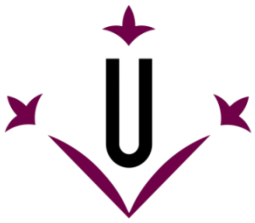

Application of innovative technologies (high-hydrostatic pressure combined with temperature and moderate-intensity pulsed electric fields) to preserve and/or improve the bioactive compounds content of pumpkin

Authors: García-Parra, J.[1], González-Cebrino, F.[1], Delgado-Adámez, J.[1], Cava, R.[2], Martín-Belloso, O.[3], Elez-Martínez, P.[3], and Ramírez, R[1,]*.

[1]CICYTEX (Centro de Investigaciones Científicas y Tecnológicas de Extremadura). Technological Agri-Food Institute (INTAEX), Avda Adolfo Suárez s/n, 06071, Badajoz, Spain.

[2] TRADINNOVAL Research Group. INBIO G+C. University of Extremadura. Campus Universitario. 10003 Cáceres, Spain

[3]Department of Food Technology, Agrotecnio Center, University of Lleida. Av. Alcalde Rovira Roure, 191. 25198 Lleida, Spain.

*Corresponding author: M. Rosario Ramírez.

Technological Agri-Food Institute (INTAEX), Avda. Adolfo Suárez s/n, 06071. Badajoz, Spain.

Tel.: +34 924 012660. Fax: +34 924 012674.

E-mail address: mariarosario.ramirez@juntaex.es / rramirez.bernabe@gmail.com

ABSTRACT

The application of novel technologies such as moderate-intensity pulsed electric fields (MIPEF) and/or high pressure thermal (HPT) treatments would improve the quality of processed pumpkin. MIPEF can be applied directly in the vegetable in order to increase the bioactive compounds content of pumpkin, in contrast, HPT treatment would be the election treatment for the preservation of purée. Traditional thermal treatment (TT) of pasteurization and sterilization was compared with equivalent HPT treatments. The effect of processing (TT vs HPT) in purées made from pumpkin pretreated with MIPEF was evaluated. Microbiological counts, enzyme inactivation (polyphenol oxidase, PPO) and bioactive compounds content (carotenoids, phenolic compounds and antioxidant activity) were analyzed in all processed purées. Regarding the pretreatment of the pumpkin, the application of MIPEF increased the content of some bioactive compounds of interest, such as carotenoids. The HPT treatment equivalent to sterilization preserved high levels of carotene compounds and antioxidant activity of pumpkin purée although this treatment importantly modified the original color of purée. However, the HPT treatment equivalent to pasteurization did not inactivate PPO enzyme in contrast to the effect reached by the equivalent TT. The bioactive compounds levels were similar or lower than by TT.

Keywords: high pressure thermal treatment; moderate-intensity pulsed electric fields; pumpkin; carotenoids; antioxidants; polyphenols; polyphenoloxidase.

INTRODUCTION

The preservation of food, both fresh and processed, has always been one of the main problems of human history. There are several traditional methods of processing, being the most used and known the thermal treatments. Usefulness of thermal treatments is primarily based on the inactivation of microorganisms and enzymes to increase the shelf-life of foods. However, degradation reactions are accelerated by temperature, so that heat treatments adversely affect nutritional compounds with health-related effects, such as vitamins, pigments, polyphenols, or antioxidant compounds (Patras et al., 2009).

Pumpkins are an appreciate vegetable crop for their pulp and seeds for human diet, mainly from the point of view of bioactive compounds, such as the carotenoids and other polyphenols compounds which contribute to the antioxidant capacity (Murkovic et al., 2002). Pumpkin purée has large amounts of carotenoids, which are pigments that give a color that ranges from yellow to red (Azevedo-Meleiro and Rodriguez-Amaya, 2007). Several studies have indicated that a higher intake of carotenoids from the diet was inversely related with a risk of degenerative and cardiovascular diseases, cataracts, macular degeneration as well as certain types of carcinomas (Ribaya-Mercado et al., 2004; Rao & Rao, 2007; Garrido et al., 2013). The minimum thermal treatment that should be applied to a pumpkin purée, should avoid at least degradation due to microorganisms, and eliminate the presence of pathogens. In this way, pH of pumpkin purée is above 4.5 and it is necessary to apply a sterilization or high pasteurization treatment to obtain a free pathogen and well-conserved product.

The trend towards healthier eating in recent years has increased consumer and industries interest for novel and alternative methods and procedures of food preservation and processing such as the emerging processing technologies (Kebede et al., 2013). High pressure thermal processing can be applied for food products sterilization or pasteurization as a function of temperature. High pressure thermal (HPT) treatments utilize a combination of high pressure (500MPa to 900MPa) and moderate-high temperature (60 to 120ºC) over a short holding time, offering several advantages like reduced thermal impact and process time, uniform and fast increase in temperature of food, and shelf-life extension of the product (Balasubramaniam et al, 2015). The rapid temperature increase during compression and temperature decrease in the

74 product upon decompression could help to reduce the severity of thermal effects encountered in
75 conventional thermal technologies (Matser et al., 2004).

76 HPT processing, in general, avoid damages in quality attributes such color or total carotene
77 content (Nguyen et al., 2007). Enzymes such as polyphenoloxidase, which are responsible for
78 biological browning reactions of phenolic compounds (Lee, 1999), was found to be partially
79 inactivated by HPT treatment on carrots (Kim et al., 2001) and pumpkins (García-Parra et al.,
80 2016). Heat-sensitive products, such as purée from pigmented fruits or vegetables, which would
81 suffer a severe loss in quality by traditional thermal processing, would be the target of this novel
82 technology.

83 Pulsed electric fields (PEF) is an emerging non-thermal food processing technology which
84 involves the application of short duration electric field pulses to foods located between two
85 electrodes (Raso & Heinz, 2006). High-intensity pulsed electric fields have been extensively
86 studied as preservation method of plant foods (Odriozola-Serrano et al., 2013; Saldana et al.,
87 2014). Recently, moderate-intensity pulsed electric fields (MIPEF) have been studied as
88 possible treatment to enhance the generation of secondary plant metabolites by inducing stress
89 reactions. It has been described that MIPEF-induced stress affects potato tissue metabolism
90 with the consequent generation of reactive oxygen species (Galindo et al., 2009). Vallverdú-
91 Queralt et al. (2013) reported that MIPEF treatments induce stress reactions in tomato fruits
92 after 24 h of refrigeration by stimulating metabolic activity and accumulating secondary
93 metabolites. Increases in phenolics and carotenoids content as well as in the antioxidant
94 capacity of MIPEF-treated tomato fruit were observed 24 h after treatments, depending on the
95 electric field strength (0.4–2 kV/cm) and number of pulses (5–30) applied (Vallverdú-Queraltet
96 al., 2012).

