity, peroxidase (POD), polyphenoloxidase
68 (PPO) and lipoxygenase (LOX) catalyse some reactions affecting sensory and
69 nutritional properties in fruit juices. HIPEF treatments from 20 to 35 kV/cm have
70 halved enzymatic activity in tomato and orange juices (Aguiló-Aguayo et al. 2010;
71 Vervoort et al. 2011). Moreover, HIPEF seems to maintain quality characteristics
72 including colour, soluble solids and viscosity as well as retain bioactive compounds of
73 fruit juices (Buckow et al. 2013; Odriozola-Serrano et al. 2008).
74 Despite of the noteworthy literature using HIPEF treatment for fruit juices quality
75 preservation, no studies comparing the effects of HIPEF and thermal treatment on
76 quality changes of mango juice have been found. Therefore, the objectives of the
77 present work were firstly to select the HIPEF treatment time capable to inactivate *L.*
78 *innocua* and to reduce enzymatic activity in mango juice while preserving its fresh-like
79 sensorial attributes. Secondly, to compare the effect of HIPEF and thermal treatments
80 on microbial stability, activity of oxidative enzymes, total carotenoids and phenolics
81 content, antioxidant capacity and physicochemical properties in mango juice throughout
82 75 days of refrigerated storage.
83
84 ## 2. MATERIAL AND METHODS
85 ### 2.1. Mango juice
86 Mangoes (*Mangifera indica L.*) *cv. Tommy Atkins* were purchased from a local
87 wholesale market (Lleida, Spain). Each fruit was washed, dried, peeled and the seed
88 was discarded. The pulp was squeezed and then centrifuged at 5400 g during 5 min at 4
89 °C (AVANTI^{TM} J-25 Beckman; Instruments Inc; Fullerton, CA) and vacuum filtered to

obtain mango juice (MJ). MJ electric conductivity (1.54 ± 0.02 mS/cm), soluble solids (12.77 ± 1.11 °Brix) and pH (3.67 ± 0.14) were measured.

2.2. HIPEF treatments

HIPEF treatments were performed using a continuous flow bench scale system (OSU-4F, Ohio State University, Columbus, OH), that generates squared wave pulses. The flow rate was 60 mL/min controlled by a speed pump (model 752210-25, Cole Palmer Instrument Company, Vermon Hills, IL). The treatment chamber device consisted of eight co-linear chambers disposed in series and each pair of chambers had a thermocouple to control temperature. The outlet treatment temperature of juice 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. Based on previous literature, constant electric field strength (35 kV/cm), pulse frequency (200 Hz) and width (4 μs) were kept to apply pulses in bipolar quadratic mode, while different treatment times were assayed (50, 100, 200, 400, 800, 1200, 1600, 1800 and 2000 μs). According to the results of microbial and enzymatic inactivation of HIPEF-treated mango juice, the treatment conditions were set for subsequently study of preservation of mango juice along the storage.

2.3. Thermal treatment

MJ was heat-treated at 90 °C for 60 s. The juice was pumped with a peristaltic pump (model D-21V, Dinko, Barcelona, Spain) and passed through a tubular stainless steel heat exchange coil system (University of Lleida, Lleida, Spain). Immediately after heating, the tubular stainless steel was immersed in an ice-water bath at 4 °C and

thereafter MJ was packaged (Odriozola-Serrano, Soliva-Fortuny, Hernández-Jover, & Martín-Belloso, 2009).

2.4. Packaging and storage

Treated MJ was bottled directly from the treatment systems in sterilized 100 mL polypropylene bottles and leaving the minimum headspace volume. Non-treated MJ was bottled thereafter the juice preparation. Once filled, the containers were tightly closed and stored in darkness under refrigeration (4 ± 1 °C) until analysis. Non-treated and treated MJ were analysed twice a week the first 3 weeks and once a week until day 75.

2.5. *Listeria innocua* culture, inoculation and enumeration

L. innocua IPL 1.17 (Institute Pasteur de Lille; Lille, France) was cultured in tryptone soy broth (TSB) with 0.6 % yeasts extract (Bioakar Diagnostic; Beauvais, France) and incubated at 35 °C with continuous agitation at 200 rpm for 15 h to obtain cells in stationary growth phase. The final concentration reached in the culture was 10^8- 10^9 colonies forming unit per mL (CFU/mL). MJ was inoculated with *L. innocua* to have an initial concentration of 10^7- 10^8 CFU/mL and then HIPEF-treated. Treated and non-treated MJ was serially diluted in saline peptone water (Bioakar Diagnostic; Beauvais, France), for *L. innocua* enumeration; the cells were spread on Palcam agar plates (Bioakar Diagnostic; Beauvais, France) and incubated at 35 °C for 24-48 h as stated by ISO 11290-2 method (1998). Colonies were counted and the results were expressed as $\log_{10}$ CFU/ mL.

2.6. Microbial evaluation during storage

Enumeration of psychrophilic microorganisms in MJ on plate count agar (PCA) (Biokar Diagnostic; Beauvais, France) was carried out after the incubation at 5 ± 1 °C for 10 days (ISO 17410, 2001 Method). Moulds and yeasts counts were determined with the ISO 7954, 1987 Method using chloramphenicol glucose agar (CGA) (Biokar Diagnostic; Beauvais, France) and incubating 2-4 days at 25 ± 1 °C. Colonies were counted and the results were expressed as $\log_{10}$ CFU/ mL. Counts below the detection limit (1.0 log CFU/mL) were considered no detectable colonies. The criterion for completing the storage study was established as the time at which a microbial population of 10^6 CFU/ mL (Salvia-Trujillo, Morales-de la Peña, Rojas-Graü, & Martín-Belloso, 2011).

2.7. Physicochemical analysis

Electric conductivity (Testo 240 conductivity-meter; Testo GmBh & Co; Lenzkirch, Germany), pH (Crison 2001 pH-meter; Crison Instruments S.A; Barcelona, Spain), soluble solid content (Atago RX-1000 refractometer; Atago Company Ltd; Japan), viscosity using a spindle SP61 at 100 rpm and 5 °C (Brookfield, Stoughton, MA) and colour (Minolta CR-400; Konica Minolta Sensing, Inc., Osaka, Japan) of MJ were measured. Colour equipment was set up for illuminate D65 and 10º observer angle and calibrated using a standard white reflector plate. MJ (10mL) were placed in petri dishes (3.5cm x 3.5cm) and colour was measured using the CIE *L*, a*, b** scale. Additionally, Hue angle (h°) was calculated as the *arctan* of the b* and a* quotient (mesure of red = 0 or 36°, yellow = 90°, green = 180°) (Hunter 1987).

