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

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Abbreviations

PG: polygalacturonase; PME: pectin methylesterase; TPC: total phenolic content; TAC: total anthocyanin content; TLC: total lycopene content; SSC: soluble solids content; CJ: control tomato juice; TAM: Total aerobic mesophilic microorganisms; AEJ: anthocyanin-enriched juice; P-AEJ: Thermally treated anthocyanin-enriched tomato juice; TS-AEJ: Thermosonicated juice; TTA: titratable acidity; C^*_{ab}: Chroma; δE: Difference from the control; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric ion reducing antioxidant power; SPC: Strawberry press cake; S.D.: Standard deviation; ANOVA: Analysis of 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 ºC at either 35 or 130 kHz resulted in higher

31 microbial inactivation when compared to thermal pasteurization at 80 ºC 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 ± 0.04

35 mg/100 mL before processing to 0.92 ± 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

1. Introduction

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

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

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

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

2. Materials and methods

2.1 Chemicals and reagents

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

2.2 Preparation of the functional anthocyanin-enriched tomato juice

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

6

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 ± 0.2 and $93.0 \pm 0.8\%$ for CJ and AEJ, respectively. The CJ and AEJ

124 were homogeneized using a T-25 digital ULTRA-TURRAX® homogenizer (IKA,

125 Staufen, Germany) at 10,000 rpm for 1 min and stored at 4 ºC 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 (80ºC, 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 ºC), 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 ºC using a ABT 101L blast chiller (Infrico, Barcelona, Spain) and stored at 4 ºC in the

137 dark until further analysis. Analyses were performed at days 1 and 7 post-processing.

138 Treatment at 60 ºC 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 ± 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 (δE) 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 ºC 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 ºBrix.

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.

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.

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.

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.

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 µmol 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 (α-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 ± 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 $p<0.05$.

3. Results and discussion

3.1 Effect of strawberry co-product inclusion into tomato juice

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

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 ºC.

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 ºC), 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 ± 0.2 log cfu/g. Thermal processing at 80 ℃ 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 ℃ 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 ℃ after 7 days of storage at 4 ℃ was higher when

275 compared to the samples treated at 20 ℃ 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 ℃, sonication at 40 ℃

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 ºC 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 ºC. The lethal effect

291 of temperature (60 ºC) 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 ºC for 1 min, especially after 7 days of storage at 4 ºC ($p<0.05$). TAM counts of AEJ

295 treated at 60 ºC with or without sonication, decreased during storage with total reductions

296 of 5.1 ± 0.1 and 5.7 ± 0.1 log cfu/g, which resulted in a final population of 3.3 ± 0.1 and

297 2.6 ± 0.1 log cfu/g at day 7, respectively. This could be due to the fact that at 60 ºC, 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 ºC 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* Enteriditis. In that study, the authors

304 reported that *Salmonella* Enteriditis was not recovered in samples subjected to

305 thermosonication at 60 ºC for more than 5 min.

306 Overall, thermosonication for 5 min at 60 ºC 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 ºC 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 ºC for 5 min at 35 or

314 130 kHz were selected for further studies. No differences were observed in the enzymatic,

315 physiochemical, 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 ºC, 1

343 min) and thermosonication (20, 40, or 60 ºC 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 ℃ and either 35 or 130 kHz resulted in better retention

360 of polyphenols when compared to thermal processing at 80 ℃ for 1 min. After 7 days of

361 storage at 4 ℃, TPC content significantly decreased in all samples, but TS-AEJ showed

362 the highest value which was 45.6 ± 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 P-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 ℃, 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 ℃ 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 ± 0.01 mg/100 mL) when compared to AEJ (1.08 ± 0.04 mg/100

384   mL) and TS-AEJ (1.06 ± 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 ℃ 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).