97 The application of novel technologies such as MIPEF and/or HPT treatments would improve the
98 quality of processed pumpkin. MIPEF would be applied directly into the vegetable in order to
99 increase the bioactive compounds content of pumpkin, in contrast, HPT treatment would be the
100 election treatment for the purée preservation. Therefore, the main objective of this study was to
101 evaluate the effect of the application of MIPEF to improve the bioactive compounds content in

pumpkin pieces and to assess the effect of HPT and TT treatments to maintain the content these compounds in pumpkin purée.

MATERIAL AND METHODS

2.1.-MATERIAL

2.1.1.-Experimental design and selection of conditions. In order to evaluate MIPEF and HPT processing, pumpkins were divided in two pre-treatments blocks, a half with no treatment (No-MIPEF) and other half with MIPEF treatment (2 kV/cm, 20 pulses). Pumpkin pieces were pretreated by MIPEF in order to improve bioactive compounds. Afterwards, thermal and HPT treatments were applied at two different intensities (equivalent to pasteurization and sterilization) in the purée manufactured from previous pumpkins. Figure 1 shows a scheme of the experimental design.

2.1.2.- Moderate-intensity pulsed electric fields processing of pumpkin. Pumpkins cv. *Butternut* at commercial ripening stage were washed, removed seeds and cut into pieces. MIPEF treatments were conducted in batch mode using an equipment manufactured by Physics International (San Leandro, CA, USA), which can deliver pulses from a capacitor of 0.1 μF with an exponential decaying waveform. A stainless steel parallel plate treatment chamber was used. A batch of pumpkin pieces was placed in the treatment chamber filled with tap water. Pumpkin pieces were treated at 2 kV/cm using 20 monopolar pulses of 4 μs at a frequency of 0.1 Hz according to a previous study (Binoti et al., 2012). MIPEF-treated pumpkins pieces were collected and immediately stored at 4 ºC for 24 h. Untreated pumpkins pieces were stored separately at 4 ºC for 24 h. Two replicates of each treatment were carried out.

2.1.3.-Pumpkin purée manufacture. MIPEF-treated and untreated pumpkin pieces (No-MIPEF) were chopped with an electric blender (Thermomix; Vorwerk, Madrid, Spain) for 5 min at maximum speed. The purée obtained had a soluble solid content of 11.7±0.3 °Brix, pH of 6.3±0.1 and the titratable acidity was 0.11±0.01 g malic acid per 100 g of sample weight. The purée was packaged, taking care to exclude as much air as possible, in plastic polyethylene (9.3 mL O_2/m^2/24h at 0 °C) pouches with 15 g of purée, which were vacuum heat-sealed (ILPRA, UM 18-GAS DIG. Barcelona, Spain). Pouches of purée were processed by HPT

immediately after being filled and closed. Pumpkin purée treated by TT was packaged in a 100 mL glass bottle.

2.1.4.-High pressure thermal processing of pumpkin purée. A multi-vessel Resato unit (FPU-100-50, serial nº 14685/42798, Roden, Netherland) was used. The equipment has 6 vessels. Three vessels were pressurized each time (1 pouch per vessel). Before the application of the treatments the samples were previously equilibrated at the initial working temperature. The equipment uses ethylene glycol as pressure-transmitting medium, and is equipped with thermostatic jacket for temperature control. The initial temperature of the assays was 36.7 ºC and 101 ºC for high pasteurization and sterilization respectively, and the pressure applied was 900 MPa. The rate of pressure build-up was 10 MPa/s. The temperature probe was on the cap of the vessel and introduced therein by contacting the pressurization medium. The adiabatic heating is responsible of the fast increase of temperature during pressure building up, while when pressure application finishes a rapid decrease of temperature is observed.

HPT treatments were compared with equivalent thermal treatment. Two conditions were selected: high pasteurization (TT: 90 ºC, 10 min. *vs.* HPT treatment: 900 MPa, 5 min, $T_{initial}$=36.7 ºC, T_{final}=58 ºC) and sterilization (TT: F_0=5 min; Z=10 ºC *vs.* HPT treatment: 900 MPa, 3 min, $T_{initial}$=101 ºC, T_{final}=121 ºC).The equivalence of the treatments was established according to the study of Timmermans et al. (2011). Processing conditions were compared based on the inactivation of the pathogen of reference for a product with pH >4.6 and a_w> 0.85 (Bull et al., 2009, Wilson & Backer, 2008).

2.1.5.- Thermal treatment of pumpkin purée. For the application of the high pasteurization, purées pouches were heated in a water bath equipped with a thermoregulator Techne TE-10A (Bibby Scientific Limited, UK) until they achieved a selected core temperature. Regarding the application of the sterilization treatment, glass bottles were treated in a semiindustrial autoclave. Purées samples core temperature profile was recorded using an Ellab Copenhagen (mod. Ctf. 84) data module.

Three pouches per treatment were analysed and were compared with non-processed purée. Therefore, a total of 30 bags were analysed. After processing, the pouches were submerged in

158 ice-water bath to rapidly reduce the purée temperature. Then, samples were frozen at -40 ºC
159 until the analyses were performed.

160 ***2.2.-METHODS***

161 ***2.2.1.-Microbiological analysis of pumpkin purée.*** Decimal dilutions of 10 g pumpkin purée
162 were prepared in sterile 0.1% (w/v) peptone solution (Merck 1.07043.1000, Merck KGaA,
163 Darmstadt, Germany). To control the product sterility the protocol proposed by Anderson and
164 Calderón (1999) was followed for food with pH higher than 4.5: (i) Incubation at 31±1 ºC for 28
165 days; (ii) Incubation at 45 ºC for 10 days; 55 ºC for 10 days; (iii) Pumpkin purée without
166 incubation (control). *Mesophilic total Counts(ISO 4833-1:2013)* were measured in plate count
167 agar (Merck) at 30 ºC for 72 h. *Moulds and yeast (ISO 21527:2008)* colonies were recorded in
168 yeast extract glucose chloramphenicol agar (Merck) at 25 ºC for 5 days. *Enterobacteriaceae*
169 *(ISO 21528-2:2004)* counts were recorded in violet red bile agar (Merck 1.01406) at 37 °C for
170 48 h. The spores of *Bacillus* (ISO 7932:2004) were determined in plate count agar (Merck) at 30
171 °C for 48 h from a peptone samples solution heated at 80 °C for 10 min. All culture media were
172 prepared following manufacturer indications. Results are expressed as colony forming units per
173 gram (CFU g^{-1}).

