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1 **Effect of thermosonication on the bioaccessibility of antioxidant**  
2 **compounds and the microbiological, physicochemical, and nutritional**  
3 **quality of an anthocyanin-enriched tomato juice**

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15

16 **Abbreviations**

17 PG: polygalacturonase; PME: pectin methylesterase; TPC: total phenolic content; TAC:  
18 total anthocyanin content; TLC: total lycopene content; SSC: soluble solids content; CJ:  
19 control tomato juice; TAM: Total aerobic mesophilic microorganisms; AEJ: anthocyanin-  
20 enriched juice; P-AEJ: Thermally treated anthocyanin-enriched tomato juice; TS-AEJ:  
21 Thermosonicated juice; TTA: titratable acidity;  $C^*_{ab}$ : Chroma;  $\delta E$ : Difference from the  
22 control; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric ion reducing antioxidant  
23 power; SPC: Strawberry press cake; S.D.: Standard deviation; ANOVA: Analysis of  
24 variance.

25 **Abstract**

26 The aim of this study was to assess the potential of thermosonication as a strategy to  
27 obtain safe and high quality tomato juice enriched in anthocyanins, formulated using  
28 strawberry processing co-products. Incorporation of strawberry press cake into the tomato  
29 juice resulted in higher polyphenolic and anthocyanin content and increased antioxidant  
30 capacity. Thermosonication for 5 min at 60 °C at either 35 or 130 kHz resulted in higher  
31 microbial inactivation when compared to thermal pasteurization at 80 °C for 1 min. In  
32 addition, thermosonication allowed increased retention of colour attributes as well as  
33 polyphenol, lycopene, anthocyanin, and antioxidant capacity retention when compared to  
34 thermal treatment. For example, the total anthocyanin content decreased from  $1.08 \pm 0.04$   
35 mg/100 mL before processing to  $0.92 \pm 0.01$  mg/100 mL after thermal pasteurization but  
36 the difference was not significant when compared with the thermosonicated juice ( $1.06 \pm$   
37  $0.03$  mg/100 mL). Although bioaccessibility of phenolic compounds after a simulated  
38 gastrointestinal digestion was lower in processed juices, thermosonicated samples  
39 showed a higher bioaccessibility when compared to the thermally-treated ones.

40

41 **Keywords:** tomato juice, anthocyanins, thermosonication, pasteurization, co-product  
42 revalorisation, functional foods

43 **1. Introduction**

44 Anthocyanins, which belong to the flavonoids subclass of polyphenols, are naturally  
45 occurring pigments which are responsible for the orange, red, violet, or blue colours of  
46 fruits and vegetables (Manach et al. 2004). Because of their peculiar chemical structure,  
47 anthocyanins can react with reactive oxygen species and present high antioxidant  
48 properties (Bueno et al. 2012). In addition, ingestion of anthocyanins and anthocyanin-  
49 rich foods has been associated with a lower risk of suffering from hypertension (Cassidy  
50 et al. 2010) and type-2 diabetes (Muraki et al. 2013). Because of their health-promoting  
51 benefits, previous studies developed foods fortified in anthocyanins. For example, Sui et  
52 al. (2016) developed a functional bread enriched in anthocyanin-rich black rice bran  
53 powder, which showed a lower digestion rate and extra health benefits. Similarly,  
54 Gültekin-Özgüven et al. (2016) developed a chocolate fortified with encapsulated  
55 anthocyanins.

56 Strawberries (*Fragaria × ananassa*) are naturally rich in anthocyanins and other  
57 phytochemicals such as phenolic acids. Because of their high content in health-promoting  
58 phytochemicals and high antioxidant activity, co-products generated during strawberry  
59 processing such as strawberry press cake or strawberry pomace are promising ingredients  
60 for food applications (Šaponjac et al. 2015). Their use as new food ingredients could open  
61 novel commercial opportunities, reduce the amount of food discarded as waste or used  
62 for low value purposes, and increase the consumption of anthocyanins and therefore  
63 promote health. However, anthocyanins show instability towards a variety of chemicals  
64 and physical parameters including pH variations, high temperatures, and light (Fernandes  
65 et al. 2018). In addition, the structure and composition of the food matrix may either  
66 enhance or prevent the release and solubilisation of anthocyanins during digestion and  
67 hence their bioaccessibility and bioavailability (Pineda-Vadillo et al. 2017).

68 Microorganisms and enzymes including polygalacturonase (PG; EC 3.2.1.15) and pectin  
69 methylesterase (PME; EC 3.1.1.11) are involved in the deteriorating modifications of fruit  
70 and fruit-based products that can cause colour, flavour, or nutritional changes.  
71 Inactivation of microorganisms and enzymes is achieved in the food industry mainly by  
72 heat treatments (Jabbar et al. 2015). However, high temperatures can result in unwanted  
73 colour changes as well as in the degradation of nutritionally interesting compounds such  
74 as polyphenols. In addition, consumers are now becoming more aware of the relationship  
75 between food, diet, and health, and this has led to increased interest in natural ingredients  
76 and development of mild processing technologies (Lafarga et al. 2018). Novel  
77 technologies with potential for being used in the food industry include high-pressure  
78 processing, pulsed electric fields, and thermosonication, a strategy that combines  
79 ultrasounds and mild temperatures. The microbial lethal effect of ultrasounds has been  
80 mainly attributed to the cavitation phenomenon (Khandpur and Gogate 2015).  
81 Cavitational effects include intense localized pressure and temperature pulse as well as  
82 high intensity shear and turbulence and these can lead to the breakage of cell walls and  
83 damage of DNA resulting in deactivation of microorganisms (Khandpur and Gogate  
84 2016). This technology has been suggested as a good alternative to thermal processing  
85 of, for example, carrot (Jabbar et al. 2015), watermelon (Rawson et al. 2011), or apple  
86 (Abid et al. 2014) juice.

87 The aim of this paper was to develop a novel tomato (*Solanum Lycopersicum* var.  
88 *canario*) juice enriched in anthocyanins using strawberry press cake and to evaluate the  
89 potential of thermosonication as an alternative to conventional thermal processing to  
90 provide a healthier, high quality, and safe product. Studied parameters included colour,  
91 pH, soluble solids content (SSC), titratable acidity (TTA), total phenolic content (TPC),  
92 antioxidant activity, and total lycopene (TLC) and total anthocyanin content (TAC).

93 Microorganisms and the activity of PG and PME were also studied. A secondary aim of  
94 this study was to determine the bioaccessibility of phenolic and antioxidant compounds  
95 using a simulated gastrointestinal digestion.

96 **2. Materials and methods**

97 **2.1 Chemicals and reagents**

98 Methanol and ferric chloride were purchased from Panreac (Barcelona, Spain). Gallic  
99 acid, ascorbic acid, hydrochloride, 2,4,6-tris(2-pyridyl)-s-triazine, 2,2-diphenyl-1-  
100 picrylhydrazyl (DPPH), tris(2-carboxyethyl)phosphine hydrochloride, potassium  
101 phosphate monobasic, potassium phosphate dibasic, sodium tetrachloropalladate, sodium  
102 acetate, sodium hydroxide, sodium chloride, peptone,  $\alpha$ -amylase (EC 3.2.1.1), pepsin (EC  
103 3.4.23.1), and sodium carbonate were purchased from Sigma-Aldrich (Steinheim,  
104 Germany). Folin-Ciocalteu's reagent was purchased from VWR (Llinars del Vallès,  
105 Spain). Buffered peptone water and plate count agar (PCA) were purchased from Biokar  
106 (Beauvais, France). All reagents used were of analytical grade. Tomatoes used for juice  
107 making were purchased locally.