2.8. Enzyme activity evaluation

Peroxidase (POD)

163 POD activity was determined using the method described by Elez-Martínez, Aguiló-
164 Aguayo, Martín-Belloso, 2006) with some modifications. The enzyme extract for POD
165 activity measurement was obtained by the homogenization of 10 mL of MJ and 20 mL
166 of sodium phosphate buffer 0.2 M at pH 6.5. The homogenate was centrifuged at
167 24000g for 15 min at 4°C (AVANTI^{TM} J-25, Beckman Instruments Inc; Fullerton, CA,
168 USA). The supernatant was filtered throughout a Whatman paper (no. 1) and the
169 resulting liquid constituted the enzymatic extract. POD activity was assayed
170 spectrophotometrically (CECIL CE 2021 spectrophotometer Cecil Instruments Ltd,
171 Cambridge, UK) in a 1 cm path cuvette by adding at 0.1 mL of enzymatic extract 2.7
172 mL of sodium phosphate buffer (0.05 M, pH 6.5), 0.1 mL phenylenediamine (1 %) and
173 0.1 mL hydrogen peroxide (1.5 %). The oxidation of p-phenylenediamine was
174 determined at 470 nm measuring the absorbance every 10 seconds during 3 min. The
175 absorbance values were referred to a sample blank containing all reagents except
176 hydrogen peroxide, which was substituted by distilled water. POD activity was obtained
177 from the slope of the linear portion of the curve. One unit of POD activity was defined
178 as the change of absorbance per minute and millilitre of enzymatic extract at 22ºC.
179 *Polyphenoloxidase (PPO)*
180 PPO activity was determined by the method of Vásquez-Caicedo et al, (2007) with
181 some modifications. For the extraction of the enzyme, 5 g of MJ were mixed with 0.5 g
182 polyvinylpolypyrrolidone (PVPP) and 4.5 g McIlvaine buffer solution (pH 6.5)
183 consisting of 35 % of 0.1 M citric acid and 75 % 0.2 M disodium phosphate. The
184 mixture was homogenised and centrifuged at 23000 g for 15 min at 4 °C (Centrifuge
185 AVANTI^{TM} J-25, Beckman Instruments Inc; Fullerton, CA). The supernatant was
186 filtered with Whatman paper (no. 1) to obtain the enzyme extract. PPO activity was
187 measured using a spectrophotometer (CECIL CE 2021; Cecil Instruments Ltd,

188 Cambridge, UK) at 400 nm by adding 100 µL enzyme extract and 3 mL of 0.5 M
189 cathecol solution and obtaining the absorbance every 10 seconds during 3 min. A blank
190 of cathecol without extract was used. The PPO activity was obtained from the slope of
191 the linear portion of the curve; one unit of PPO activity was defined as a change of one
192 unit of absorbance per minute and millilitre of enzyme extract at 22 °C.
193 *Lipoxigenase (LOX)*
194 LOX activity was determined by the method described by Anthon & Barrett (2003) with
195 modifications. The enzyme extract was obtained by mixing 20 mL of MJ with 5 mL of a
196 solution containing 0.5 M phosphate buffer (pH 6.5) and 0.5% Triton X-100 and
197 centrifuging 10 min at 10000 g at 4 °C (Centrifuge AVANTI^{TM} J-25, Beckman
198 Instruments Inc; Fullerton, CA). The pellet was discarded and the supernatant was
199 filtered with Whatman paper (N_o. 1). The LOX activity of the enzyme was measured by
200 mixing 2 mL phosphate buffer 0.1 M (pH 6.5), 40 µL linoleic acid and adding 100 µL
201 enzymatic extract. The reaction was measured with a spectrophotometer (CECIL CE
202 2021; Cecil Instruments Ltd, Cambridge, UK) at 234 nm each 10 seconds during 3 min.
203 The activity was calculated from the slope of the linear portion of the curve. A blank
204 was prepared with 2 mL phosphate buffer 0.1 M mixed with 1 mL linoleic. One unit of
205 LOX activity was defined as a change of one unit of absorbance per minute and per
206 millilitre of enzyme extract at 22 °C.
207 Enzymatic activity was expressed as percentage of residual activity (RA %) which was
208 calculated by the quotient between the enzyme activity of treated (*AEt*) and the non-
209 treated (AE_O) MJ.
210

211 **2.9. Bioactive compounds and antioxidant activity determination**

212 *Total carotenoids*

The determination of total carotenoids was performed according to Robles-Sánchez, Rojas-Graü, Odriozola-Serrano, González-Aguilar, & Martín-Belloso (2009). MJ (5 mL) were added to 20 mL of tetrahydrofuran (THF) and homogenized with an Ultra-Turrax T 25 basic (IKA® WERKE, Germany). An aliquot was filtered throughout a N_o 1 Whatman paper. Total carotenoids were measured spectrophotometrically (CECIL CE 2021 spectrophotometer; Cecil Instruments Ltd, Cambridge, UK) at 470 nm, quantified using β-carotene as an external standard and expressed as μg of β-carotene equivalent per MJ/mL.

Total phenolic

The content of total phenolic compounds (TP) was determined according to the Folin-Ciocalteu colorimetric method described by Singleton, Orthofer, & Lamuela-Raventós (1998) with slight modifications. MJ (0.5 mL) was mixed and homogenised with saturated sodium carbonate solution (10 mL) and Folin-Ciocalteu reagent (10 mL). After one hour in dark storage, absorbance was measured at 765 nm (CECIL CE 2021 spectrophotometer; Cecil Instruments Ltd, Cambridge, UK). TP content was calculated on the basis of a standard curve of gallic acid and expressed as μg of gallic acid equivalent (GAE) per MJ mL.

Antioxidant Capacity

Antioxidant capacity was determined by a radical-scavenging activity (RSA) assay evaluated as bleaching of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. MJ (10 mL) was centrifuged at 3500 g, 20 min and 4°C in a Centrifuge AVANTI^{TM} J-25 (Beckman Instruments Inc; Fullerton, CA, USA). The reaction mixture constituted of 10 μL of supernatant, 3.9 mL of methanolic DPPH (0.0025 gL^{-1}) and 90 μL of distilled water was carried out. The samples were shaken vigorously and kept in the dark for 30 min. The absorption of the samples was measured with a spectrophotometer (CECIL CE

2021 Cecil Instruments Ltd, Cambridge, UK) at 515 nm against a blank of methanol without DPPH (Odriozola-Serrano et al. 2008). The results were expressed as percentage of DPPH inhibition as shown in equation 1 where A_o is de absorbance of DPPH reagent and *As* is the absorbance of the MJ sample reaction with DPPH.

$$DPPH\ inhibition\,(\%) = \frac{Ao - As}{Ao} \cdot 100 \qquad \text{eq.1}$$

2.10. Sensory evaluation

A total 30 non-trained panellists participated in the sensory test of treated and non-treated MJ at day of processing. A hedonic scale from 0 (dislike) to 10 (extremely like) was used to rate the colour, flavour and overall acceptance. MJ (30 mL) processed by HIPEF (35 kV, 1800 µs, 200Hz, 4µs), heat (90ºC, 60 s) and non-treated (NT) were served at 16 ± 1 °C in transparent cup coded with three digits randomly numbered. Moreover, a glass containing potable water and a piece of non-salted cracker were provided to panellists to eliminate the residual taste between samples (Mosqueda-Melgar, Raybaudi-Massilia, & Martín-Belloso, 2012)

2.11. Statistical analysis

All the treatments were assayed in duplicate and two replicate analyses were carried out for each sample to obtain the mean values and standard deviations (SD) for each analysed parameter. The analysis of variance (ANOVA) and Least Significant Differences (LSD) was performed in order to find statistical differences ($p \leq 0.05$). All statistical analyses were conducted with Statgraphics plus Centurion XV software Version 15.1.02 (StatPoint Technologies, Inc.).