174 ***2.2.2.- Polyphenol oxidase (PPO) enzyme activity of pumpkin purée.*** A pumpkin purée
175 sample (5 g) was mixed with 10 mL of the extraction solution (0.2 M phosphate buffer, 1 M
176 NaCl, 4% polyvinylpolypyrrolidone, 1% Triton X-100, adjusted to pH 6.5) for 3 min and
177 centrifuged (Centrifuge Allegra 25R, Beckman Coulter, Inc.) at 22,300xg at 4 °C for 30 min. The
178 supernatant was filtered through a Whatman#1 paper to obtain the enzyme extract. The activity
179 of the enzyme was determined spectrophotometrically adding 100 µL of the enzyme extract to a
180 3 mL of 0.1 M catechol in 0.05 M sodium phosphate buffer (pH = 6.5) solution. The tests were
181 carried out by recording the color changes at 400 nm for 1 min using an UV-Vis
182 spectrophotometer (UV-2450, Shimadzu, Japan). The relative activity (RA %) of PPO was
183 calculated by equivalence $RA = {}^{A}/_{A_0} * 100$ where A and A_0 are the absorbance of the pressure-
184 treated and control samples, respectively.

185 ***2.2.3.-Instrumental color of pumpkin purée.*** A Konica Minolta Spectrophotometer CM- 3500d
186 was used to measure the instrumental color. A petri plate was filled with purée samples, taking

187 care to exclude air bubbles, and was placed into the 30 mm aperture masks. Reflectance
188 measurements were made using geometry d/8 (diffuse illumination and 8° viewing angle) with
189 the primary illuminant D65. In addition, the Chroma (C) was calculated ($C = \sqrt{a^{*2} + b^{*2}}$) as well
190 as Hue (H) angle ($H = \tan^{-1}\frac{b^*}{a^*}$).

191 Total color differences (ΔE) were calculated using the equation (1):

192 (1) $$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$$

193 where L^*_0, a^*_0, b^*_0 are the control values for unprocessed purées (in order to evaluate changes
194 after processing).

195 ***2.2.4.-Carotenoids determination in pumpkin purée.*** Carotenoids (lutein,α-carotene and β-
196 carotene) were extracted with acetone in conditions of restricted lighting, following the method
197 described by Bohoyo-Gil et al., (2012), using an Ultra High Performance Liquid Chromatograph
198 Agilent 1290 (Agilent Technologies, CA, USA) with a Zorbax Eclipse Plus C18 (column 50x2.1
199 mm; 1.8 µm particle size) coupled to a DAD detector (measuring at 460 nm). The gradient
200 mobile phase was a mix of acetonitrile/methanol 85:15 (solvent A) and
201 acetonitrile/methanol/ethyl acetate 60:20:20 (solvent B) containing 0.1% BHT and 0.05% TEA:
202 initially of 100% solvent A for 4 min, then 5 min with 100% solvent B and finally 100% solvent A
203 until the end of the run. Flow rate was 0.3 mL/min and column temperature was maintained at
204 28 ºC using a column oven. Carotenoids were identified and quantified by their retention times
205 and the coinjection of standard solutions. Results are expressed as mg·100g^{-1} of sample
206 weight.

207 ***2.2.5.-Total polyphenols content of pumpkin purée.*** Five grams of purée were centrifuged
208 (Centrifuge Allegra 25R, Beckman Coulter Inc., USA) at 22300xg and 4 ºC for 15 min and
209 filtered by glass wool. To determinate total polyphenols content (TPC) Folin-Ciocalteau reagent
210 was used according to the method of Singleton and Rossi (1965). The absorbance was
211 measured at 760 nm using gallic acid as a standard. Results were expressed as mg Gallic Acid
212 Equivalent (GAE) 100g^{-1} fresh weight.

2.2.6.-Total antioxidant activity of pumpkin purée. The antioxidant capacity was measured using ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) diammonium salt (Sigma) radical cation assay. A UV-Vis spectrophotometer (UV-2450, Shimadzu, Japan) at 750 nm was used for measurements. The results were expressed as mg of Trolox 100 g^{-1} of fresh weight.

2.2.7.-Statistical analysis. Three samples per treatment were analyzed (n=3). One analysis of variance (ANOVA) of the effect of treatment was performed using SPSS, Version 17.0 (SPSS Inc., Chicago, IL). HSD Tukey's test was applied to compare the mean values when ANOVA showed significant differences. Mean values with standard error of the mean (SEM) and standard deviation are reported. Correlations were estimated with the Pearson test at $p<0.05$. The relationships between parameters were assessed by the calculation of Principal Components Analysis (PCA).

RESULTS AND DISCUSSION

3.1.-Changes in microbiological counts of pumpkin purée.

Table 1 shows the results of microbiological counts in pumpkin purée with different processing conditions. Microbiological analyses were performed to evaluate the efficacy of treatments on the inactivation of microorganisms. Untreated pumpkin purées (MIPEF and No-MIPEF) had between 4-2 log $CFUg^{-1}$ of the principal microbial counts such as mesophilic, moulds and yeasts, *Enterobacteriaceae*, and *Bacillus sporulated*. Therefore, MIPEF-treated and untreated pumpkins pieces had similar loads of vegetative microorganisms. The effect of MIPEF on pumpkin pieces did not affect the microbial counts of the purée obtained from them. MIPEF treatments applied did not have enough intensity to inactivate microorganisms (Soliva-Fortuny et al., 2009), so this pretreatment did not affect microbial counts. However, the load of *Enterobacteriaceae* and sporulated bacillus was higher in purées obtained from pumpkins treated with MIPEF than those obtained from No-MIPEF pumpkins. The cause could be the contamination of these pieces during de application of the PEF.

Processing conditions (TT and HPT) applied were effective to reduce microbial counts. In fact, most processed purées counts were below the detection limit of the method (1 log $CFUg^{-1}$). This suggests that treatments eliminated all vegetative forms of microorganisms. Pasteurization and

241 sterilization by TT vs. HPT treatment showed similar microorganisms' inactivation, which agrees
242 with the equivalent intensity of the treatments applied.

243 In the case of the sterilization treatments, the spores were also inactivated, reaching
244 commercial sterilization. Sterility test described by Anderson and Calderón (1999) was applied
245 in this study to determine if the commercial sterilization was reached at those conditions.
246 Sterilization processes are applied in order to produce shelf stable products at room
247 temperature by inactivating spores rather than only vegetative microorganisms (Knockaert et
248 al., 2012).