4. Conclusions

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

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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 ℃ for 1 min; TT-5: Thermal processing at either 60, 40,

606	20 ℃ for 5 min; TS-5: Thermosonication (at either 35 or 130 kHz) at 60, 40, or 20 ℃ for

607	5 min. Values represent the mean of three independent experiments ± 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 ± 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 ± 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
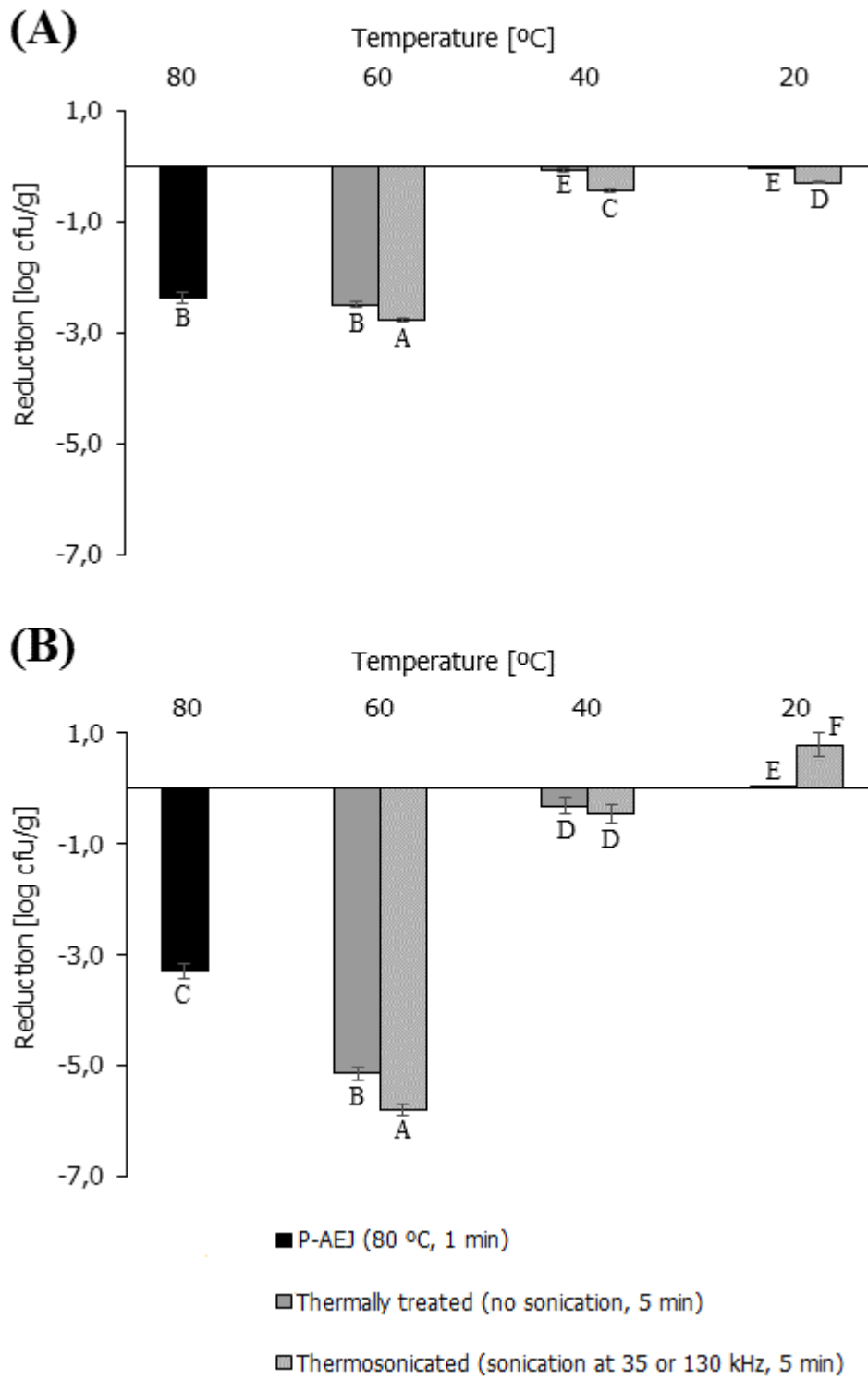
620	criterion for statistical significance was $p<0.05$.