3. RESULTS AND DISCUSSION

263 **3.1. Effect of HIPEF treatment on mango juice**

264 ***3.1.1. L. innocua* inactivation**

265 A maximal reduction of *L. innocua* survival of 5.7 log units was achieved after applying
266 HIPEF (35 kV/ cm, 200 Hz, 4μs) for 800 μs to MJ (Figure 1). The longer is the HIPEF
267 treatment time up to 800 μs, the higher the decrease of microbial population. As
268 described in Figure 1, no significant differences in the *L. innocua* inactivation levels
269 were appreciated at HIPEF treatments from 800 to 2000 μs. According to
270 microbiological criteria proposed by FDA (2004) for fruit juices, 5 log reductions of
271 target microorganisms should be accomplished for obtaining safe product. Similarly,
272 Mosqueda-Melgar, Raybaudi-Massilia, & Martín-Belloso (2007) achieved 5 log
273 reduction of *L. innocua* population in melon juice treated by HIPEF (35 kV/cm, bipolar
274 square wave, 4 μs pulses and 200 Hz) as treatment times increased up to 1250 μs.
275 Previous research explained the effect of increasing HIPEF treatment time on microbial
276 inactivation by the formation of membrane pores triggering to membrane destabilization
277 and cell rupture (Vega-Mercado et al. 1997). Although the efficacy of HIPEF (20
278 kV/cm, 90 Hz and 130 L/h) against *L. innocua* was also proved in orange juice
279 (Timmermans et al. 2014), less studies have been found to reduce more than 5 log at
280 800 μs. The low pH (4.1) and conductivity (1.71 mS/cm) of MJ could cause *L. innocua*
281 cells more sensible to damage. Indeed, Amiali, Ngadi, Raghavan, & Nguyen, (2006)
282 reported that lowering ionic concentration cause an increase of the treatment chamber
283 resistance, which could enhance the microbial inactivation levels. Wouters, Dutreux,
284 Smelt, & Lelieveld (1999) observed better reduction of *L. innocua* in solutions with low
285 pH (4.0) and conductivity (2.7 mS/cm) than in alkaline solutions.

286 **3.1.2. Enzyme activity**

HIPEF treatment applied for 1800 μs reduced at 70, 53 and 44 % PPO, LOX and POD activity in the MJ, respectively (Figure 2). Differently, at treatment times below 1800 μs, when no significant reduction of enzymatic activity was observed, various deleterious reactions affecting loss of nutritive value and yellow colour might occur in MJ.

A reduction of the RA as increasing HIPEF treatment time has been also reported by Aguiló-Aguayo, Sobrino-López, Soliva-Fortuny, & Martín-Belloso (2008) and Aguiló-Aguayo et al. (2010), who reached 10 and 30 % of RA for PPO and LOX, respectively, in strawberry treated by HIPEF (35 kV/ cm, 1000 μs, 200 Hz and 4μs). HIPEF treatment, that is known to conduct to cell electroporation, might benefit the contact between enzyme and substrate released from the cell, hence, no complete inactivation was achieved in MJ (Huang et al. 2012). The effect of HIPEF at 1800 μs might cause an irreversible conformational change of the globular protein chain of enzymes in MJ. An enzyme denaturation might be a feasible reason for enzymatic activity reduction (Luo et al. 2010).

The studied oxidative enzymes followed similar pattern of inactivation. Nevertheless, differences on the RA between LOX and the other oxidative enzymes in MJ at the longest treatment time were observed (Figure 2). This could indicate a different level of HIPEF effect on the enzymatic structure. PPO and POD structure contains a prosthetic group, thereby, the influence of electric fields on changing the structure of copper-containing enzyme has been reported scarcely since it can be considered tightly bound organic molecules (Sharma et al. 2013). Otherwise, conformational changes in LOX structure, with no prosthetic group, could occur easily. Moreover, other authors have reported that charges separation of tertiary structure occurred in LOX native conformation leading almost complete inactivation of LOX, when long treatments and

312 high voltage are used in enzymatic solution, but not in PPO (Luo et al. 2010). In
313 agreement with the available scientific literature, electrochemical effect of HIPEF may
314 affect the local electrostatic fields in proteins and disrupt electrostatic interactions of
315 peptide chains leading to conformational changes in enzymes (Buckow et al. 2013).
316 Therefore, HIPEF treatment had greatest degree of activity reduction on LOX compared
317 with PPO and POD in HIPEF-treated MJ at 2000 μs.

318 **3.1.3. Physicochemical parameters**

319 HIPEF treatment had no significant effect ($p \geq 0.05$) on pH and conductivity of MJ
320 when different treatment times were applied. Average values in pH and conductivity of
321 treated-MJ were 4.1 ± 0.1 and 1.71 ± 0.01 mS/cm, respectively. In a similar way,
322 Zhang, Gao, Zhang, Shi, & Xu (2010) and Aguilar-Rosas, Ballinas-Casarrubias,
323 Nevarez-Moorillon, Martin-Belloso, & Ortega-Rivas, (2007) reported that both HIPEF-
324 processed longan and apple juice, did not show pH differences with the non-treated
325 products. Other reports indicated no change of conductivity after HIPEF treatment
326 (Mosqueda-Melgar et al., 2012; Vega-Mercado et al., 1997). Although no effect of
327 HIPEF treatment time on TSS content or viscosity of MJ was observed, differences
328 between HIPEF-treated and non-treated MJ were detected. Non-treated MJ (10.8 ± 0.7
329 °Brix and 4.0 ± 0.3 mPa·s) presented lower average values of TSS and viscosity
330 compared with HIPEF-treated (35 kV/ cm, 200 Hz, 4μs and 2000 μs) MJ (12.9 °Brix ±
331 0.6 and 5.4 mPa·s ± 1.1). Cserhalmi, Sass-Liss, Tóth-Markus & Lechner (2006) and
332 Falade, Babalola, Akinyemi, & Ogunlade (2004) reported an increase in TSS and
333 viscosity of citrus juices treated by HIPEF (28 kV/cm, 100 μs, 2 μs-bipolar pulses at
334 100 Hz), which were attributed to the breakdown cell effect releasing soluble solids
335 from the cell. Moreover, changes in HIPEF-treated MJ compared with the non-treated
336 might be also attributed to a decline of the pectinolitic enzyme activity, which could

337 enable to maintain pectin content in MJ and hence higher TSS and viscosity (Espachs-
338 Barroso et al. 2006).
339 Figure 3 shows a non-significant changes of L* value of MJ from 50 to 2000 μs.
340 Similarly, the h° value was maintained in the range of 74.5 to 73.9 in HIPEF-treated MJ
341 as treatment time increased (Figure 3). Thus, HIPEF treatment preserved characteristic
342 colour of MJ. The significant reduction of enzymatic activity in HIPEF-treated MJ
343 might prevent quality degrading oxidative reactions (Pathare et al. 2012). The present
344 results are aligned with previous studies, where colour of HIPEF-treated orange (Cortés
345 et al. 2008) and carrot juice (Quitão-Teixeira et al. 2007) were preserved as in fresh
346 juices. Carrot, orange and mango juice have similar yellow colour tonality, which could
347 be mainly attributed to carotenoid compounds. Thus, yellow colour might be preserved
348 whether great content of natural pigments such as carotenoids is maintained.
349 **3.2. Sensory evaluation of mango juice**
350 Figure 4 shows the influence of HIPEF (35 kV/cm, 1800 μs, 200 Hz, 4 μs) and TT (90
351 ˚C, 60 s) on sensorial attributes (colour, flavour and overall acceptance) of MJ
352 compared with the non-treated. Similar overall acceptance and flavour between treated
353 and non-treated MJ were observed. Mosqueda-Melgar, Raybaudi-Massilia, Martín-
354 Belloso, (2012) observed no differences in flavour and overall acceptance comparing
355 fresh fruit juices and those treated by HIPEF and TT. On the other hand, colour values
356 in HIPEF and thermally-treated MJ were alike. Nevertheless, significant differences ($p \leq 0.05$) in colour perception of non-treated MJ (5.6 ± 1.6) compared with the HIPEF-
358 treated (7.2 ± 1.8) were detected. The reduction of oxidative enzyme activity in HIPEF-
359 treated MJ might avoid the loss of colour. Also, the possible release of natural pigments
360 due to the electroporation effect in HIPEF treatment could explain the great colour score
361 of HIPEF-treated MJ given by the consumers.