249 Pumpkin purées processed by a high pasteurization (TT or HPT) would need to be preserved at
250 refrigeration temperature after processing and their shelf-life would be of approximately 6 weeks
251 (ECFF, 2006), while sterile purées (TT or HPT) could be stored at room temperature. Both
252 tested pressure–temperature combinations resulted in microbial stable pumpkin based
253 products.

254 *3.2.-Relative activity of Polyphenoloxidase (PPO) enzyme of pumpkin purée.*

255 Figure 2 shows relative PPO activity (RA %) respect to pumpkin purées obtained from No-
256 MIPEF untreated pumpkin pieces. Similar initial values of PPO activity were found in untreated
257 pumpkin (No MIPEF vs. MIPEF). As expected, MIPEF treatments had no effect on PPO activity,
258 since the applied field intensity was low and enzymes were not inactivated (minimum 25 kV/cm /
259 3 µs at 60 ºC to obtain less than 10 RA% of PPO, Schilling et al., 2008). The objective of the
260 MIPEF was to induce stress in the cell to produce a greater amount of bioactive compounds
261 (Vallverdú-Queralt et al., 2013).

262 After high pasteurization, similar values of PPO activity were found after HPT treatment
263 compared to untreated purée (in MIPEF and No-MIPEF pumpkins), while TT reduced the initial
264 activity of the enzyme to the 50%. Similar trends in PPO activity were observed in purées
265 obtained from MIPEF-treated or untreated pumpkin pieces.

266 Differences in the effect of HPT and TT treatment of pasteurization and sterilization may be
267 associated with the unequal inactivation of PPO after both treatments. The activity of this
268 enzyme was significantly reduced with TT, in both pasteurization and sterilization. However, in

HPT pasteurization treatment, initial enzyme activity remained unchanged while it was more inactivated in the HPT sterilization treatment.

Another strategy to reduce the PPO resistance to HPT pasteurization could be the reduction of the pH of the purée. However, the modification of the pH of the purées comprises the utilization of chemicals such as ascorbic or citric acids, and that, nowadays is not in line with consumer's demands. PPO enzyme stability is generally pH-dependent, so this enzyme is quite resistant to high pressure treatment in a fruit and vegetable homogenate with pH ≥6.0 (Terefe et al., 2014). In addition, threshold pressure for inactivation of avocado PPO at room temperature decreased from 850 MPa (pH 8.0) to 450 MPa (pH 4.0) while natural pH of avocado is around 6 (Weemaes et al., 1998). Nevertheless, the inactivation of enzymes by high pressure or other physical agent depends on intrinsic factors such as the source of the enzyme, pH, and media composition (Terefe et al., 2014).

Enzyme inactivation due to high pressure has been widely discussed in multiples vegetable matrices (Terefe et al., 2014), but the unpredictable nature of enzymes under a high-pressure environment restricts in the possibility of predicts their behavior in other foods (Chakraborty et al. 2014). The thermostability of enzymes is increased under specific conditions of pressure and temperature in HPT treatments, allowing enzyme-catalyzed reactions to take place at moderately high temperature and faster rate (Ludikhuyze et al., 2003).

In contrast to thermal pasteurization, the application of HPT treatment of high pasteurization did not inactivate enzymes which could limit the shelf-life of processed products. Therefore, if enzyme inactivation is desired in pressure-pasteurized products, other treatment like additive (ascorbic acid to decrease pH) or blanching may be given prior to pressure pasteurization (García-Parra et al., 2014). However, the use of temperature in high-pressure treatments is essential to obtain a significant inactivation of these enzymes as it may increase the shelf-stability of the products.

García-Parra et al., (2016) reached an adequate inactivation of PPO (~50%) in pumpkin purée at the same pressure conditions (900 MPa) and with initial temperatures of 60, 70, 80 ºC (in the current study initial temperature was 36.7 ºC). Therefore, HPT pasteurization of pumpkin purée

297 would be recommendable to be performed at higher initial temperatures than 60 ºC to allow a
298 higher inactivation of PPO.

299 *3.3.- Instrumental color changes in pumpkin purée.*

300 The influence of the treatments applied on the overall changes of color in pumpkin purée is
301 presented in Table 2. Pumpkin purées obtained from MIPEF-treated or untreated pumpkin
302 pieces showed similar values of the CIEL*a*b* values, so that the application of MIPEF did not
303 alter the initial color of the pumpkin pieces. In contrast, the application of different preservation
304 treatments modified the initial color of pumpkin purée.

305 Purée lightness (CIEL*) was significantly reduced after processing. Both treatments
306 (pasteurization and sterilization by TT and HPT processing) reduced the lightness purées (lower
307 values of CIE L*) and the reddish hue of the purées (lower CIE a* values). HPT treatments of
308 sterilization increased CIE b* values. The treatment that produced the most drastic color
309 changes in the parameters CIEL*a*b* values was HPT sterilization treatment which reduced
310 importantly lightness and redness (L* and a*) and increased CIE b* (yellowness). Previous
311 studies have evaluated the effect of high pressure at room/refrigerated temperature (HPP) or TT
312 on pumpkin; however the intensity of the treatments applied was not comparable with those
313 applied in the current study. Contador et al., (2012) found a decrease of CIEL*a*b* values after
314 processing pumpkin purée at 600 MPa/ 5 min/ 10 ºC compared to untreated purée. In the same
315 way, Zhou et al., (2014), found a reduction in CIE a* and CIE b* values after processing
316 pumpkin purée by HPP (450-550 MPa/ 15-10 min respectively, at room temperature) and TT
317 (85 ºC/ 5 min) compared to untreated samples. García-Parra et al., (2016) found that HPT
318 treatment in pumpkin purée at 900 MPa/ Ti=60 ºC/ 1 min (more intense than high pasteurization
319 conditions in current study) and at 900 MPa/ Ti=80 ºC/ 1 min (less intense than sterilization
320 conditions) increased CIE L*, and did not modify CIE a* and b*.

321 Color is perhaps the most important quality index for pumpkin purée, because it directly affects
322 the consumers' perception of the product. The significant differences in individual color
323 parameters cannot indicate whether a difference in color is perceptible by humans or not. For
324 that reason, total color differences (ΔE) were calculated to evaluate overall color changes from
325 the untreated purée (Table 3). Differences in perceivable color can be classified analytically as

not noticeable (0 to 0.5), slightly noticeable (0.5 to 1.5), noticeable (1.5 to 3.0), well visible (3.0 to 6.0), and great (6.0 to 12.0). Therefore, ΔE values of at least 3 indicate a difference of color perceptible by most people (Cserhalmi et al., 2006). Since ΔE values after processing pumpkin purée were around 7-8 (even 13), so great color changes were found after processing especially after HPT treatment.

