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.

Abstract

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

Keywords: tomato juice, anthocyanins, thermosonication, pasteurization, co-product 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 ºC 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

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 ± 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

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

2.5 Physicochemical characteristics

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

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

SSC was measured at 20 ºC with a handheld refractometer (Atago Co. Ltd., Tokio, Japan). Measurements were performed in triplicate per treatment, sampling day, and replicate and results were expressed in ºBrix.

2.6 Total phenolic content (TPC)

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

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

2.7 Antioxidant activity: FRAP and DPPH· scavenging activity

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

2.8 Total anthocyanin content (TAC)

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

2.9 Total lycopene content (TLC)

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

2.10 Enzymatic activity

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

both enzymes was expressed as PG or PME units per mL. PME and PG units were defined as the amount of enzyme required to release 1 μmol of carboxyl or reducing groups per min.

2.11 Simulated gastrointestinal digestion

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

2.12 Statistical analysis

Results are expressed as mean ± standard deviation (S.D.). A multifactorial design with storage period and treatment factors was used to analyse the results. Data were analysed using analysis of variance (ANOVA) with JMP 13 (SAS Institute Inc., Cary, USA). Where significant differences of storage period or treatment time were found, a Tukey pairwise comparison of the means was conducted to identify where the sample differences 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

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

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

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

3.2 Effect of conventional thermal processing and thermosonication on juice microbiological quality

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

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

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

3.4 Total phenolic content and antioxidant activity

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

359 thermosonication for 5 min at 60 ºC and either 35 or 130 kHz resulted in better retention
360 of polyphenols when compared to thermal processing at 80 ºC for 1 min. After 7 days of
361 storage at 4 ºC, 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 ºC, 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 ºC 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 ºC 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 ºC 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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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 ºC for 1 min; TT-5: Thermal processing at either 60, 40,
606 20 ºC for 5 min; TS-5: Thermosonication (at either 35 or 130 kHz) at 60, 40, or 20 ºC 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**

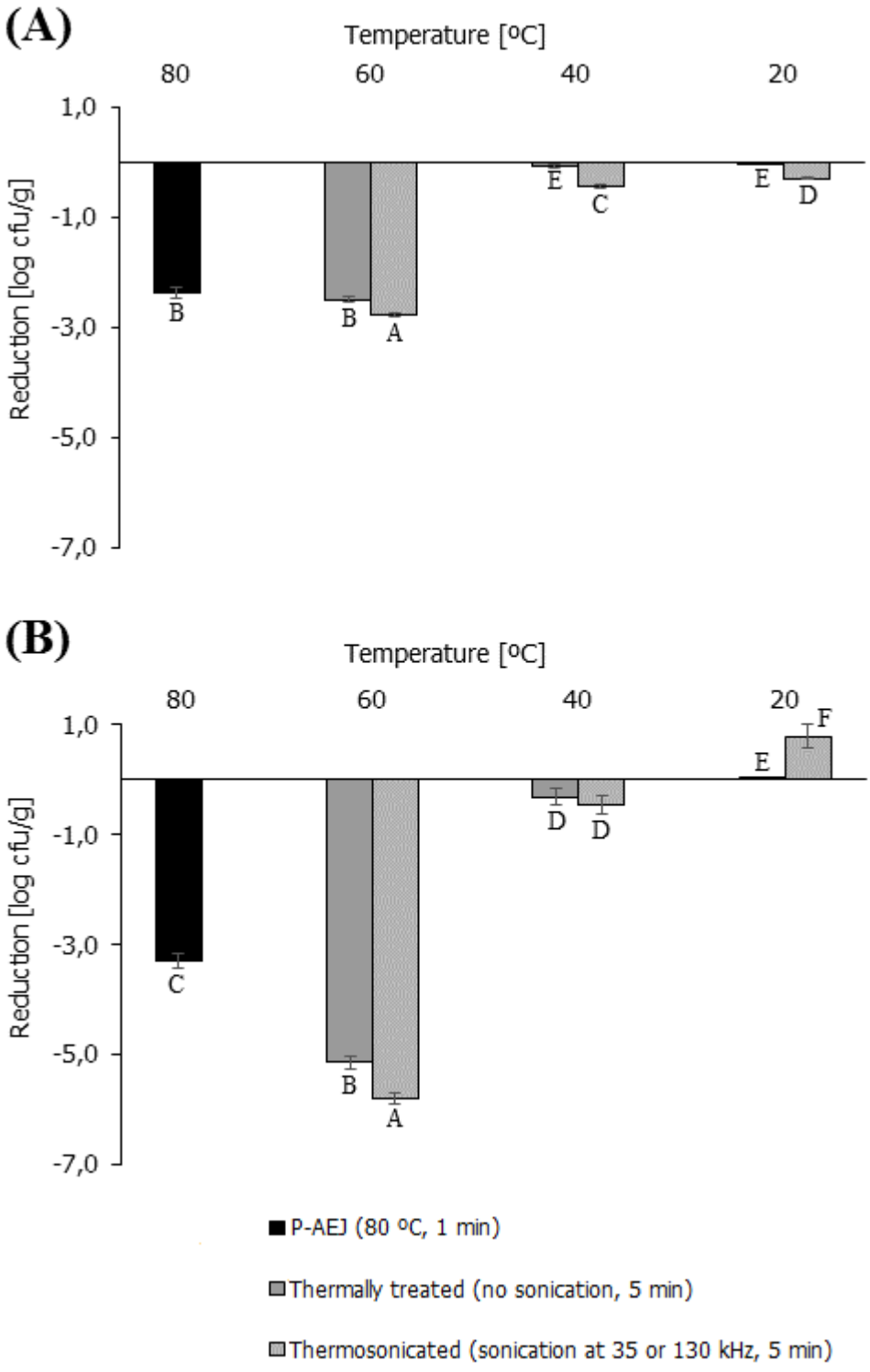
622 Values represent the mean of three independent experiments ± S.D. Capital letters
623 indicate significant differences between treatments at the same sampling day. Lower case