362 Since HIPEF-treated MJ at 1800 μs led to a significant reduction of *L. innocua*
363 population and enzymatic activity as well as fresh-like physicochemical characteristics,
364 sensory evaluation of MJ treated by HIPEF and TT at day of processing, and further
365 quality analysis along the storage were carried out at 35 kV/cm, 1800 μs, 200 Hz, 4 μs.
366
367 **3.3. Storage stability of mango juice**
368 **3.3.1 Microbial evaluation**
369 Initial counts of moulds and yeasts in non-treated MJ were 4.22 ± 0.58 $\log_{10}$ CFU/mL,
370 while those of psychrophilic bacteria were 1.74 ± 0.15 $\log_{10}$ CFU/mL. HIPEF or TT
371 effectively reduced microbial loads of the juice up to the detection limit just after
372 processing (day 0) (Figure 5). During storage, it was observed that moulds and yeasts
373 population increased earlier than psychrophilic bacteria in treated and non-treated MJ.
374 No microbial growth in HIPEF-treated MJ was detected during the first two weeks of
375 storage, while the TT-MJ did not show microbial growth along the entire storage time.
376 Microbial counts for HIPEF-treated and thermal-treated MJ was lower than 6 $\log_{10}$
377 CFU/mL until day 59 and 75, respectively, whereas non-treated MJ exceed those counts
378 at day 23.
379 Diverse studies have suggested that microorganisms are inactivated because of
380 electroporation and electrofusion phenomena during the HIPEF treatment (Buckow et
381 al. 2013). Nevertheless, a microbial growth in HIPEF-treated MJ could be attributed to
382 a non-complete inactivation of microorganisms (Mosqueda-Melgar et al., 2007). HIPEF
383 treatment enabled to extend the lag phase of MJ microbial population, hence, the
384 recovery of injured microorganisms and germination of those sporulated was delayed.
385 Timmermans et al., (2011) and Elez-Martínez, Soliva-Fortuny, Martín-Belloso, (2006)
386 observed no growth of moulds and yeasts in HIPEF-treated orange juice at 25 and 35

kV/ cm, respectively, during 20 and 56 days. Although, Timmermans et al., (2011) achieved similar microbial stability compared with the present study, it must be noted that the treatment temperature used was 56 °C, whereas present results were obtained without exceeding 40 °C.

3.3.2. Enzyme activity

At the beginning of storage, RA of HIPEF-treated (35 kV/cm for 1800 μs with bipolar pulses of 4 μs at 200Hz) MJ were 70.0 ± 5.1; 69.9 ± 4.9 and 46.3 ± 10.2 % for PPO, LOX and POD, respectively. The application of thermal treatment to MJ significantly reduced activity of PPO and POD up to 55.5 ± 0.5 and 20.7 ± 1.0 at day 0 (table 1). The PPO and POD molecular structure, which contains a prosthetic group in their structure, has been reported to be specially affected by pH, temperature and electric fields (Luo et al. 2010). Otherwise, RA_{LOX} after thermal treatment increased at day of processing, LOX appeared to be less thermo-sensible. During storage, a severe increase of RA_{POD} in non-treated MJ was observed, whereas PPO and LOX activities were slightly reduced. Probably, the increase of POD activity might be assigned to the cell release of POD substrate (organic hydroperoxides), which enable the enzyme-substrate contact (Vervoort et al. 2011).

Both electrochemical and thermal effects associated with HIPEF and TT could result in changes in the structure and conformation of enzymes, which may lead to inactivation (Huang et al. 2012; Timmermans et al. 2011). However, the appearing of isoenzymes and uncomplete inactivation might explain the fluctuations of enzymatic activity in TT and HIPEF-treated MJ along the storage. RA of PPO and POD in MJ treated by TT and HIPEF had a drastically decrease from day 16 until the end of storage. Among oxidative enzymes, RA_{POD} of 25.1 ± 3.5 % (day 75) and 17.0 ± 4.4 % (day 49) was the lowest in MJ treated by TT and HIPEF, respectively. Consistently, literature has reported that

412 POD seemed to be more susceptible to HIPEF than other enzymes and is associated
413 with the modification of the α-helix structure (Leong and Oey 2014). These results are
414 inconsistent with the complete POD inactivation during 56 days reported by Elez-
415 Martínez, Soliva-Fortuny, et al. (2006) in orange juice after HIPEF treatment (35 kV/cm
416 for 1000 μs with bipolar pulses of 4 μs at 200 Hz). However, other authors described a
417 progressive decrease of RA_{POD} in HIPEF-treated orange juice (23 kV/cm, 90 Hz,
418 monopolar pulses of 2 μs and 130 L/h) along 58 days (Vervoort et al. 2011).
419 In contrast, significant RA_{LOX} reduction in treated MJ required long storage time. Both
420 TT and HIPEF treatments reduced significantly more than a 50 % the initial activity of
421 LOX at the end of storage. Similar to other studies a retarded decrease of the RA_{LOX}
422 was observed (Espachs-Barroso et al. 2006; Zhao et al. 2007). According to Aguiló-
423 Aguayo, Soliva-Fortuny & Martín-Belloso (2010), LOX protein chain could undergo
424 changes and a development of resistant isoforms in HIPEF-treated fruit juices. Thus, the
425 conformational changes in LOX structure might delay the reduction of the activity
426 throughout storage time.
427 It is known that HIPEF and thermal enzyme inactivation mechanisms are related to the
428 unfolding of proteins due to changes in their secondary structure (Salvia-Trujillo et al.,
429 2011). Also, a weak affinity of enzyme-substrate complex might describe the decrease
430 of RA in HIPEF-treated MJ during the storage. Another hypothesis for reducing
431 enzymatic activity in HIPEF-treated MJ throughout storage would be the formation of
432 aggregates as a result of a strong polarization of the protein molecules and hydrophobic
433 interactions or covalent bonds (Luo et al. 2010). Therefore, the protein aggregation
434 along the storage could reduce the enzymatic reaction by avoiding the substrate from
435 fitting the active site of the enzyme.
436 **3.3.3. Physicochemical parameters**