108 **2.2 Preparation of the functional anthocyanin-enriched tomato juice**

109 Strawberry press cake obtained after juice making was frozen, freeze-dried using a  
110 Crydos-50 freeze-dryer (Telstar, Barcelona, Spain), and stored at -20 °C until further use.  
111 The freeze-dried strawberry press cake was labelled as SPC. Two different types of juices  
112 were prepared: the control tomato and the anthocyanin-enriched tomato juice. Control  
113 tomato juice and anthocyanin-enriched juice were labelled as CJ and AEJ, respectively.  
114 The CJ was prepared using an Infinity Cold Press Revolution Juicer (Groupe SEB Iberica,  
115 Barcelona, Spain). Preliminary trials were carried out to establish the maximum SPC  
116 inclusion level that did not significantly affect the organoleptic properties of the juice.  
117 Following these trials, tomato juice containing SPC at concentrations ranging from 40 to  
118 50 g/L obtained the highest acceptability scores (data not shown). Therefore, the AEJ was  
119 prepared by incorporating 100 g of SPC, suspended in distilled water at a SPC:water ratio  
120 of 1:3 (w/v), into CJ until a final SPC concentration of 45 g/L (35 g of SPC, 135 mL of

121 water, and 865 mL of CJ per 1000 mL of AEJ). The amount of water in which the SPC  
122 was resuspended was calculated to achieve a comparable water content in both juices,  
123 determined as  $93.1 \pm 0.2$  and  $93.0 \pm 0.8\%$  for CJ and AEJ, respectively. The CJ and AEJ  
124 were homogenized using a T-25 digital ULTRA-TURRAX<sup>®</sup> homogenizer (IKA,  
125 Staufen, Germany) at 10,000 rpm for 1 min and stored at 4 °C during a 7-day period.

### 126 **2.3 Juice processing**

127 Juice processing was carried out at the pilot plant facilities of IRTA Fruitcentre, Lleida,  
128 Spain. Aliquots of 100 mL of AEJ were introduced in triplicate into 100 mL clear glass  
129 flasks and were either left untreated (control, AEJ), thermally treated (80°C, 1 min; P-  
130 AEJ), or thermosonicated using a TI-H 20 stainless steel ultrasonic bath (Elma  
131 Schmidbauer GmbH, Singen, Germany). Effective ultrasonic power was 250 W and the  
132 tank internal dimensions and capacity were 330/300/200 mm (W/D/H) and 16.8 L,  
133 respectively. Thermosonication parameters studied included temperature (20, 40, or 60  
134 °C), processing duration (0, 5, or 10 min), and ultrasonic frequencies (0, 35, or 130 kHz)  
135 at constant mode. Immediately after processing, samples were chilled to approximately 4  
136 °C using a ABT 101L blast chiller (Infrico, Barcelona, Spain) and stored at 4 °C in the  
137 dark until further analysis. Analyses were performed at days 1 and 7 post-processing.  
138 Treatment at 60 °C with ultrasounds at either 35 or 130 kHz for 5 min, which were found  
139 to be the optimum conditions, were abbreviated as TS-AEJ.

### 140 **2.4 Microbiological analysis**

141 Total aerobic mesophilic microorganisms (TAM) were determined before and after  
142 processing. Briefly, 25 g of sample were mixed in triplicate with 225 mL of buffered  
143 peptone water in a 400 mL sterile full-page filter bag (Bagpage, Interscience, Saint Nom,  
144 France). The mixture was homogenized in a Masticator Basic 400 (IUL, Barcelona,  
145 Spain) at 8.5 strokes per s for 90 s. Serial decimal dilutions were made in duplicate in

146 saline peptone (sodium chloride 8.5 g/L, peptone 1 g/L) and plated on plate count agar  
147 Petri dishes (PCA, Biokar Diagnostics, France). Plates were incubated at  $30 \pm 1$  °C for 3  
148 days. Colony forming units (cfu) were counted and results were expressed as log cfu/g.  
149 Reductions were calculated by subtracting the TAM population after treatment (log cfu/g)  
150 from the initial one.

## 151 **2.5 Physicochemical characteristics**

152 Colour parameters were determined using a Minolta CR-200 colorimeter (Minolta INC,  
153 Tokyo, Japan). CIE values were recorded in terms of  $L^*$  (lightness),  $a^*$  (redness,  
154 greenness), and  $b^*$  (yellowness/blueness). Calibration was carried out using a standard  
155 white tile (Y:92.5, x:0.3161, y:0.3321) provided by the manufacturer and the D65  
156 illuminant, which approximates to daylight. Chroma ( $C^*_{ab}$ ) and difference from the  
157 control ( $\delta E$ ) were calculated following the methodology described by Wibowo et al.  
158 (2015). Results are the average of 10 measurements per treatment, sampling day, and  
159 replicate.

160 The pH of the samples was measured using a Basic 20 pH meter (Crison Instruments  
161 S.A., Barcelona, Spain). To measure TTA, 10 mL of juice were diluted in 10 mL of  
162 distilled water and were titrated with 0.1 N sodium hydroxide up to pH 8.2. Results are  
163 the average of three measurements per treatment, sampling day, and replicate and were  
164 expressed as g of malic acid per L.

165 SSC was measured at 20 °C with a handheld refractometer (Atago Co. Ltd., Tokio, Japan).  
166 Measurements were performed in triplicate per treatment, sampling day, and replicate and  
167 results were expressed in °Brix.

## 168 **2.6 Total phenolic content (TPC)**

169 The TPC was determined by the Folin Ciocalteu method as described by Altisent et al.  
170 (2014) using a GENESYS™ 10S-UV Vis spectrophotometer (Thermo Fisher Scientific,

171 MA, USA). TPC was determined in triplicate for each treatment, sampling day, and  
172 replicate and results were expressed as mg of gallic acid equivalents per 100 mL.

### 173 **2.7 Antioxidant activity: FRAP and DPPH· scavenging activity**

174 Antioxidant activity was assessed using two different methods: the ferric ion reducing  
175 antioxidant power (FRAP) and the DPPH scavenging activity assays following the  
176 methodologies previously described by Plaza et al. (2016) and Hidalgo et al. (2010),  
177 respectively. Antioxidant activity was determined in triplicate for each treatment,  
178 sampling day, and replicate and results were expressed as mg of ascorbic acid equivalents  
179 per 100 mL.

### 180 **2.8 Total anthocyanin content (TAC)**

181 The TAC was determined following the methodology previously described by Meyers et  
182 al. (2003) using a spectrophotometer. TAC was determined in triplicate for each  
183 treatment, sampling day, and replicate and results were expressed as mg of cyanidin 3-  
184 glucoside equivalents per 100 mL.