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

Figure 5. Resistance of (A) polyphenols and antioxidant activity, assessed using (B) FRAP and (C) DPPH· assays, to a simulated gastrointestinal digestion

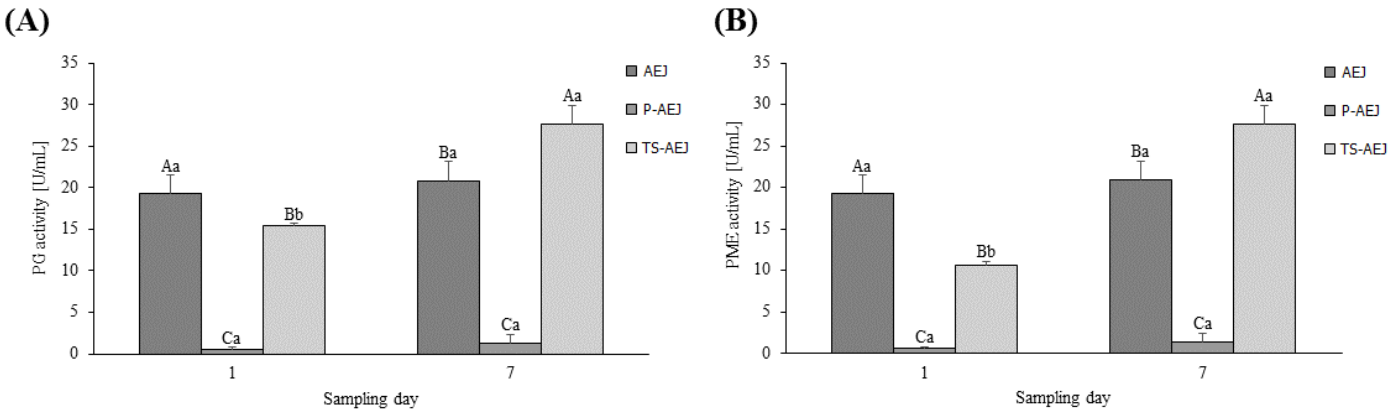
Values represent the mean of three independent experiments ± S.D. Capital letters indicate significant differences between treatments at the same phase of digestion. Lower case letters indicate significant differences between digestive phases for the same treatment. The criterion for statistical significance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