High pasteurization produced similar color changes after TT or HPT treatment, however, when a sterilization treatment was applied, color changes were more intense by HPT treatment than by TT. The similar results of TT and HPT treatment in the pasteurization contrast with the differences in the PPO inactivation. If the inactivation of the PPO had been complete, the instrumental color parameters found in the pumpkin puree would be higher and/or better. Nguyen et al., (2007) found the maximum color changes in carrots (ΔE=6) at 700 MPa/ 121 ºC/ more than 10 min, that was lower than color changes values found in conventional thermal treatment at 121 ºC. Combined treatment of lower pressure and temperature (500 MPa/ 95 ºC/ more than 10 min) achieved a lower color difference.

ΔE was higher in current study than previous after applying HPT treatment to pumpkin purée (300, 600, 900 MPa / Ti= 60, 70, 80 ºC/ 1min) (García-Parra et al., 2016). This is an unexpected result, because in other products, HPT treatment, maintains better the original color of the vegetables compared to thermal treatment (Oey et al. 2008). However, it should be noted that all HPT treatment applied at those conditions reached an adequate inactivation of PPO, which could partially explain the differences with HPT pasteurization treatment in the current study. In addition the higher value of ΔE in HPT sterilization treatment respect to the study of García-Parra et al., (2016) could be in part caused to the most intense processing conditions applied to pumpkin in the present study.

3.4.- Changes in carotenoids of pumpkin purée.

Carotenoids found in pumpkin purée were lutein, α-carotene and β-carotene (Table 4), being the last one the most abundant. Purée made from MIPEF pre-treated pumpkin pieces and stored at 4 ºC for 24h showed high contents of carotenoids (lutein, α- and β-carotene). This would indicate that the application of MIPEF would be efficient to induce an abiotic stress and thus increase the production of some bioactive compounds. Vallverdú-Queralt et al., (2013)

355 found an increment of α-carotene and lutein in juices made of tomatoes which were pretreated
356 with MIPEF. This behavior was attributed to MIPEF-induced stress.

357 Regarding the effect of processing in pumpkin purées, carotene levels were similar in the
358 pasteurized purée by TT and HPT treatment. Sterilized purées showed lower carotenoids
359 content in purees sterilized by TT than those treated by HPT processing, especially in those
360 purées obtained from non-MIPEF treated pumpkin pieces.

361 Release of carotenoids from chromoplasts of the cells plants was enhanced as a consequence
362 of thermal processing or pressure-induced structural changes at cell membrane (Knockaert et
363 al., 2011). Contador et al. (2012) reported an increase in the extraction of carotenoids in
364 acidified pumpkin purée treated by HPP (600 MPa/ 5 min/ 10 ºC). When pressure is combined
365 with moderate temperatures, carotenoid contents are also well preserved or even increased.
366 Tauscher, (1998) found in carrot based products a very low carotene loss (less than 5%) at 600
367 MPa/75 ºC/40 min. In this respect, Sánchez et al., (2014) found that carotenoids content of
368 commonly consumed vegetables (carrot, tomato or red pepper) was not significantly influenced
369 by HPT treatment (625 MPa/ 5 min/ 70 ºC and 625 MPa/ 5 min/ 117 ºC). At higher treatment
370 temperatures, Vervoort et al. (2012) did not point out significant changes in α-carotene and β-
371 carotene contents in carrot after high pressure sterilization at 700 MPa and 124.8 ºC for 3 min.
372 Nguyen et al. (2007) also reported a 92% carotene retention in carrot after 700 MPa and 121 ºC
373 treatment for 1 min. In pumpkin purée, García-Parra et al., (2016) found important increases of
374 carotenes after processing at 900 MPa/ Ti= 60, 70, 80 ºC/ 1min, which is in line with HPT
375 treatments of pasteurization and sterilization in the current study.

376 *3.5.- Total polyphenols content (TPC) and total antioxidant activity (TAA) of pumpkin purée.*

377 The effect of treatment on TPC and TAA of pumpkin purée is shown in table 5. TPC were
378 slightly increased in pumpkin purée when a MIPEF pretreatment was applied to pumpkin
379 pieces. In contrast, TAA was significantly reduced in pumpkin with MIPEF.

380 TPC and TAA in pumpkin purées changed differently depending on the treatment applied
381 (HPT/TT). In general, the effect was different for high pasteurization or for sterilization. In the
382 high pasteurization process, HPT treatment presented lower values of TPC and TAA than TT. In

383 contrast, in the sterilization process, HPT treatment presented higher values of TPC and TAA
384 than TT.

385 Compounds with antioxidant activity in pumpkin would be carotenoids and phenolic compounds.
386 García-Parra et al., (2016) analyzed the changes in individual polyphenols (syringic and
387 chlorogenic acids) content in pumpkin after HPT (900 MPa/ Ti= 60, 70, 80 ºC/ 1min) and found
388 important increases of these compounds especially at the most intense conditions (Ti= 80 ºC).
389 This would agree with the better preservation of TPC compounds after HPT sterilization than
390 HPT pasteurization. According to Oey et al., (2008), the increase in TAA after HPT treatment
391 seems to be related to increased extractability of antioxidant compounds of the vegetable matrix
392 rather than an absolute increase. Moreover, Dini et al., (2013), suggested that biological
393 structures were altered due to thermal treatments, which could make possible the conversion of
394 insoluble phenolic compounds into more soluble forms. In addition, the different inactivation of
395 PPO by the TT or HPT treatment could have affected the stability of bioactive compounds such
396 as polyphenols, which are easily oxidized by the action of the enzyme, and thus reducing TPC
397 and TAA of pumpkin purée. HPT pasteurization treatment was not sufficient to inactivate the
398 enzymes, while the sterilization treatment achieved enzyme inactivation and this agree with the
399 lower TPC and TTA of HPT than TT for a high pasteurization treatment. In contrast for
400 sterilization the inverse results were found, which explain the importance of the inactivation of
401 PPO to preserve the antioxidant components of pumpkin.

402 Pasteurization by TT could initially release a large number of phenolic compounds already
403 present, which contribute to the TAA (Miglio et al., 2008). However, a higher temperature
404 treatment, such as sterilization, could eventually degrade the compounds released. In this case,
405 the HPT process results in an increase in TPC and TAA, which may be due to the shorter
406 treatment time and an adiabatic process. Anyway, differences between HPT pasteurization and
407 sterilization clearly depend on inactivation of PPO due to his role as substrate in degradation
408 reactions.