### 185 **2.9 Total lycopene content (TLC)**

186 The TLC was determined following the methodology previously described by Fish et al.  
187 (2002) using a spectrophotometer. TLC was determined in triplicate for each treatment,  
188 sampling day, and replicate and results were expressed as mg of lycopene per 100 mL.

### 189 **2.10 Enzymatic activity**

190 The activity of the enzyme PG was determined following the methodology of Sila et al.  
191 (2008) with brief modifications as described by Zudaire et al. (2018). In addition, the  
192 activity of the enzyme PME was determined following the method described by Plaza et  
193 al. (2016) with some modifications as described in Zudaire et al. (2018). The activity of

194 both enzymes was expressed as PG or PME units per mL. PME and PG units were defined  
195 as the amount of enzyme required to release 1  $\mu$ mol of carboxyl or reducing groups per  
196 min.

### 197 **2.11 Simulated gastrointestinal digestion**

198 A simulated gastrointestinal digestion of AEJ, P-AEJ, and TS-AEJ was performed at day  
199 7 post-processing following the methodology previously described by Minekus et al.  
200 (2014). The methodology consists of three sequential stages including oral ( $\alpha$ -amylase,  
201 pH 7.0), gastric (pepsin, pH 3.0), and intestinal (pancreatin and fresh bile, pH 7.0) phases.  
202 Digestions and determinations of TPC and antioxidant activity were carried out after  
203 gastric and intestinal phases and determined in triplicate for each treatment and replicate.

### 204 **2.12 Statistical analysis**

205 Results are expressed as mean  $\pm$  standard deviation (S.D.). A multifactorial design with  
206 storage period and treatment factors was used to analyse the results. Data were analysed  
207 using analysis of variance (ANOVA) with JMP 13 (SAS Institute Inc., Cary, USA).  
208 Where significant differences of storage period or treatment time were found, a Tukey  
209 pairwise comparison of the means was conducted to identify where the sample differences  
210 occurred. The criterion for statistical significance was  $p < 0.05$ .

### 211 3. Results and discussion

#### 212 3.1 Effect of strawberry co-product inclusion into tomato juice

213 Strawberries are rich sources of anthocyanins (Ma et al. 2018) and as expected,  
214 incorporation of SPC into tomato juice resulted in increased TAC ( $p<0.05$ ). The TAC of  
215 CJ and untreated AEJ at day 1 was  $0.09 \pm 0.01$  and  $1.08 \pm 0.04$  mg/100 mL, respectively.  
216 In addition, the AEJ showed a lower TLC ( $2.02 \pm 0.10$  mg/100 mL) when compared to  
217 CJ ( $2.38 \pm 0.07$  mg/100 mL,  $p<0.05$ ), because of the dilution of the lycopene found in CJ  
218 after addition of water and strawberry co-products. AEJ also showed higher TPC and  
219 antioxidant activity when compared with the control ( $p<0.05$ ). The TPC of the CJ and  
220 AEJ was  $24.03 \pm 1.02$  and  $57.25 \pm 2.39$  mg/100 mL respectively ( $p<0.05$ ). FRAP and  
221 DPPH· values of AEJ were  $73.01 \pm 0.82$  and  $51.84 \pm 4.05$  mg/100 mL. These were higher  
222 than those of CJ, which were  $31.26 \pm 1.86$  and  $24.39 \pm 1.24$  mg/100 mL respectively  
223 ( $p<0.05$ ). Several studies demonstrated the bioactive properties of anthocyanin-rich  
224 extracts and foods (Ma et al. 2018; Zhao et al. 2015). Results reported in the current paper  
225 compared well with those obtained in previous studies, which demonstrated that  
226 anthocyanin-rich products and extracts could increase the health benefits of foods and  
227 show potential for being used as novel ingredients for the development of functional  
228 foods. Kamiloglu et al. (2017) showed that enrichment of cake flour with black carrot  
229 pomace, at concentrations ranging from 50 to 150 g/kg, caused a dose-dependent increase  
230 in anthocyanins, total phenolics, and total antioxidant capacity. Pineda-Vadillo et al.  
231 (2016) also reported increased *in vitro* antioxidant activity of dairy and egg products  
232 enriched with grape extracts rich in anthocyanins and other polyphenols. Anthocyanin-  
233 rich ingredients can increase the health benefits of foods beyond their polyphenolic  
234 content and antioxidant capacity. Indeed, Sui et al. (2016) recently reported that

235 enrichment of bread with an anthocyanin-rich extract from black rice reduced the  
236 digestibility rate of the product providing it with extra health benefits.

237 Colour attributes and other physiochemical parameters, listed in Table 1, were also  
238 affected after incorporation of SPC into CJ. The  $L^*$  value was higher in AEJ when  
239 compared to CJ ( $p < 0.05$ ). This denotes a lighter appearance of the juice after  
240 incorporation of SPC into the tomato juice. In addition, incorporation of SPC into the  
241 tomato juice also resulted in increased red hue ( $p < 0.05$ ). No differences were observed in  
242  $C_{ab}^*$  values, which means that that CJ and AEJ had a comparable colour intensity.  $\delta E$   
243 combines the change in  $L^*$ ,  $a^*$ , and  $b^*$  values to quantify the colour deviation from a  
244 standard reference sample, in this case, to compare the colour difference between CJ and  
245 AEJ. Those samples with  $\delta E > 3$  display a visible colour deviation (Wibowo et al. 2015).  
246 As expected, both juices exhibited a visible colour deviation. Incorporation of SPC into  
247 the tomato juice also resulted in decreased pH ( $p < 0.05$ ). The opposite trend was observed  
248 for TTA and SSC ( $p < 0.05$ ). Finally, a separation layer was observed during storage of CJ  
249 (not measured). However, incorporation of SPC into CJ gave no phase separation during  
250 storage for 7 days at 4 °C.

251 Overall, incorporation of SPC into tomato juice, at the concentration evaluated herein,  
252 resulted in a stable product with a significantly higher nutritional quality. Some  
253 physicochemical properties such as SSC, TTA, pH, or colour were significantly affected  
254 after addition of SPC into the CJ.

### 255 **3.2 Effect of conventional thermal processing and thermosonication on juice** 256 **microbiological quality**

257 In order to assess the effect of different thermosonication conditions on the  
258 microorganisms on the juice, the survival rates of TAM counts were analysed.  
259 Preliminary trials were carried out at different temperatures (20, 40, or 60 °C), durations