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

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

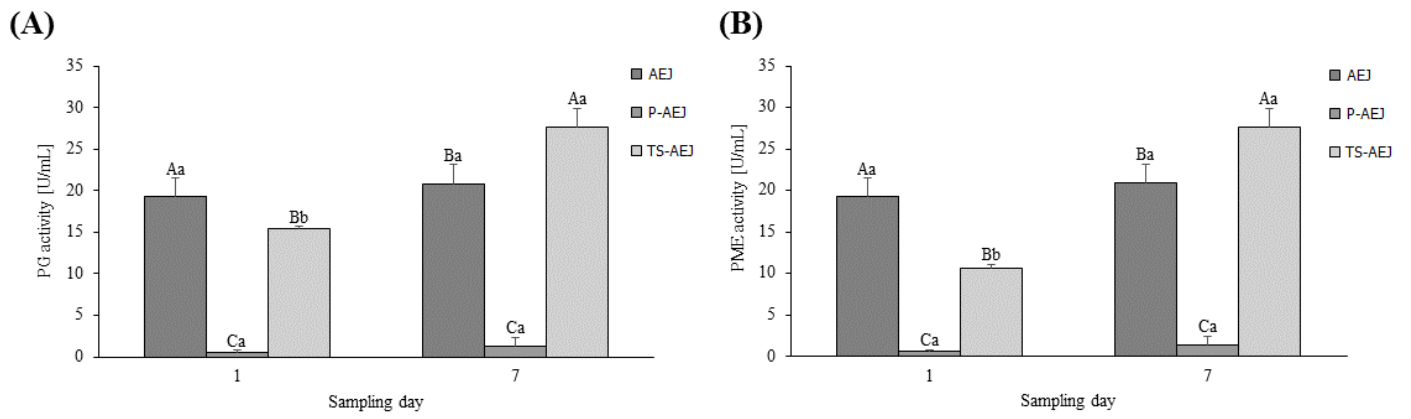623	indicate significant differences between treatments at the same sampling day. Lower case

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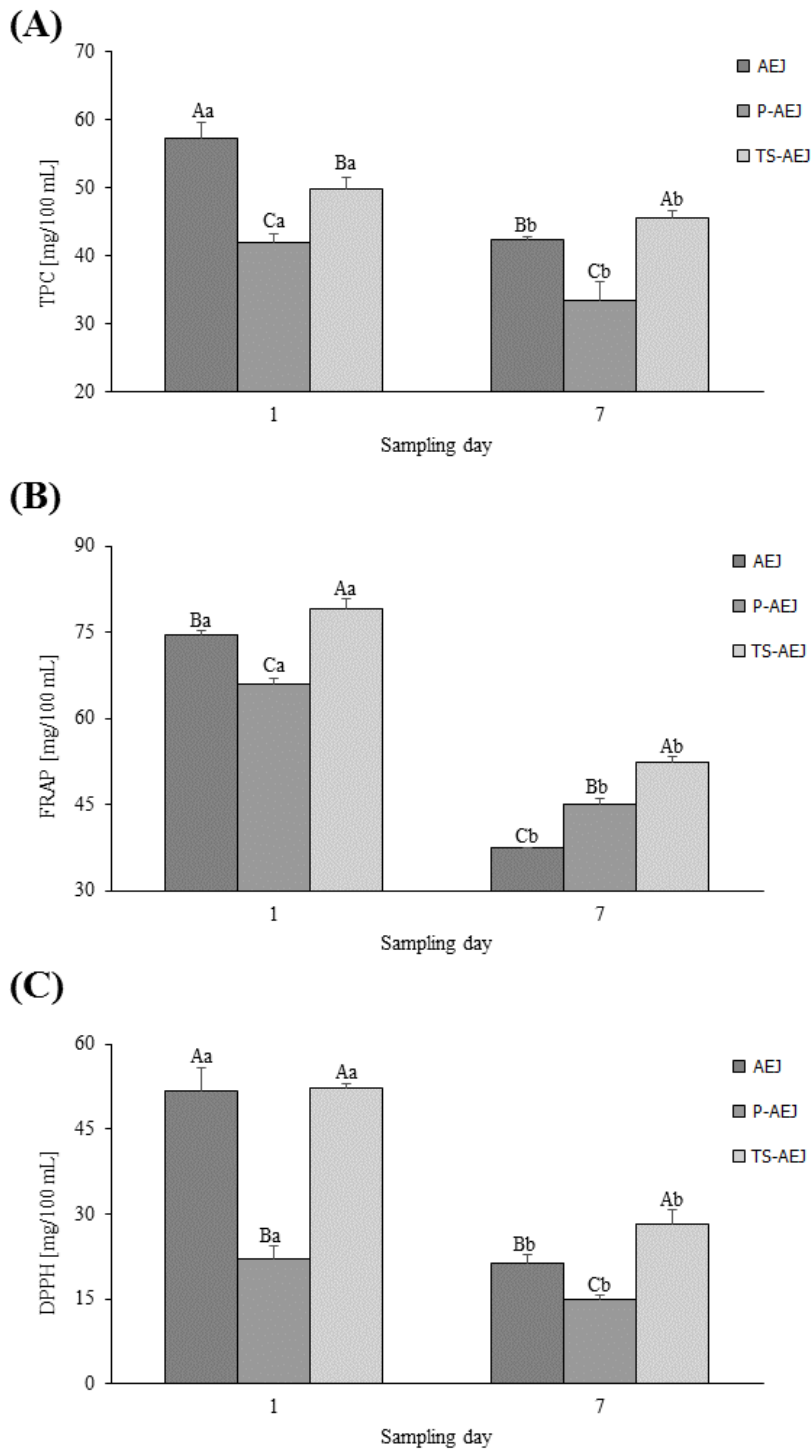
625 criterion for statistical significance was $p<0.05$.