409 Changes in color of purées after processing are difficult to explain and could not be only caused
410 by the inactivation of PPO. Probably several interconnected causes may affect visual color
411 modifications such as i) PPO inactivation which produces an enzymatic browning of purée (low

412 CIE L*, a*, b*); ii) non-enzymatic browning as a consequence of heating which increases
413 Maillard reaction; iii) degradation (isomerization, oxidation)/release of carotenoids pigments
414 during processing (reduction of CIE a*, b*); iii) others (activity of other oxidative enzymes,
415 interactions with other components present in the vegetable like ascorbic acid), etc. In this
416 study, although color changes were more marked in sterilized purée by HPT treatment, they
417 also showed an important increase of CIE b*, which could be associated to the preservation of
418 certain carotenes after processing.

419 Maillard reaction is an important chemical pathway providing pleasant flavor, taste, and color
420 compounds to cooked foods. The reaction occurs between amines and carbonyl compounds,
421 particularly reducing sugars and it is very dependent of temperature of processing. Brown
422 pigments such as melanoidines are formed as a result of this reaction (Fogliano et al., 1999). In
423 the case of pumpkin, due to its composition rich in sugars, these reactions may play an
424 important role in global changes in furan formation from sugars and amino acids from pumpkin
425 after processing (Limacher et al., 2008). In this respect, Kebede et al., (2017) have reported
426 than the combination of pressure with temperature in HPT treatment to did not favor Maillard
427 reaction development.

428 All treatments applied were effective to inactivate microorganisms present in pumpkin purée.
429 Differences in the effect of the pasteurization or sterilization when compare TT or HPT
430 treatments on the different parameters analyzed could be caused by the resistance of the PPO
431 to the HPT treatment of pasteurization. Probably if the activity of PPO would be similar after
432 processing by TT or HPT treatments, results of bioactive compounds or color of purées after
433 pasteurization would have been different. The equivalence of both treatments was chosen on
434 microbiological basis, however, the activity of enzymes such as the PPO should be also taken
435 into account and that should be similar after processing to compare both treatments at similar
436 conditions.

437 Some studies suggest that changes in the color of pumpkin products may occur because of the
438 degradation of carotenoids (Dutta et al., 2006). Significant inverse correlations ($r=-0.519$;
439 $p<0.01$) were noted between total carotenoids content and CIEL*.The increase in CIE L* value

was related with the occurrence of non-enzymatic browning reactions that also took place together with oxidation and isomerization of β-carotene (Gliemmo et al., 2009).

A PCA was performed to examine the relationship between some selected traits in the samples. Principal components PC1 and PC2 explained 44.6% and 24.1% of the variation respectively. Figure 2A shows the loading plot of the different variables (coefficients of the eigenvectors) for PC1 and PC2, representing the quality parameters measured in pumpkin purée. The distribution of the data on PC1 and PC2 (Figure 2B) showed two separate groups of points. On the negative axis of PC1, initial pumpkin pieces (No MIPEF and MIPEF) were located and associated with purées processed by high pasteurization HPT treatment (made from No-MIPEF and MIPEF pumpkin pieces) near to parameters such as RA % PPO, while purées sterilized by TT (No MIPEF and MIPEF) were located on the positive axis of PC1.

In the present paper, the best global conditions of processing for pumpkin purée would be HPT sterilization, in spite of the greater color modifications. These processing conditions allowed the total inactivation of microorganisms and spores which would allow the preservation of purée at room temperatures. In addition, the high inactivation of PPO and the high bioactive compounds content (carotenes, polyphenols and antioxidant activity) of processed purée at those conditions would permit to improve the quality of processed purée.

4.-CONCLUSIONS

The application of MIPEF in the pumpkin pieces had beneficial effects because it increased the content of some bioactive compounds of interest, such as carotenoids. That pretreatment could increase some bioactive compounds of vegetables before processing, although much research needs to be performed for the optimization of these pretreatments. In addition, the effects of processing (thermal treatment or high pressure thermal treatment) applied to preserve the purée made from these vegetables have also great importance for the preservation of bioactive compounds in the final purée. The sterilization treatment by HPT treatment achieved the best preserved levels of carotene compounds and antioxidant activity of pumpkin purée. However, this treatment importantly modified original color of purée. The pasteurization by HPT treatment did not inactivate PPO enzyme in contrast to the effect reached by an equivalent TT. This fact could have reduced the final quality of these purées. PPO inactivation should be taken into

account when applying HPT treatments because not all treatments would be advantageous compared to the standard TT. So, when traditional TT and HPT treatment are compared, not only equivalent conditions in microbiological basis are necessary to be established, an equivalent enzyme inactivation should be also taken into account. In this sense, the inactivation of enzymes such as the PPO could be of great relevance for the successful application of this novel technology.