260 (5 or 10 min), and frequencies (0, 35, or 130 kHz). Khandpur and Gogate (2015)  
261 suggested that a controlled application of ultrasounds is required in order to maximize the  
262 degree of microbial inactivation and minimize the loss of nutrient quality and avoid to  
263 stimulate enzymes. In the current study, the thermosonication process was not optimised,  
264 and further studies are needed in order to select the conditions that would permit higher  
265 antimicrobial effects and higher retention of bioactive compounds. A response surface  
266 methodology varying frequency, temperature, duration, and power would allow to obtain  
267 optimum conditions. No effect on microbial inactivation was observed with respect to  
268 frequencies or duration. However, differences were observed with respect to temperature  
269 and the combined effect of temperature and ultrasounds ( $p<0.05$ ). Initial TAM count of  
270 AEJ was  $6.3 \pm 0.2$  log cfu/g. Thermal processing at 80 °C for 1 min resulted in reductions  
271 in the total aerobic mesophilic organisms count of 2.4 and 3.3 log cfu/g at days 1 and 7  
272 (Figure 1A and 1B, respectively). Operating at 20 °C had no effect on the microbial load  
273 of the samples when compared with the untreated juice. Moreover, the microbial load of  
274 samples sonicated for 5 min at 20 °C after 7 days of storage at 4 °C was higher when  
275 compared to the samples treated at 20 °C for 5 min and not sonicated ( $p<0.05$ ). The  
276 observed increase could be caused by a liberation of carbohydrates and other compounds  
277 which promote the growth of the microorganisms that survived to the process, as the  
278 application of ultrasounds for assisting extraction of phytochemicals and other organic  
279 compounds from plant material has been widely published. In addition, sonication can  
280 disaggregate microbial cell aggregates resulting in more than one cfu from each initial  
281 cfu. Although no lethal effect was observed when operating at 20 °C, sonication at 40 °C  
282 resulted in a low but significant reduction in the TAM count (Figure 1;  $p<0.05$ ).  
283 Reductions ranged between 0.40 and 0.46 log cfu/g at day 1 and 0.18 and 0.64 log cfu/g  
284 at day 7 depending on the frequencies and process durations used. Thermal treatment of

285 the juice at 40 °C for 5 min, with no sonication, resulted in a no reduction in the TAM  
286 count at day 1 and a reduction of 0.31 log cfu/g at day 7, suggesting a synergetic effect  
287 of temperature and ultrasounds. It has been suggested that ultrasounds enhance the  
288 sensitivity of microorganisms to heat, pressure, and acidic conditions due to acoustic  
289 cavitation and modifications in their cell membrane (Bermúdez-Aguirre and Barbosa-  
290 Cánovas 2012). The same trend was observed when processing at 60 °C. The lethal effect  
291 of temperature (60 °C) combined with ultrasounds (35 or 130 kHz for 5 or 10 min) was  
292 higher when compared to that of sonication or thermal processing alone ( $p<0.05$ ).  
293 Observed reductions were even bigger than those obtained after thermal processing at 80  
294 °C for 1 min, especially after 7 days of storage at 4 °C ( $p<0.05$ ). TAM counts of AEJ  
295 treated at 60 °C with or without sonication, decreased during storage with total reductions  
296 of  $5.1 \pm 0.1$  and  $5.7 \pm 0.1$  log cfu/g, which resulted in a final population of  $3.3 \pm 0.1$  and  
297  $2.6 \pm 0.1$  log cfu/g at day 7, respectively. This could be due to the fact that at 60 °C, some  
298 injured microorganisms did not survive storage due to the harsh environment encountered  
299 in the AEJ (low pH and temperature, high acidity, and no oxygen). Similar results were  
300 observed after thermosonication (20 kHz, 750 W) at 60 °C of carrot (Jabbar et al. 2015)  
301 or apple (Abid et al. 2013) juice. Results were also in line with those reported by Kiang  
302 et al. (2013) who evaluated the effect of thermosonication (25 kHz, 200 W) on the human  
303 pathogens *Escherichia coli* O157:H7 and *Salmonella* Enteritidis. In that study, the authors  
304 reported that *Salmonella* Enteritidis was not recovered in samples subjected to  
305 thermosonication at 60 °C for more than 5 min.

306 Overall, thermosonication for 5 min at 60 °C and either 35 or 130 kHz allowed a higher  
307 reduction in the microbial load of AEJ when compared to a pasteurization treatment at 80  
308 °C for 1 min. The observed reduction was especially higher at day 7 ( $p<0.05$ ). In addition,

309 the combined antimicrobial effect of temperature and ultrasounds was higher when  
310 compared to both strategies alone.

### 311 **3.3 Effect of conventional thermal processing and thermosonication on juice** 312 **enzymatic and physicochemical quality**

313 Based on microbiological results, thermosonication treatments at 60 °C for 5 min at 35 or  
314 130 kHz were selected for further studies. No differences were observed in the enzymatic,  
315 physicochemical, and nutritional properties of juices treated by either 35 or 130 kHz and  
316 therefore, results shown in this section are the average of both treatments.  
317 Thermosonication and cold storage had no effect on the pH, TTA, and SSC of the juice  
318 when compared to the fresh untreated juice (Table 1). Similar results were published  
319 previously (Jabbar et al. 2015; Abid et al. 2013; Walkling-Ribeiro et al. 2009). As  
320 mentioned previously, those samples with  $\delta E > 3$  displayed a well visible colour deviation  
321 (Cserhalmi et al. 2006). Therefore, according to Cserhalmi et al. (2006) colour deviations  
322 caused by thermosonication were not visible for any of the sampling days assayed.  
323 Thermal processing resulted in no differences in colour 24 h after processing (P-AEJ,  
324 Table 1), but differences were visible at day 7 ( $\delta E > 3$ ), suggesting a better retention of  
325 physicochemical properties in the thermosonicated juice when compared to the thermally  
326 treated one. Probably, colour changes were caused by a degradation of pigments such as  
327 lycopene and anthocyanins caused by temperature and storage.

328 Endogenous enzymes found in fruits are responsible for changes in their postharvest  
329 quality. Enzymes like PG and PME are involved in breakdown of pectin and other cell  
330 wall materials, resulting in products with reduced viscosity and undesirable organoleptic  
331 properties (Chakraborty et al. 2015). The effect of thermosonication and thermal  
332 processing on the activity of the enzymes PG and PME in the AEJ is shown in Figure 2.  
333 The activity of both enzymes after processing showed a similar trend. Thermal processing

334 significantly reduced the activity of both PG and PME at days 1 and 7 when compared to  
335 the untreated control ( $p<0.05$ ). Thermosonication of the juice also decreased the activity  
336 of PME at day 1 ( $p<0.05$ ) but the observed decrease was significantly lower when  
337 compared to conventional thermal processing ( $p<0.05$ ). Enzymatic inactivation by  
338 thermosonication has been attributed to the combined effect of temperature and to the  
339 chemical and mechanical effects induced by cavitation and high shear forces produced by  
340 bubble implosions with acoustic field (Ercan and Soysal 2011). Free radicals produced  
341 by sonication can also oxidize enzymes reducing their activity (Terefe et al. 2009).  
342 Similar results were reported by Jabbar et al. (2015) after thermal processing (80 °C, 1  
343 min) and thermosonication (20, 40, or 60 °C for 5 or 10 min) of carrot juice. In that study,  
344 the authors assessed the enzymatic activity after processing and not during storage. In the  
345 current paper, the activity of both PG and PME increased in TS-AEJ at day 7 and was  
346 even higher than that measured in AEJ ( $p<0.05$ ). Results obtained in the current paper  
347 suggest that thermosonication has a lower enzyme inactivation capacity when compared  
348 to conventional pasteurization. However, previous studies suggested that the inactivation  
349 of enzymes by thermosonication is time-dependent (Rithmanee and Intipunya 2012;  
350 Ercan and Soysal 2011; Jabbar et al. 2015). Therefore, although long processing times  
351 are not feasible at industrial scale, further studies could assess the effect of longer  
352 thermosonication processes on the activity of both PG and PME of the AEJ developed  
353 herein.