632 **Figure 1**

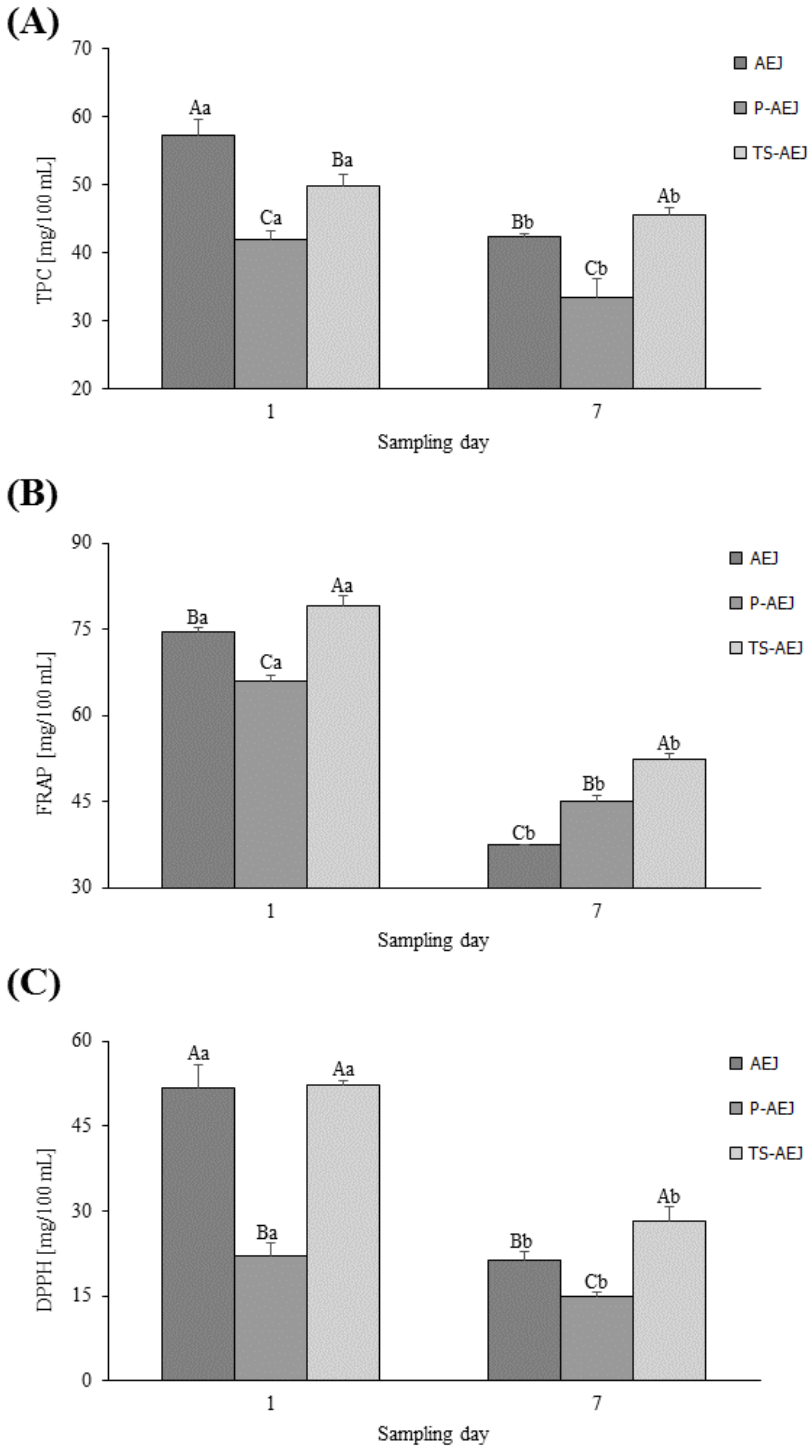


633

634 **Figure 2**

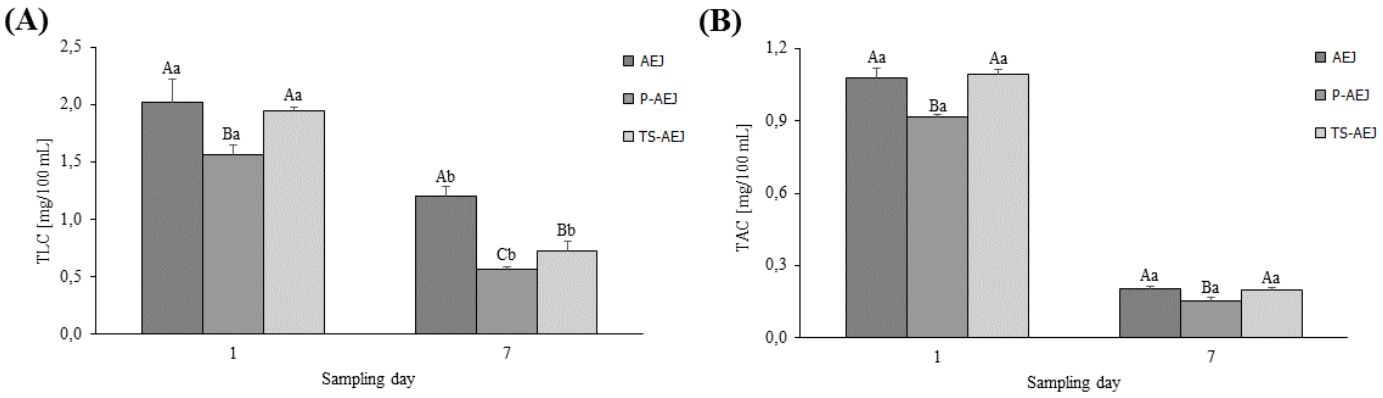

(A)
PG activity [U/mL]
35
30
25
20
15
10
5
0
Aa
Ca
Bb
Ba
Ca
Aa
1
7
Sampling day
AEJ
P-AEJ
TS-AEJ
(B)
PME activity [U/mL]
35
30
25
20
15
10
5
0
Aa
Ca
Bb
Ba
Ca
Aa
1
7
Sampling day
AEJ
P-AEJ
TS-AEJ