5.-ACKNOWLEDGEMENTS

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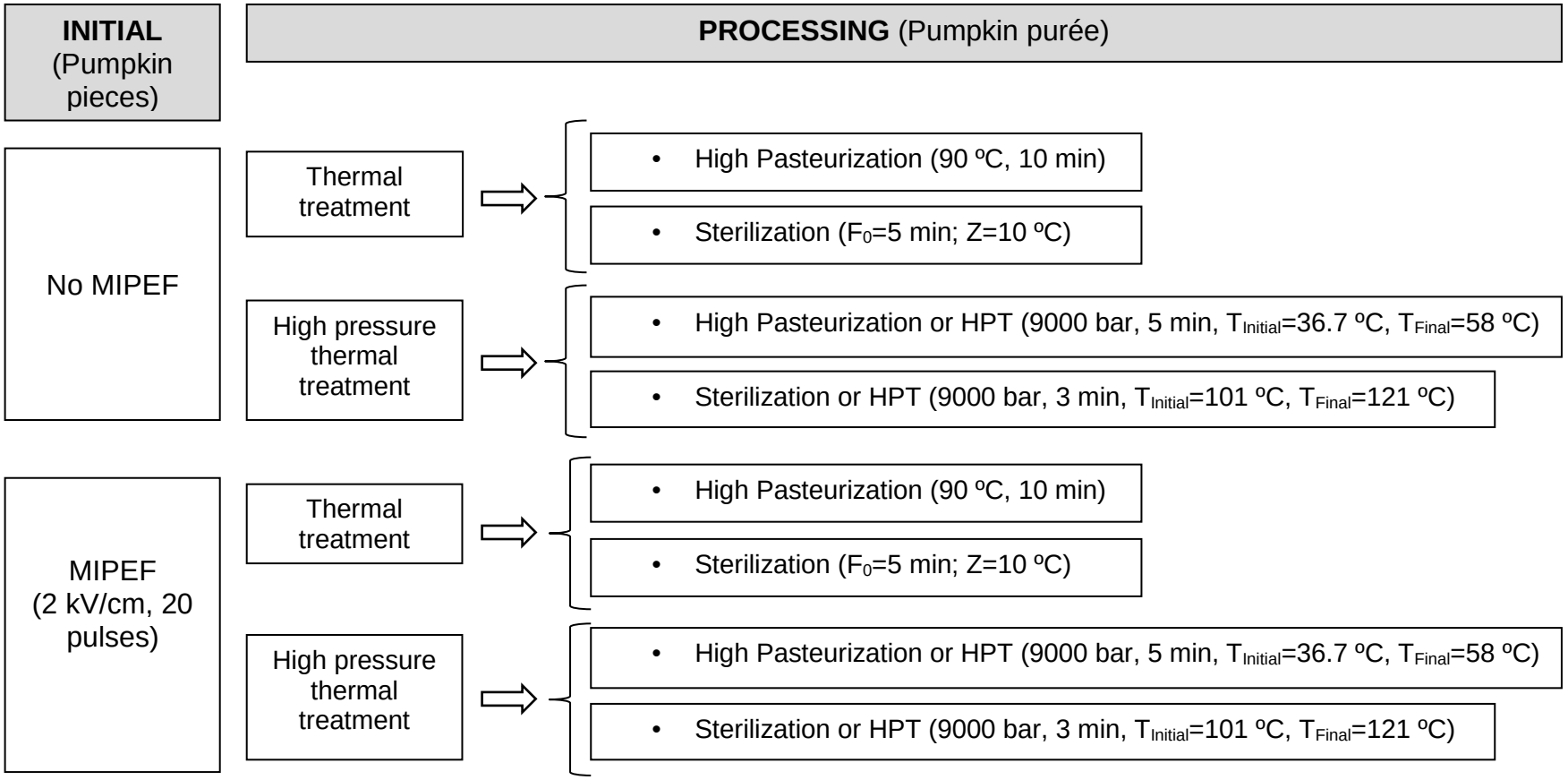
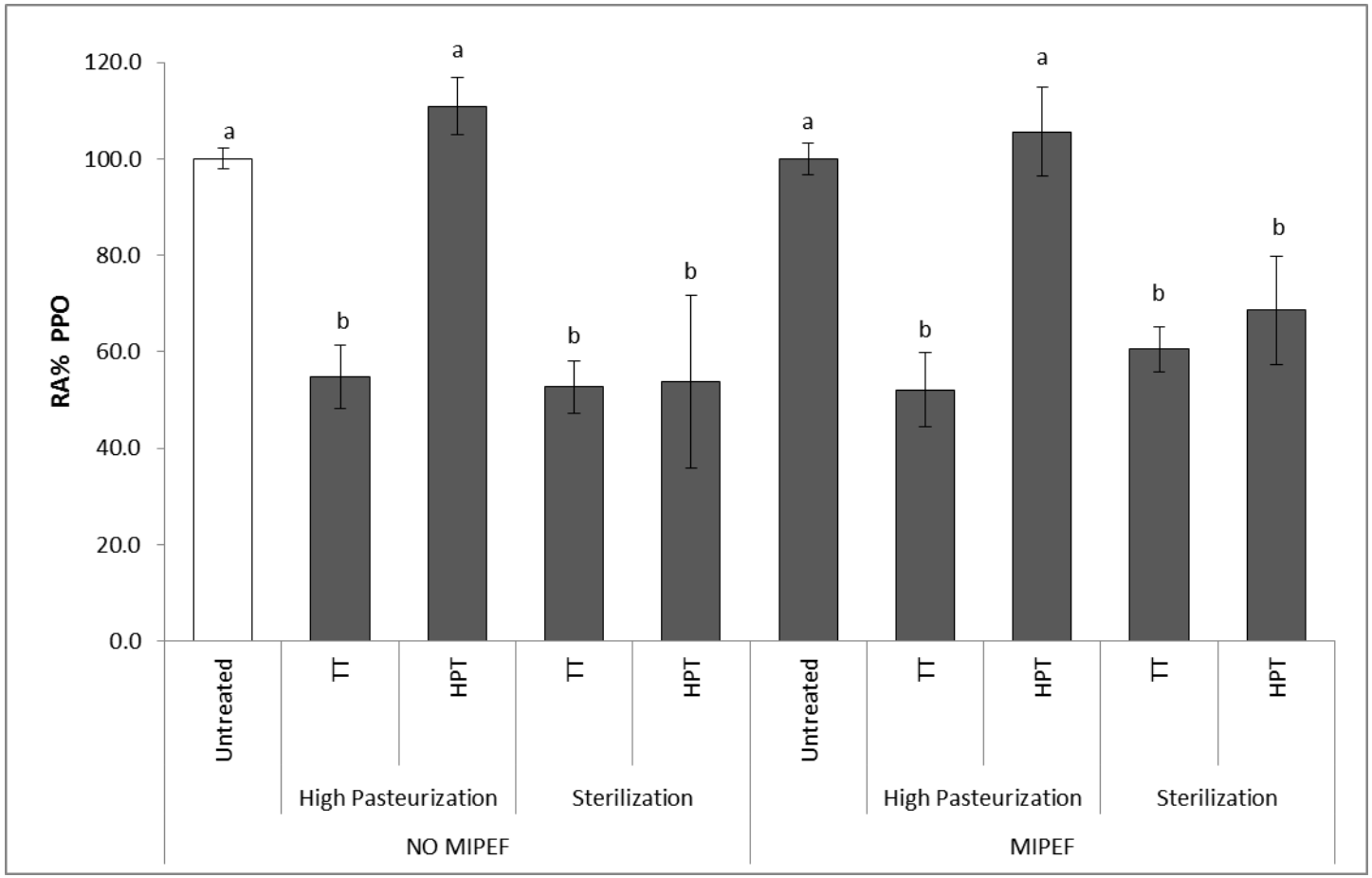
Figure 1. Experimental design.



MIPEF: Moderate-intensity of pulsed electric fields. HPT: high pressure thermal treatment.

Figure 2. Relative polyphenol oxidase activity (% respect to the control) of purées manufactured with pumpkin non-treated and treated by MIPEF and processed by high-pasteurization and sterilization by thermal treatment or by HPT (high pressure thermal treatment).



Means and standard deviations are represented. a, b: Different letters indicate significant statistical differences (Tukey's Test, $p<0.05$). MIPEF: moderate-intensity pulsed electric fields. TT: thermal treatment.HPT: high pressure thermal treatment.