#### 354 **3.4 Total phenolic content and antioxidant activity**

355 Figure 3 shows the effect of thermosonication on the TPC and antioxidant activity of the  
356 AEJ. The TPC of both P-AEJ and TS-AEJ was lower when compared to that of untreated  
357 AEJ ( $p<0.05$ ; Figure 3A). However, the TPC of the thermally treated juice was lower  
358 when compared to that of the thermosonicated juices ( $p<0.05$ ). This means that

359 thermosonication for 5 min at 60 °C and either 35 or 130 kHz resulted in better retention  
360 of polyphenols when compared to thermal processing at 80 °C for 1 min. After 7 days of  
361 storage at 4 °C, TPC content significantly decreased in all samples, but TS-AEJ showed  
362 the highest value which was  $45.6 \pm 1.1$  mg/100 mL ( $p < 0.05$ ).

363 Results obtained for antioxidant activity correlated well with those obtained for TPC. No  
364 differences were detected in the antioxidant potential of AEJ and TS-AEJ at day 1 when  
365 assessed using the DPPH· assay (Figure 3C). Thermosonication resulted in increased  
366 FRAP values when compared to the control ( $p < 0.05$ ; Figure 3B), probably caused by a  
367 higher amount of antioxidant compounds in the water:methanol extracts as ultrasounds  
368 have been repeatedly used to increase the extraction of bioactive compounds from foods  
369 (Barba et al. 2016; Chemat et al. 2017). Both FRAP and DPPH· values of the P-AEJ were  
370 lower when compared to AEJ and TS-AEJ ( $p < 0.05$ ), supporting previous results which  
371 suggested that thermosonication resulted in better retention of nutritional properties when  
372 compared to thermal processing (Escudero-López et al. 2016; Chen et al. 2015; Khandpur  
373 and Gogate 2015, 2016).

### 374 **3.5 Total anthocyanin and lycopene content**

375 The TLC (Figure 4A) of P-AEJ was lower than that of the AEJ and TS-AEJ at days 1 and  
376 7 ( $p < 0.05$ ). No differences were observed between the TLC of the CJ and the TS-AEJ,  
377 suggesting that thermosonication at 60 °C, at 35 or 130 kHz, for 5 min had no effect on  
378 the lycopene content of the juice. Lycopene has a strong red colour and the observed  
379 degradation of lycopene after thermal processing could explain the measured colour  
380 change in P-AEJ when compared to AEJ. In addition, the TLC of all samples decreased  
381 during storage at 4 °C for 7 days ( $p < 0.05$ ).

382 A similar trend was detected for the TAC (Figure 4B), which was significantly lower  
383 ( $p < 0.05$ ) for P-AEJ ( $0.92 \pm 0.01$  mg/100 mL) when compared to AEJ ( $1.08 \pm 0.04$  mg/100

384 mL) and TS-AEJ ( $1.06 \pm 0.03$  mg/100 mL). No differences were observed between the  
385 TAC of the AEJ and TS-AEJ, suggesting no degradation of anthocyanins caused by  
386 thermosonication. However, the TAC of all samples decreased during storage at 4 °C for  
387 7 days, and the observed decrease in TAC was higher for P-AEJ (83.4%) when compared  
388 to AEJ and TS-AEJ: 81.2 and 81.6%, respectively ( $p < 0.05$ ). Cano-Lamadrid et al. (2017)  
389 also experienced significant reductions in the anthocyanin content during cold storage of  
390 a fermented milk product enriched in anthocyanins using pomegranate juice.

### 391 **3.6 *In vitro* gastrointestinal digestion**

392 Previous studies demonstrated that the amount of health-promoting compounds released  
393 by foods during digestion, especially during the intestinal phase, might be higher than the  
394 one expected from common water-organic extracts (Pérez-Jiménez and Saura-Calixto  
395 2005). However, other papers suggested that polyphenols are degraded during digestion  
396 and that their bioaccessibility could be limited (Zudaire et al. 2017). In the current study,  
397 both the TPC and the antioxidant capacity, assessed using the FRAP or DPPH· method,  
398 decreased during the simulated digestion (Figure 5;  $p < 0.05$ ). The TPC of the P-AEJ  
399 (Figure 5A) after the intestinal phase of digestion was lower when compared to that of  
400 AEJ and TS-AEJ (Figure 5A;  $p < 0.05$ ). Results suggest that both processing technologies  
401 limit the bioaccessibility of phenolic compounds. However, the observed decrease in  
402 bioaccessibility was higher after thermal processing when compared to thermosonication.  
403 Similar results were observed with respect to antioxidant activity. Antioxidant capacity,  
404 measured as FRAP (Figure 5B) or DPPH (Figure 5C) after the intestinal phase was lower  
405 in processed P-AEJ and TS-AEJ when compared to the untreated AEJ ( $p < 0.05$ ).  
406 However, no significant differences were observed between both processed samples  
407 besides a slightly higher FRAP value after the intestinal phase in P-AEJ ( $p < 0.05$ ).  
408 Polyphenols are highly sensitive to alkaline conditions (Chen et al. 2014). Therefore, after

409 the intestinal digestion phase, polyphenols could have been degraded by the alkaline pH,  
410 thus leading to the observed loss in the TPC and the antioxidant capacity as previously  
411 reported by Bermúdez-Soto et al. (2007).

#### 412 **4. Conclusions**

413 The anthocyanin-enriched tomato juice developed herein showed not only higher  
414 nutritional properties but also improved physiochemical properties, which were  
415 comparable to those of currently commercialized fruit juices. Moreover, this enriched  
416 tomato juice has the advantage of using strawberry co-products, increasing its added value  
417 and sustainability. Results obtained in the current paper support previous studies which  
418 suggested that thermosonication could be used to minimize the degradation of phenolic  
419 compounds during processing and retain the antioxidant capacity of fruit juices. Fruit  
420 processing by either a conventional thermal treatment or by thermosonication resulted in  
421 a lower amount of phenolic compounds in the extracts obtained using water and methanol  
422 and also in the enzymatic extracts obtained after a simulated gastrointestinal digestion.  
423 Moreover, microbial inactivation is of key importance in order to produce safe products.  
424 Thermosonication at either 35 or 130 kHz for 5 min at 60 °C resulted in higher reductions  
425 in the total aerobic mesophilic organisms count when compared to a conventional  
426 pasteurization process. Therefore, based on the results reported herein, we can conclude  
427 that thermosonication could be used a suitable strategy to obtain healthier and safer juices.  
428 Optimization of the thermosonication conditions using a response surface methodology  
429 could improve the retention of bioactive and nutritious compounds and the observed  
430 lethal effects.

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438

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602 **Figure captions**

603 **Figure 1. Effect of processing on the total aerobic mesophilic microorganisms count**  
604 **at days 1 (A) and 7 (B)**

605 TT-1: Thermal processing at 80 °C for 1 min; TT-5: Thermal processing at either 60, 40,  
606 20 °C for 5 min; TS-5: Thermosonication (at either 35 or 130 kHz) at 60, 40, or 20 °C for  
607 5 min. Values represent the mean of three independent experiments  $\pm$  S.D. Different  
608 letters indicate significant differences between treatments at the same sampling day. The  
609 criterion for statistical significance was  $p < 0.05$ .