626 **Figure 5. Resistance of (A) polyphenols and antioxidant activity, assessed using (B)**

627 **FRAP and (C) DPPH· assays, to a simulated gastrointestinal digestion**

628 Values represent the mean of three independent experiments ± 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**

(A)

Temperature [°C]

80   60   40   20

(B)

Temperature [°C]

80   60   40   20

■ P-AEJ (80 °C, 1 min)

▨ Thermally treated (no sonication, 5 min)

▨ Thermosonicated (sonication at 35 or 130 kHz, 5 min)

633

634   **Figure 2**

(A)

(B)

636 **Figure 3**

(A)

(B)

(C)
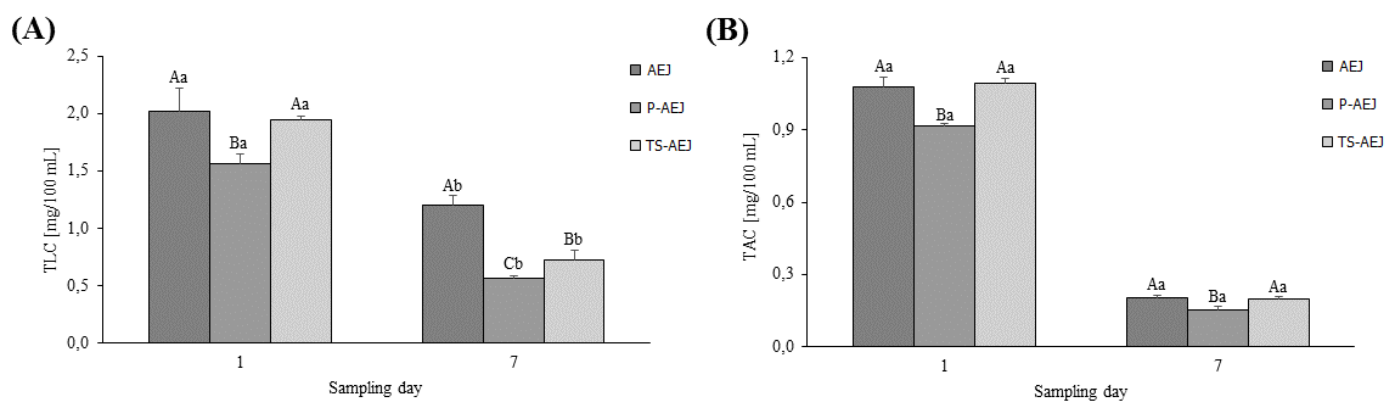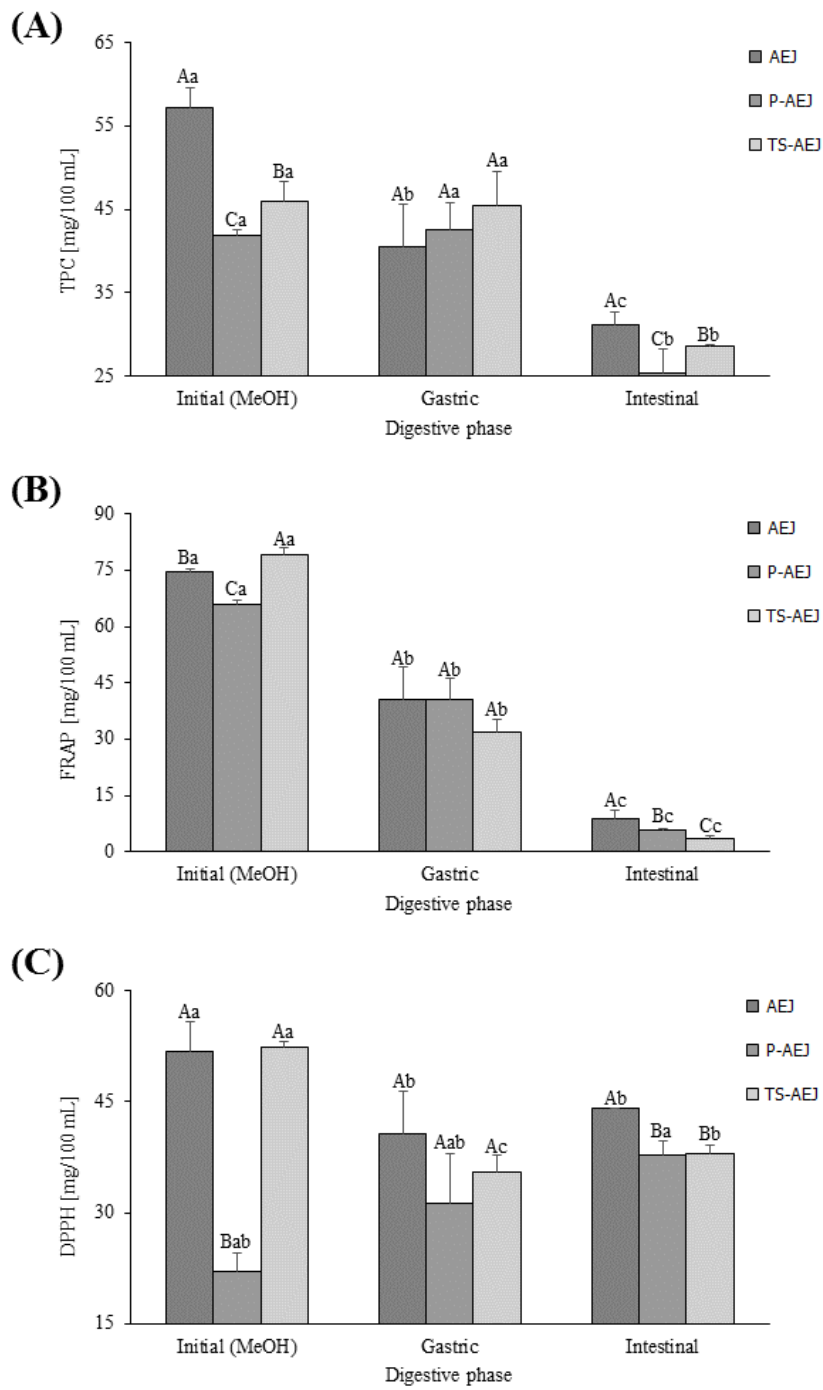

637

638   **Figure 4**

640   **Figure 5**

(A)

(B)

(C)

641

Table 1. Effect of processing on the physicochemical quality of the anthocyanin-

643   **enriched tomato juice**

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

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

647   Values represent the mean of three independent experiments $\pm$ 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 $p < 0.05$.