Table 1. Microbiological counts (log CFU g^{-1})of purées manufactured with pumpkins non-treated and treated by MIPEF and processed by high-pasteurization and sterilization by thermal or HPT treatment (high pressure thermal treatment).

	No MIPEF					MIPEF						
	Untreated	High Pasteurization		Sterilization		Untreated	High Pasteurization		Sterilization		SEM	p
		TT	HPT	TT	HPT		TT	HPT	TT	HPT		
Mesophilic	4.3a	<1.0b	<1.0b	<1.0b	<1.0b	4.2a	<1.0b	<1.0b	<1.0b	<1.0b	0.2	0.001
Moulds and yeasts	3.8a	<1.0b	<1.0b	<1.0b	<1.0b	3.8a	<1.0b	<1.0b	<1.0b	<1.0b	0.2	0.001
Enterobacteriaceae	3.8b	<1.0c	<1.0c	<1.0c	<1.0c	3.9a	<1.0c	<1.0c	<1.0c	<1.0c	0.2	0.001
Sporulated Bacillus	2.2b	<1.0c	<1.0c	<1.0c	<1.0c	3.4a	<1.0c	<1.0c	<1.0c	<1.0c	0.1	0.001

<1 log UFC g^{-1} means below the detection limit of the method. SEM: standard error mean. a, b, c: Different letters in the same row indicate significant statistical differences (Tukey's Test, $p<0.05$). MIPEF: moderate-intensity pulsed electric fields. TT: thermal treatment. HPT: high pressure thermal treatment.

Table 2. Instrumental color of purées manufactured with pumpkin (No-MIPEF and MIPEF) and processed by high-pasteurization and sterilization by thermal or HPT treatment (high pressure thermal treatment).

	No MIPEF					MIPEF						
	Untreated	High Pasteurization		Sterilization		Untreated	High Pasteurization		Sterilization		SEM	p
		TT	HPT	TT	HPT		TT	HPT	TT	HPT		
CIE L	52.8ab	51.9bc	51.7bc	51.2c	48.1d	52.7ab	53.6a	51.4c	50.9c	49.1d	0.3	0.001
CIE a*	35.2a	31.7b	28.5c	28.6c	24.7d	35.2a	32.8b	28.9c	27.7c	24.9d	0.7	0.001
CIE b*	65.3c	58.1d	70.4ab	68.5bc	70.5ab	65.0c	58.4d	66.5bc	68.5bc	73.4a	0.9	0.001
Chroma	74.2ab	66.2c	76.0ab	74.2ab	74.7ab	74.0b	67.0c	72.6b	73.8b	77.5a	0.7	0.001
Hue	61.7c	61.4c	68.0b	67.3b	70.7a	61.5c	60.7c	66.4b	68.0b	71.2a	0.7	0.001

SEM: standard error mean. a, b, c, d: Different letters in the same row indicate significant statistical differences (Tukey's Test, $p<0.05$). MIPEF: moderate-intensity pulsed electric fields. TT: thermal treatment. HPT: high pressure thermal treatment.

Table 3. Instrumental color changes (ΔE) of purées manufactured with pumpkinnon-treated and treated by MIPEF and processed by high-pasteurization and sterilization by thermal or HPT treatment (high pressure thermal treatment).

	No MIPEF				MIPEF			
	High Pasteurization		Sterilization		High Pasteurization		Sterilization	
	TT	HPT	TT	HPT	TT	HPT	TT	HPT
ΔE (untreated-processed purée by thermal treatment or HPT equivalent)	8.08	8.48	7.46	12.55	7.11	6.63	8.54	13.81

MIPEF: moderate-intensity pulsed electric fields.TT: thermal treatment. HPT: high pressure thermal treatment.

Table 4. Individual carotenes content (mg $100g^{-1}$)of purées manufactured with pumpkinnon-treated and treated by MIPEF and processed by high-pasteurization and sterilization by thermal or HPT treatment (high pressure thermal treatment).

	No MIPEF					MIPEF						
	Untreated	High Pasteurization		Sterilization		Untreated	High Pasteurization		Sterilization		SEM	p
		TT	HPT	TT	HPT		TT	HPT	TT	HPT		
Lutein	0.34b	0.23cd	0.21cd	0.27bc	0.26bc	0.50a	0.16d	0.23cd	0.30bc	0.22cd	0.0	0.001
α-carotene	0.95bc	0.88c	0.83c	0.83c	1.45a	1.32ab	0.88c	1.03bc	1.01bc	1.15abc	0.0	0.001
β-carotene	2.22bc	2.21bc	2.04bc	2.03bc	3.72a	2.98ab	1.86c	2.51bc	2.43bc	2.93ab	0.1	0.001
Carotenes Total	3.44bc	3.25bc	3.00c	3.04c	5.44a	4.73ab	2.77c	3.70bc	3.67bc	4.30abc	0.2	0.001

SEM: standard error mean. a, b, c, d: Different letters in the same row indicate significant statistical differences (Tukey's Test, $p<0.05$). MIPEF: moderate-intensity pulsed electric fields. TT: thermal treatment. HPT: high pressure thermal treatment.

683 Table 5. Total polyphenols content (mg galic acid $100g^{-1}$ fresh weigh) andtotal antioxidant activity (TAA, mg Trolox $100g^{-1}$ fresh weigh) of purées manufactured
684 with pumpkinnon-treated and treated by MIPEF and processed by high-pasteurization and sterilization by thermal or HPT treatment (high pressure thermal
685 treatment).

	No MIPEF					MIPEF						
	Untreated	High Pasteurization		Sterilization		Untreated	High Pasteurization		Sterilization		SEM	p
		TT	HPT	TT	HPT		TT	HPT	TT	HPT		
TPC	32.00ab	29.03bc	24.87d	26.78cd	34.44a	33.41a	32.56a	26.30cd	27.68cd	33.20a	0.6	0.001
TAA	81.34bc	81.09bc	67.12e	72.10d	83.67b	66.46e	80.25bc	79.86bc	76.85c	93.22a	1.4	0.001

686 SEM: standard error mean. a-e: Different letters in the same row indicate significant statistical differences (Tukey's Test, $p<0.05$). MIPEF: moderate-intensity
687 pulsed electric fields. TT: thermal treatment. HPT: high pressure thermal treatment.

Figure 2. PCA. (Figure A) Loading plots after principal component analysis of the variables in the plane defined by two first components (PC#1: principal component 1; PC#2: principal component 2). (Figure B) Score plot after principal component analysis of the individuals in the plane defined by two first principal components.