437 pH and TSS values remained stable throughout the storage and no statistical differences
438 among treatments were observed. pH average values for non-treated, TT and HIPEF-
439 treated MJ were 3.7 ± 0.1, 3.76 ± 0.04 and 3.7 ± 0.1, respectively. The mean values of
440 TSS for non-treated, TT and HIPEF-treated MJ were 9.4 ± 0.9, 10.72 ± 0.52 and 8.53 ±
441 1.62, respectively. In contrast to the obtained results, Timmermans et al., (2011)
442 observed a TSS increase in HIPEF-treated orange juice (23 kV/cm and 90 Hz) after 58
443 days of refrigerated storage. Differences might be attributed to the use of lower electric
444 field compared with that of the present study; hence, less reduction of enzymatic
445 activity might lead deleterious quality process as increment of turbidity and TSS.
446 L* values of the non-treated, HIPEF-treated and TT MJ at day 0 were 39.78 ± 0.01,
447 38.87 ± 0.52 and 40.34 ± 0.25, respectively (table 2). During storage, non-treated MJ
448 rapidly declined L*, whereas a slightly decreased in HIPEF-treated MJ was observed.
449 L* values of thermal-treated MJ were preserved along the storage. On the other hand,
450 initial h° values of non-treated (106.57 ± 0.26), TT (107.4 ± 0.6) and HIPEF-treated
451 (108.03 ± 0.38) MJ were not significantly different. Along the storage, h° of non-treated
452 MJ decreased; hence loss of yellow colour might occur. TT and HIPEF treatment
453 maintained similar h° in MJ throughout the storage. The loss of L* and h° could be
454 associated with the formation of dark colour compounds and reduction of yellow colour
455 in beverages due to the non-enzymatic browning reactions (Pathare et al. 2012).
456 According to other studies, the loss of colour in non-treated MJ might be related with
457 the oxidative reactions mostly triggered by residual activity of POD and PPO
458 (Timmermans et al. 2011; Wibowo et al. 2015). In this sense, the increase of RA_{POD}
459 observed in non-treated MJ probably conducted the deterioration of colour. Differently,
460 all treated MJ significantly reduced the activity of POD and PPO; hence, enzymatic

461 browning was avoided. Therefore, treated MJ preserved the yellow colour of fresh
462 mango juice.

463 **3.3.4. Bioactive compounds and antioxidant activity**

464 The effects of processing and storage time on bioactive compounds and antioxidant
465 activity of MJ are shown in Figure 6. Considering total carotenoid content, TT and
466 HIPEF-treated MJ showed a decrease of 17 and 13 %, respectively, compared with non-
467 treated MJ at the beginning of the storage (Figure 6a). Carotenoids compounds are
468 thermo-labile; hence, heat processing leads to significant higher losses in TT MJ than
469 those HIPEF-treated. Differently, an electroporation on the cell membrane, which
470 enable the releasing of carotenoids among other compounds, in HIPEF-treated MJ could
471 occur. Oxidative reactions promoted by enzymes, light or oxygen could affect rapidly
472 the carotenoids released in TT or HIPEF-treated MJ, which could explain the
473 subsequently decline of carotenoids content (Soliva-Fortuny, Balasa, Knorr & Martín-
474 Belloso, 2009). According to Odriozola-Serrano, Soliva-Fortuny, Hernández-Jover, &
475 Martín-Belloso (2009) oxidation may occur by self-oxidation, where alkylperoxyl
476 radicals are formed and these radicals attack the double bonds resulting in formation of
477 epoxides. Thus, the severity of oxidation depends on the structure of carotenoids and the
478 environmental conditions. However, during storage period, HIPEF-treated MJ reached
479 2.2 times more carotenoids than those heat-treated (Figure 6a). Similarly, other studies
480 described great retention of carotenoids in HIPEF-treated compared to heat-treated
481 orange juice during storage (Buckow et al. 2013). Total phenolic compounds in MJ
482 varied from 560.1 ± 17.9 (non-treated) to 333.8 ± 27.8 (HIPEF-treated) and 529.6 ±
483 15.4 (TT) μg of gallic acid/ mL at processing day (day 0). Similarly to Santhirasegaram,
484 Razali, George, & Somasundram, (2015), no significant difference in the phenolics
485 concentration after thermal treatment compared with non-treated MJ was observed

immediately after processing. Although other authors have also reported that after HIPEF treatment he phenolic content is reduced, the mechanism is not well known (Rawson et al. 2011). The interaction with other compounds such as solutes resulting from the high electric field and long treatment time applied could create aggregations reducing the content of phenolic compounds (Soliva-Fortuny et al. 2009).

Total phenolics decreased in non-treated MJ along the storage (Figure 6b). Otherwise, total phenolic compounds concentration increased in MJ treated by HIPEF throughout the storage. Indeed, HIPEF-treated MJ (683.79 ± 0.50 µg GAE/mL) showed the highest phenolics concentration compared with TT MJ at day 59. Phenolic compounds are formed in plant products via the action of phenylalanine ammonia-lyase (PAL) in the phenylpropanoid metabolism (Patthamakanokporn et al. 2008). This response is initiated when the plant recognizes a stimulus at the cellular level. It could be hypothesized that HIPEF induced PAL activity and 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 phosphorylates PAL, which regulates the phenylpropanoid metabolism (Vallverdú-Queralt et al. 2012). On the other hand, the loss of phenolic compounds in non-treated fruit juice was also observed by Patthamakanokporn, Puwastien, Nitithamyong, & Sirichakwal (2008) who attributed the decrease of phenolics during the storage to deleterious enzymes such as PPO. After analyzing the data obtained in this work, it was observed that there was a negative correlation (r= - 0.74) between the activity of PPO and the content of phenolics. This result seems to indicate the importance of TT and HIPEF treatment in reducing RA_{PPO}. Thus, decreasing of PPO activity, which uses phenolic compounds for the oxidative processes to trigger on

510 quinones, was mainly associated with increasing in phenolics (Cheema and
511 Sommerhalter 2015).
512 Initial antioxidant capacity in MJ was 20.4 ± 1.3, 18.7 ± 0.4 and 17.9 ± 0.4 % of DPPH
513 inhibition for HIPEF, TT and non-treated MJ, respectively. The enhancement of radical
514 scavenging activity in HIPEF-treated MJ might be attributed to the stress response of
515 antioxidant compounds. During storage, the antioxidant capacity of MJ depleted
516 irrespective of the treatment applied (Figure 6c). It is remarkable that both total
517 carotenoids content and antioxidant capacity rapidly decreased in TT and non-treated
518 MJ along the storage. Our results for HIPEF-treated MJ were in accordance with
519 Odriozola-Serrano et al. (2008) who observed a significant loss of antioxidant capacity
520 as storage time increased in HIPEF-treated tomato juice (35 kV/ cm, 100 Hz and 1500
521 μs of treatment time). In many plant species, a good relationship between antioxidant
522 activity and total phenolics was noted. Contrarily, no correlation between total phenolic
523 compounds and antioxidant capacity in treated MJ was observed. Thus, antioxidant
524 capacity in MJ during refrigerated storage could be related to other bioactive
525 compounds such as vitamin C, which could be easily affected by oxidative deleterious
526 reactions (Buckow et al. 2013).
527
528 4. **CONCLUSIONS**
529 HIPEF treatment at 35 kV/cm, 4 μs- bipolar pulses, 200 Hz and 1800 μs proved to be
530 feasible in the reduction of *L. innocua* population to pasteurization levels in mango
531 juice while enzymatic activity of PPO, LOX and POD was reduced up to 70, 53 and 44
532 % RA, respectively, and fresh-like physicochemical properties maintained. The native
533 flora stability of HIPEF-treated mango juice was assured throughout 59 days at 4 °C.
534 On the other hand, LOX activity of HIPEF- treated mango juice was halved along the

storage. Also, the POD and PPO enzymatic activity in HIPEF-treated mango juice was lower than in those untreated throughout storage. The reduction of PPO enabled a significant increase of the phenolic content in HIPEF-treated mango juice during 59 days. Differently, antioxidant capacity and carotenoid content of all evaluated mango juices decreased gradually throughout storage period. However, bioactive compounds in mango juice were better retained after HIPEF than thermal treatments. The beneficial effect of the HIPEF treatment was noticeable over the storage period with enhanced phenolic content and maintaining fresh-like characteristics of mango juice.