610 **Figure 2. Effect of processing on the activity of (A) PG and (B) PME**

611 Values represent the mean of three independent experiments  $\pm$  S.D. Capital letters  
612 indicate significant differences between treatments at the same sampling day. Lower case  
613 letters indicate significant differences between sampling days for the treatment. The  
614 criterion for statistical significance was  $p < 0.05$ .

615 **Figure 3. Effect of processing on the (A) TPC and antioxidant activity when assessed**  
616 **using the (B) FRAP and (C) DPPH· assays**

617 Values represent the mean of three independent experiments  $\pm$  S.D. Capital letters  
618 indicate significant differences between treatments at the same sampling day. Lower case  
619 letters indicate significant differences between sampling days for the treatment. The  
620 criterion for statistical significance was  $p < 0.05$ .

621 **Figure 4. Effect of processing on the (A) TLC and (B) TAC**

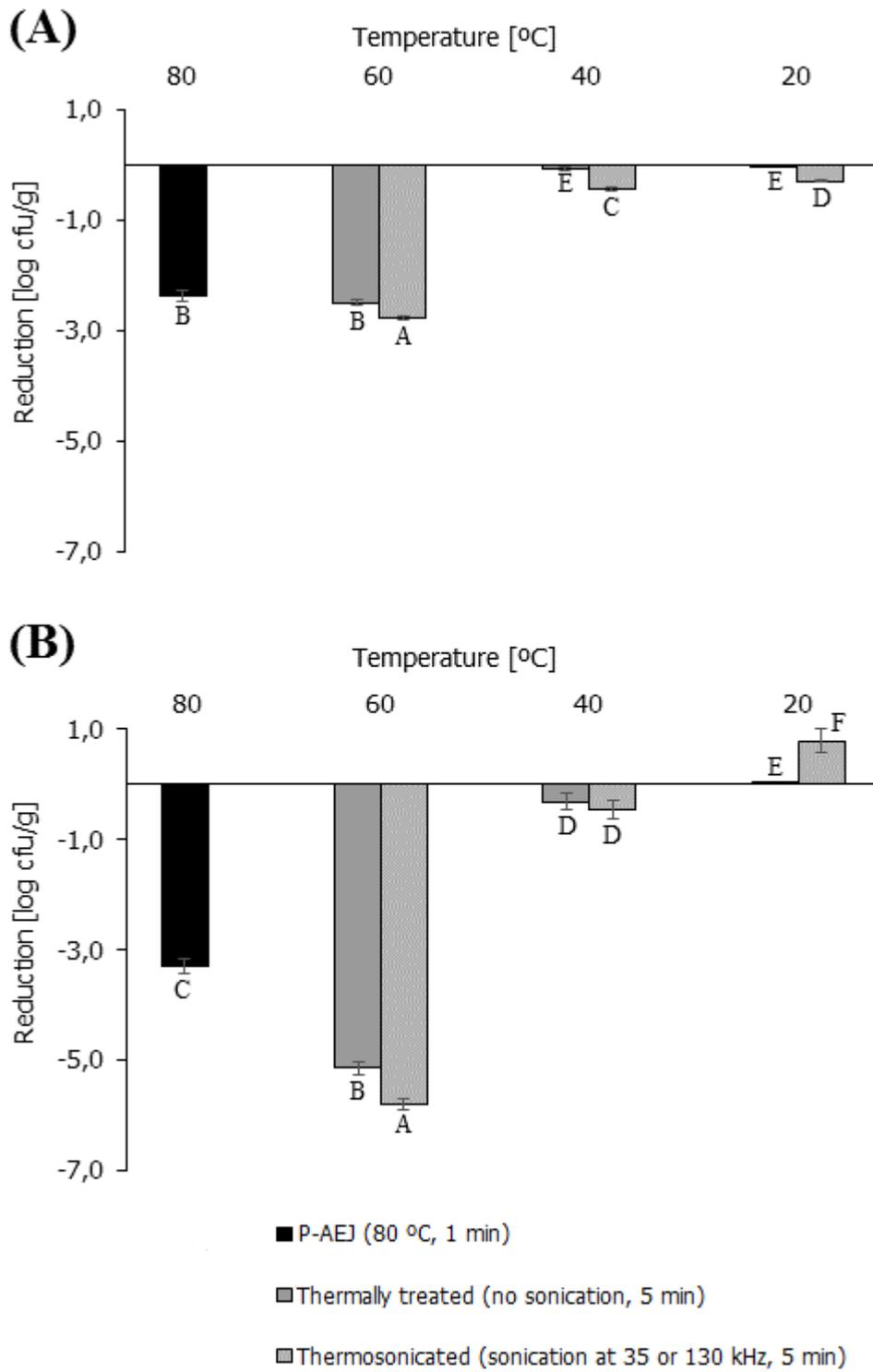
622 Values represent the mean of three independent experiments  $\pm$  S.D. Capital letters  
623 indicate significant differences between treatments at the same sampling day. Lower case

624 letters indicate significant differences between sampling days for the treatment. The  
625 criterion for statistical significance was  $p < 0.05$ .

626 **Figure 5. Resistance of (A) polyphenols and antioxidant activity, assessed using (B)**  
627 **FRAP and (C) DPPH $\cdot$  assays, to a simulated gastrointestinal digestion**

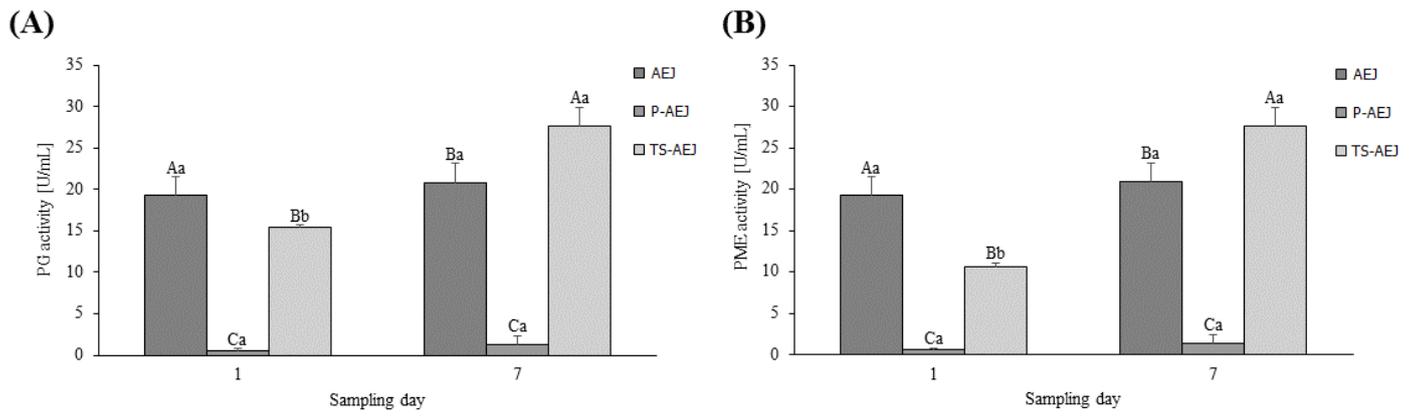
628 Values represent the mean of three independent experiments  $\pm$  S.D. Capital letters  
629 indicate significant differences between treatments at the same phase of digestion. Lower  
630 case letters indicate significant differences between digestive phases for the same  
631 treatment. The criterion for statistical significance was  $p < 0.05$ .