636 **Figure 3**

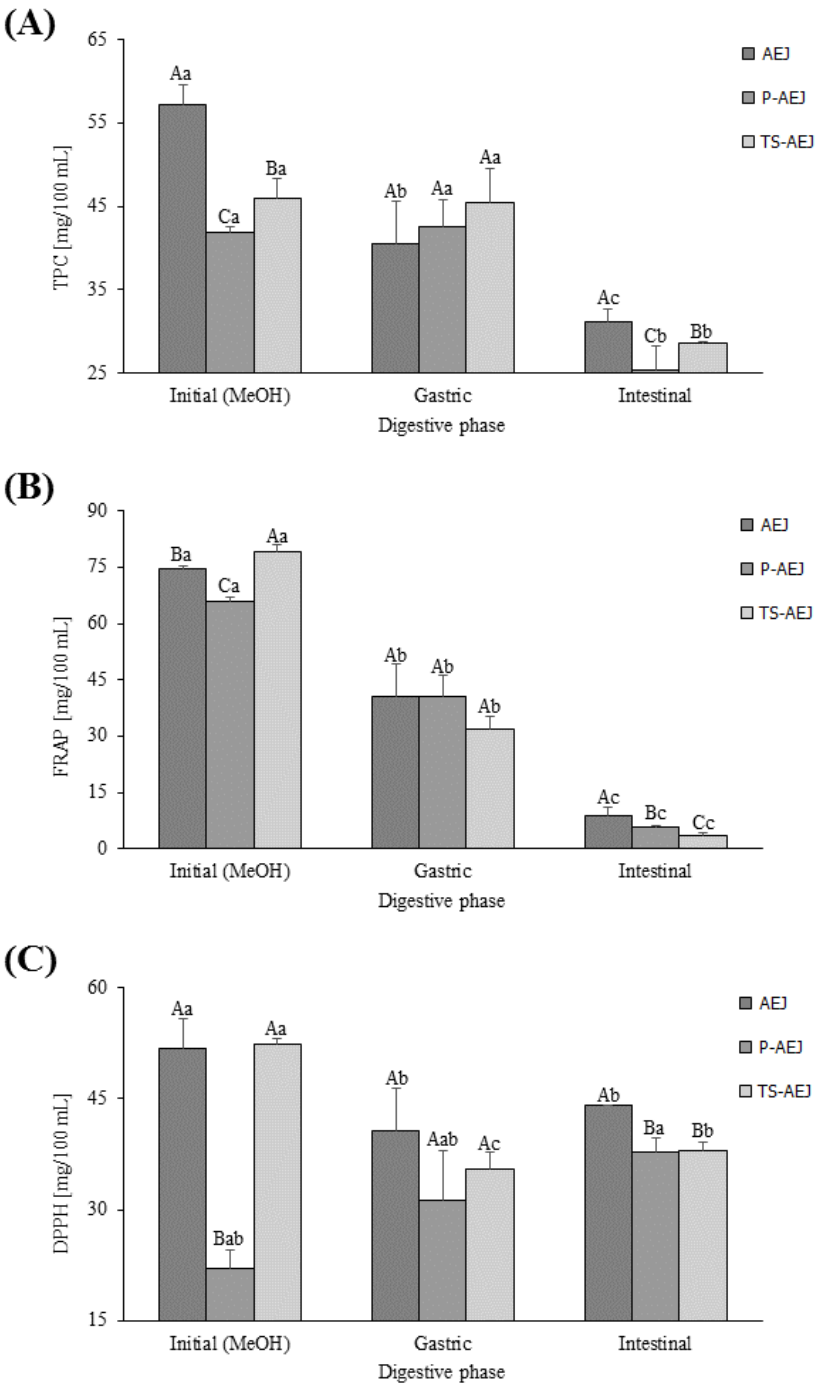


637

638 **Figure 4**


(A)
TLC [mg/100 mL]
2,5
2,0
1,5
1,0
0,5
0,0
Aa
Ba
Aa
Ab
Cb
Bb
1
7
Sampling day
AEJ
P-AEJ
TS-AEJ
(B)
TAC [mg/100 mL]
1,2
0,9
0,6
0,3
0,0
Aa
Ba
Aa
Aa
Ba
Aa
1
7
Sampling day
AEJ
P-AEJ
TS-AEJ

640 **Figure 5**



641

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

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

Values represent the mean of three independent experiments ± S.D. Capital letters indicate significant differences between juices at the same sampling day. Lower case letters indicate significant differences between different sampling days for the same juice. The criterion for statistical significance was $p<0.05$.