ACKNOWLEDGMENTS

This work was supported by the University of Lleida (Spain) and financed by Tecnológico de Monterrey, Mexico (Research Chair Funds CAT-200 and CDB081).

548
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FIGURES

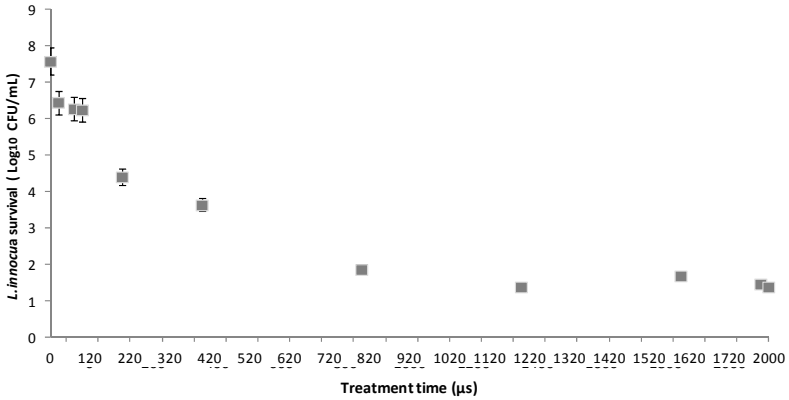

Figure 1: Survival of *L.innocua* population inoculated in mango juice treated by HIPEF (35 kV/cm, 4-µs bipolar pulses at 200 Hz) at different times (µs).

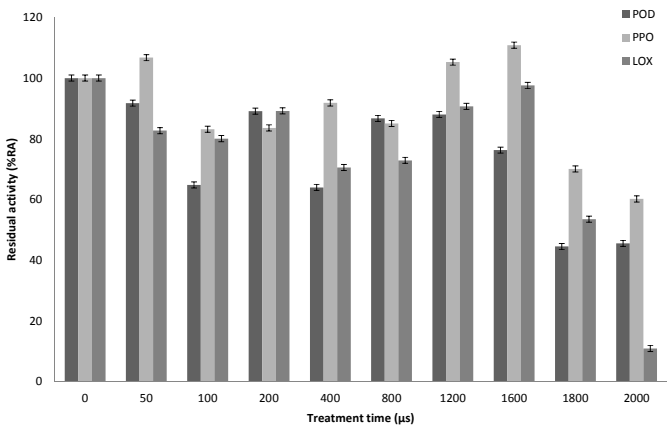

Figure 2: Effect of HIPEF (35 kV/cm, 4-µs bipolar pulses at 200 Hz) at different treatment time in residual activity of oxidative enzymes: peroxidase (POD) (♦), polyphenoloxidase (PPO) (■) and lipoxigenase (LOX) (▲).

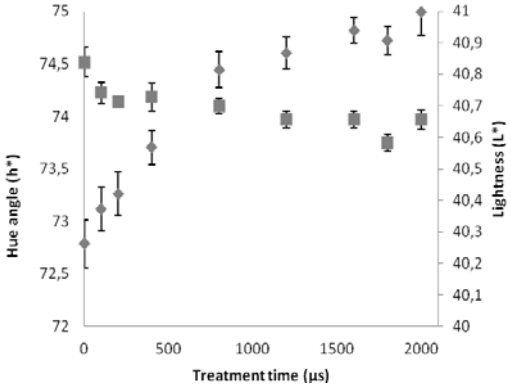

Figure 3: Colour parametres Lightness (L*) (♦) and hue angle (h°) (■) of mango juice treated by HIPEF (35 kV/cm, 4-µs bipolar pulses at 200 Hz) at different treatment times.

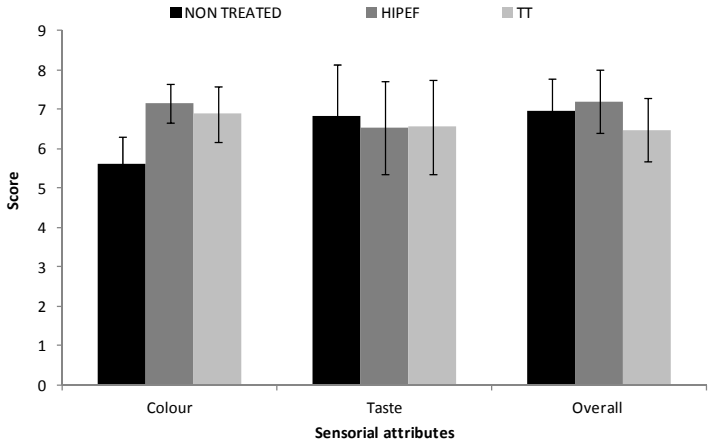

Figure 4: Effect of high intensity pulsed electric fields treatment (HIPEF) (35 kV/cm, 1800 µs, 4-µs bipolar pulses at 200 Hz), thermal treatment (TT) (90 ° C, 60 s) and non-treated conditions on sensorial attributes: colour, taste and overall acceptance of mango juice.

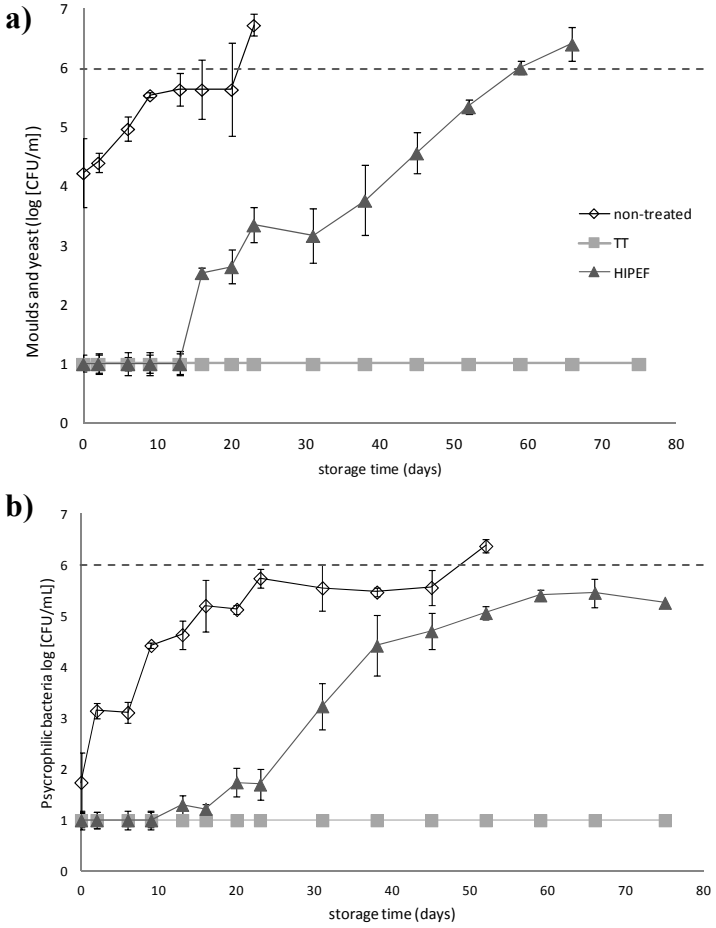

Figure 5: Moulds and yeasts (a) and psychrophilic bacteria (b) growth in mango juice treated by HIPEF treatment (35 kV/cm, 1800 µs, 4-µs bipolar pulses at 200 Hz) or thermal treatment (90 ° C, 60 s) compared with the non-treated throughout storage at 4 °C during 75 days. Limit of microbial shelf-life at 6 log CFU/mL (----).