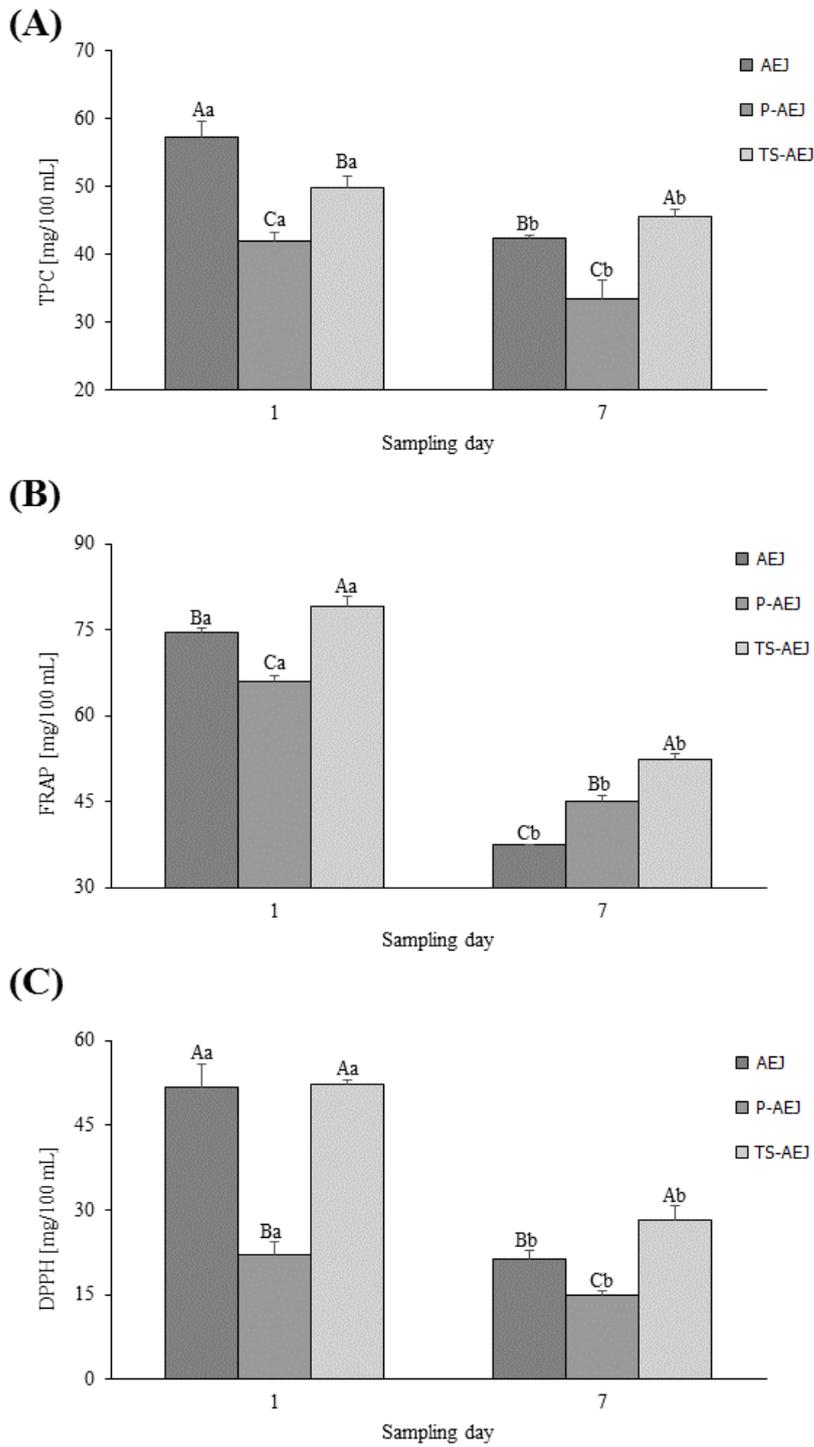
632 **Figure 1**



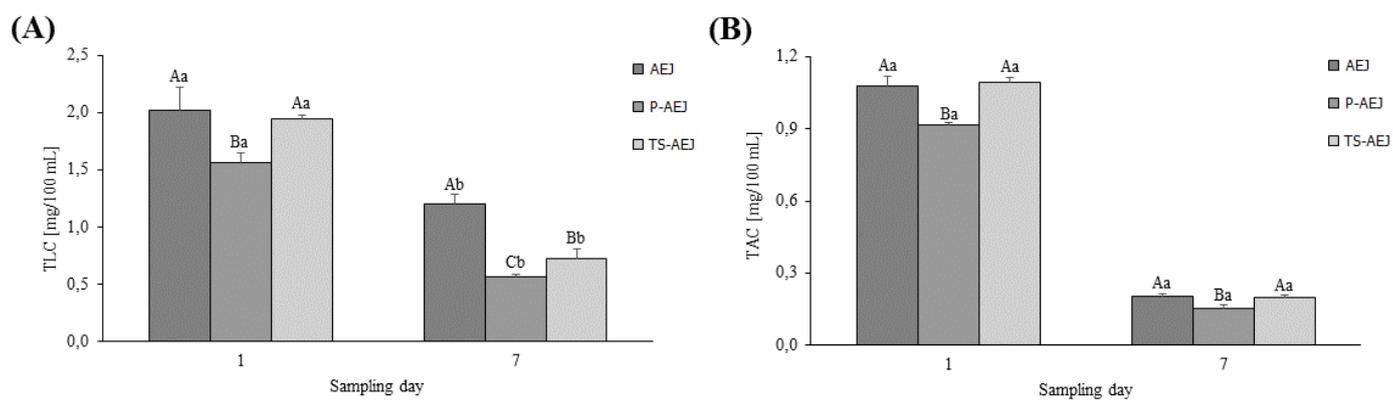
633

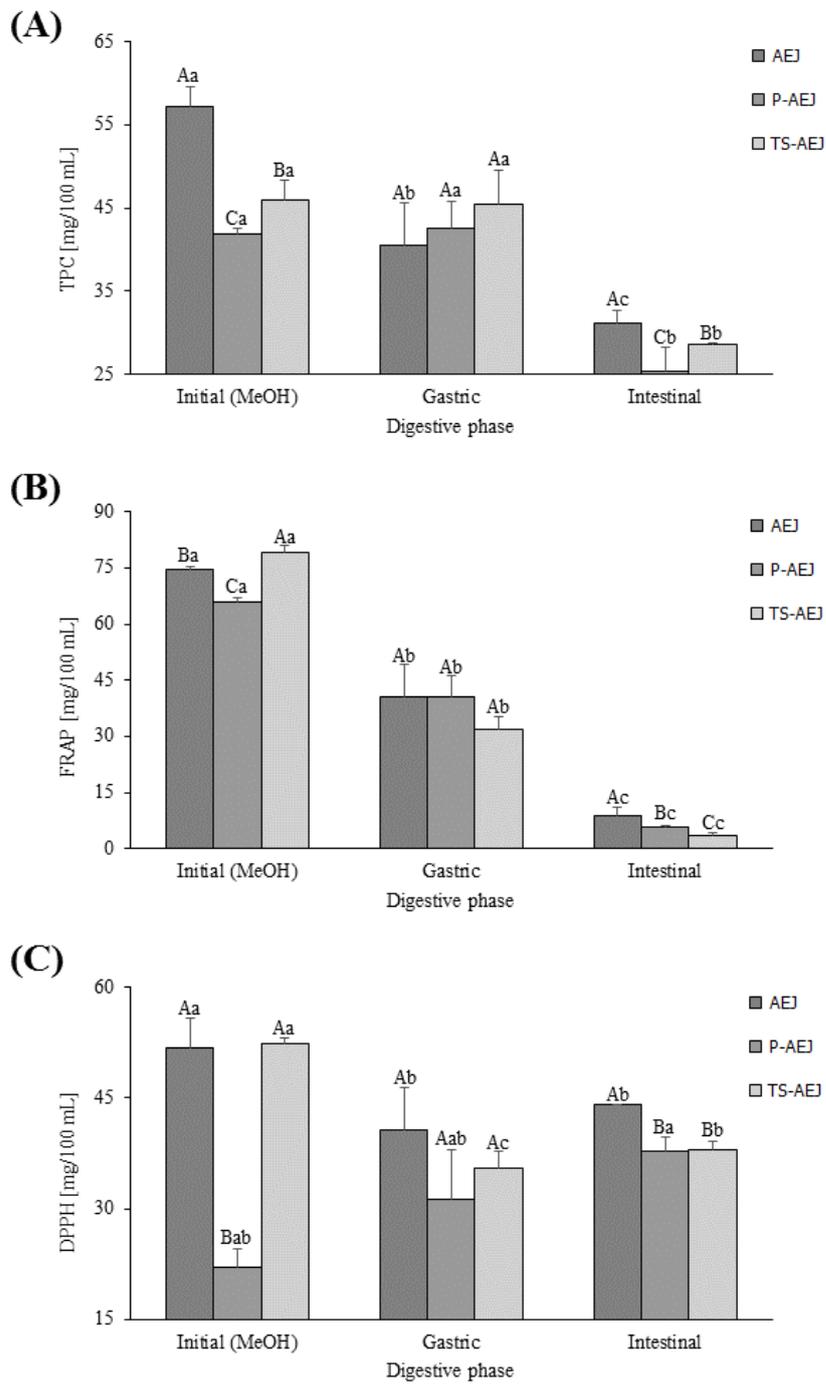
634 **Figure 2**





638 **Figure 4**





642 **Table 1. Effect of processing on the physicochemical quality of the anthocyanin-**  
 643 **enriched tomato juice**

	<b>CJ</b>	<b>AEJ</b>	<b>P-AEJ</b>	<b>TS-AEJ</b>
<b>Day 1</b>				
<b><i>L</i>*</b>	41.88 ± 1.06 <sup>D</sup>	44.79 ± 0.07 <sup>Ba</sup>	44.15 ± 0.33 <sup>Ca</sup>	45.53 ± 0.19 <sup>Aa</sup>
<b><i>a</i>*</b>	10.91 ± 0.66 <sup>D</sup>	13.87 ± 0.08 <sup>Aa</sup>	12.70 ± 0.16 <sup>Ca</sup>	12.94 ± 0.08 <sup>Ba</sup>
<b><i>b</i>*</b>	16.21 ± 0.83 <sup>A</sup>	14.50 ± 0.14 <sup>Ba</sup>	14.40 ± 0.24 <sup>Ba</sup>	14.65 ± 0.20 <sup>Ba</sup>
<b><i>C</i>*<sub>ab</sub></b>	19.54 ± 1.05 <sup>A</sup>	20.06 ± 0.15 <sup>Aa</sup>	19.20 ± 0.26 <sup>Aa</sup>	19.55 ± 0.11 <sup>Aa</sup>
<b>ΔE</b>	4.4 ± 0.0	-	1.4 ± 0.3	1.2 ± 0.1
<b>pH</b>	4.25 ± 0.01 <sup>A</sup>	3.94 ± 0.02 <sup>Ba</sup>	3.91 ± 0.02 <sup>Ba</sup>	3.91 ± 0.02 <sup>Ba</sup>
<b>TTA (g/L)</b>	3.26 ± 0.09 <sup>B</sup>	4.53 ± 0.44 <sup>Aa</sup>	4.56 ± 0.05 <sup>Aa</sup>	4.70 ± 0.33 <sup>Aa</sup>
<b>SSC (°Brix)</b>	5.03 ± 0.06 <sup>B</sup>	6.60 ± 0.20 <sup>Aa</sup>	6.67 ± 0.25 <sup>Aa</sup>	6.60 ± 0.30 <sup>Aa</sup>
<b>Day 7</b>				
<b><i>L</i>*</b>	-	40.90 ± 0.40 <sup>Bb</sup>	43.60 ± 0.16 <sup>Aa</sup>	41.59 ± 0.26 <sup>Bb</sup>
<b><i>a</i>*</b>	-	11.28 ± 0.07 <sup>Bb</sup>	12.09 ± 0.12 <sup>Ab</sup>	11.36 ± 0.12 <sup>Ba</sup>
<b><i>b</i>*</b>	-	11.66 ± 0.20 <sup>Bb</sup>	13.53 ± 0.14 <sup>Ab</sup>	11.74 ± 0.08 <sup>Bb</sup>
<b><i>C</i>*<sub>ab</sub></b>	-	16.22 ± 0.19 <sup>Bb</sup>	18.15 ± 0.03 <sup>Ab</sup>	16.34 ± 0.13 <sup>Bb</sup>
<b>ΔE</b>	-	-	3.4 ± 0.1	0.7 ± 0.3
<b>pH</b>	-	3.92 ± 0.01 <sup>Ba</sup>	3.96 ± 0.04 <sup>Aa</sup>	3.88 ± 0.03 <sup>Ba</sup>
<b>TTA (g/L)</b>	-	4.26 ± 0.07 <sup>Ba</sup>	4.50 ± 0.47 <sup>ABa</sup>	4.55 ± 0.05 <sup>Aa</sup>
<b>SSC (°Brix)</b>	-	6.40 ± 0.10 <sup>Ba</sup>	6.63 ± 0.06 <sup>Aa</sup>	6.67 ± 0.06 <sup>Aa</sup>

644 CJ: Control tomato juice; AEJ: Anthocyanin-enriched juice; P-AEJ: AEJ pasteurised at  
 645 80 °C for 1 min; TS-AEJ: AEJ thermosonicated at 60 °C and either 35 or 130 kHz for 5  
 646 min.

647 Values represent the mean of three independent experiments ± S.D. Capital letters

648 indicate significant differences between juices at the same sampling day. Lower case

649 letters indicate significant differences between different sampling days for the same juice.

650 The criterion for statistical significance was  $p < 0.05$ .