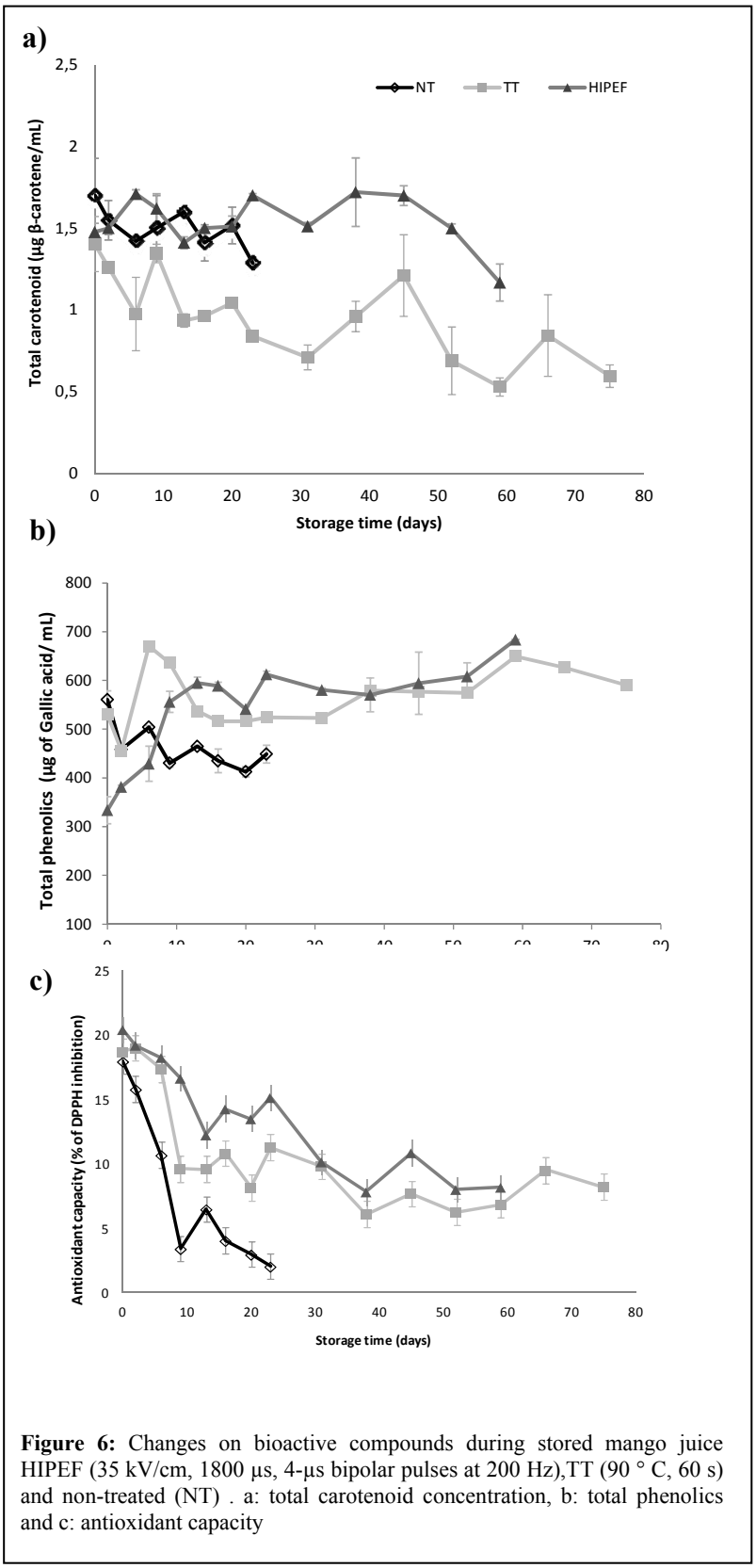

Figure 6: Changes on bioactive compounds during stored mango juice HIPEF (35 kV/cm, 1800 µs, 4-µs bipolar pulses at 200 Hz),TT (90 ° C, 60 s) and non-treated (NT) . a: total carotenoid concentration, b: total phenolics and c: antioxidant capacity

TABLES

Table 1: Effect of HIPEF (35 kV/cm, 1800 μs and 200Hz) and TT (90 °C 60 s) on residual activities (RA) of polyphenoloxidase (PPO), peroxidase (POD) and lipoxigenase (LOX) enzymes in mango juice throughout 75 days of storage at 4˚C.

	RA_{PPO} (%)			RA_{POD} (%)			RA_{LOX} (%)		
Days	NT	TT	HIPEF	NT	TT	HIPEF	NT	TT	HIPEF
0	100 ± 1.0 ^{aA}	55.5 ± 0.5 ^{deE}	70.0 ± 5.1 ^{cdC}	100 ± 5.9 ^{bA}	20.7 ± 1.0 ^{aB}	46.3 ± 10.2 ^{aC}	100 ± 11.1 ^{abA}	120.9 ± 26.7 ^{aB}	69.9 ± 4.9 ^{aB}
2	61.1 ± 6.4 ^{bA}	66.1 ± 1.0 ^{fgA}	39.2 ± 21.9 ^{defB}	127.1 ± 0.0 ^{bA}	37.2 ± 8.9 ^{dB}	56.0 ± 0.0 ^{bC}	91.5 ± 4.4 ^{bA}	143.7 ± 29.1 ^{aB}	119.5 ± 8.4 ^{bC}
6	42.7 ± 0.0 ^{efA}	63.8 ± 15.1 ^{efgB}	92.0± 8.7 ^{bcC}	128.21 ± 12.8 ^{aA}	46.2 ± 16.6 ^{eB}	32.2 ± 8.3 ^{cC}	109.9 ± 12.4 ^{aA}	155.4 ± 11.3 ^{aB}	163.1 ± 22.0 ^{bB}
9	45.4 ± 0.0 ^{deA}	78.3 ± 20.3 ^{ghB}	98.7 ± 14.1 ^{jC}	127.4 ± 32.0 ^{cdA}	40.2 ± 3.5 ^{eB}	34.5 ± 6.1 ^{deC}	94.4 ± 7.1 ^{bA}	84.5± 13.7 ^{aB}	109.9 ± 0.1 ^{cC}
13	57.2 ± 1.7 ^{cA}	72.4 ± 1.6 ^{fgB}	106.8 ± 19.1 ^{jC}	134.5 ± 3.3 ^{bcA}	40.0 ± 11.6 ^{eB}	23.7 ± 3.2 ^{efC}	72.3 ± 7.4 ^{cA}	92.7 ± 17.3 ^{aB}	102.6 ± 2.4 ^{cdC}
16	51.4 ± 8.5 ^{cdA}	96.9 ± 20.8 ^{hB}	56.0 ± 16.0 ^{iA}	154.5 ± 12.1 ^{bcA}	40.1 ± 6.4 ^{eB}	20.7 ± 0.0 ^{efC}	49.3 ± 12.9 ^{efA}	76.5 ± 20.3 ^{bA}	103.6 ± 3.7 ^{cdA}
20	38.7 ± 0.0 ^{efA}	52.1 ± 0.0 ^{cdB}	52.1 ± 0.0 ^{iC}	168.7 ± 21.7 ^{dA}	28.8 ± 1.8 ^{bcdB}	22.0 ± 3.9 ^{defB}	44.6 ± 10.4 ^{fghA}	77.3 ± 0.0 ^{aB}	105.7 ± 0.0 ^{cC}
23		38.7 ± 11.9 ^{bA}	38.3 ± 11.9 ^{defgA}		32.6 ± 11.4 ^{cdB}	24.3 ± 2.3 ^{deB}		75.4 ± 2.4 ^{aB}	92.9 ± 5.7 ^{eC}
31		56.9 ± 2.6 ^{deB}	51.9 ± 11.2 ^{hiB}		22.0 ± 0.0 ^{bB}	22.4 ± 2.4 ^{defB}		75.9 ± 0.0 ^{aB}	108.7 ± 9.6 ^{cC}
38		40.4 ± 7.7 ^{bcB}	45.2 ± 3.7 ^{fghiB}		22.4 ± 3.2 ^{bB}	20.2 ± 7.4 ^{efB}		74.6 ± 13.3 ^{aB}	96.3 ± 3.9 ^{deC}
45		60.6 ± 9.37 ^{efB}	46.8± 14.3 ^{defgA}		24.8 ± 0.8 ^{bcB}	26.8 ± 3.1 ^{dB}		80.0 ± 0.0 ^{aB}	80.3 ± 1.5 ^{fB}
52		48.8 ± 15.9 ^{bcdB}	28.2 ± 4.8 ^{cdeA}		28.6 ± 16.2 ^{cdB}	23.5 ± 0.8 ^{deB}		52.3 ± 13.8 ^{aB}	55.8 ± 11.4 ^{gAB}
59		52.1 ± 6.1 ^{cdB}	45.7 ± 11.8 ^{ghiB}		24.8 ± 4.7 ^{bcB}	17.0 ± 4.4 ^{fgB}		49.6 ± 7.7 ^{aB}	43.4 ± 6.3 ^{ghB}
66		17.9 ± 11.2 ^{aB}			21.9 ± 1.9 ^{bB}			51.6 ± 11.5 ^{aA}	
75		12.1 ± 8.7 ^{aB}			25.1 ± 3.5 ^{bcB}			40.6 ± 6.1 ^{aB}	

NT: non-treated mango juice

Values represent the mean ± standard deviation. Values in a column followed by the same lower case letter and in a row followed by the same upper case letter are not significantly different ($p > 0.05$).

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Table 2: Effect of HIPEF (35 kV/cm, 1800 µs and 200Hz) and TT (90°C 60 s) on lightness (L*) and hue angle (h°) colour parameters s in mango juice throughout 75 days of storage at 4˚C.

	L*			h˚		
Days	NT	TT	HIPEF	NT	TT	HIPEF
0	38.79 ± 0.01 ^{Aa}	40.34 ± 0.25 ^{Ba}	38.87 ± 0.52 ^{Aa}	106.57 ± 0.26 ^{Aa}	107.4 ± 0.6 ^{Aa}	108.03 ± 0.38 ^{Aa}
2	32.52 ± 0.21 ^{Ab}	37.78 ± 0.00 ^{CBb}	38.73 ± 0.01 ^{Ca}	107.97 ± 1.31 ^{Aa}	110.3 ± 0.5 ^{Ab}	108.75 ± 0.11 ^{Aa}
6	32.21 ± 0.21 ^{Ab}	37.59 ± 0.09 ^{Cb}	38.56 ± 0.05 ^{Ca}	104.00 ± 2.31 ^{Aa}	110.6 ± 0.7 ^{Ab}	108.86 ± 0.16 ^{Aa}
9	32.05 ± 0.01 ^{Ab}	37.15 ± 0.61 ^{Cb}	36.28 ± 3.03 ^{Ca}	103.90 ± 2.80 ^{Ab}	109.98 ± 0.47 ^{Ab}	110.08 ± 0.57 ^{Aa}
13	31.91 ± 0.05 ^{Ab}	37.32 ± 0.03 ^{Cb}	32.76 ± 0.11 ^{Cb}	103.22 ± 3.24 ^{Ab}	109.64 ± 0.66 ^{Ab}	108.52 ± 0.09 ^{Aa}
16	31.91 ± 0.01 ^{Ab}	37.07 ± 0.04 ^{Cb}	32.7 ± 0.0 ^{Cb}	102.3 ± 3.4 ^{Ab}	108.96 ± 0.78 ^{Ab}	107.27 ± 0.05 ^{Aa}
20	31.6 ± 0.2 ^{Ab}	37.00 ± 0.46 ^{Cb}	31.9 ± 0.3 ^{Cb}	101.2 ± 3.8 ^{Cb}	107.04 ± 1.61 ^{Ab}	105.75 ± 0.77 ^{Bb}
23	31.13 ± 1.35 ^{Ab}	36.90 ± 0.39 ^{Cb}	32.43 ± 0.21 ^{Cb}	100.4 ± 4.1 ^{Cb}	107.3 ± 1.3 ^{Ab}	104.86 ± 0.07 ^{Bb}
31		36.9 ± 0.1 ^{Cb}	32.52 ± 0.01 ^{Cb}		112.3 ± 1.5 ^{Dc}	109.9 ± 0.2 ^{Aa}
38		36.82 ± 0.05 ^{Cb}	32.42 ± 0.09 ^{Cb}		112.6 ± 1.6 ^{Dc}	109.4 ± 0.2 ^{Aa}
45		36.22 ± 0.57 ^{Cb}	32.63 ± 0.01 ^{Cb}		110.7 ± 1.3 ^{Dc}	108.09 ± 0.02 ^{Aa}
52		36.8 ± 0.0 ^{Cb}	32.3 ± 0.2 ^{Cb}		111.2 ± 2.2 ^{Dc}	105.86 ± 0.09 ^{Ab}
59		35.77 ± 1.73 ^{Cb}	31.8 ± 0.2 ^{Cc}		111.8 ± 1.2 ^{Dc}	105.67 ± 0.09 ^{Ab}
66		38.3 ± 1.3 ^{Cb}			126.3 ± 1.7 ^{Dd}	
75		40.8 ± 0.0 ^{Ca}			102.5 ± 1.8 ^{De}	

NT: Non-treated mango juice
Values represent the mean ± standard deviation. Values in a column followed by the same lower case letter and in a row followed by the same upper case letter are not significantly different