

# High pressure and thermal pasteurization effects on sweet cherry juice microbiological stability and physicochemical properties

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This study evaluated high pressure processing (P1 - 400 MPa/5 min; P2 - 550 MPa/2 min) and thermal pasteurization (TP - 70°C/30 s) effects on sweet cherry juice's microbiological and physicochemical parameters, during four weeks of refrigerated storage. All treatments reduced the microbiological load to undetectable levels not affecting total soluble solids and titratable acidity. The pH increased with all treatments, however, it decreased during storage. Phenols were differently affected: TP increased them by 6%, P1 had no effect while P2 decreased them by 11%. During storage, phenols in control and TP samples decreased by 26% and 20%, P1 samples decreased them by 11% whereas P2 showed no variation. TP had no effect on anthocyanins, while pressure treatments increased them by 8%. Anthocyanins decreased during storage, particularly in the control and P1 (decreasing 41%). All treatments had no effect on antioxidant activity until the 14th day, thereafter high pressure processing samples showed the highest antioxidant activity.

**Keywords:** sweet cherry juice; high pressure processing; thermal pasteurization; phenols; anthocyanins; antioxidant activity

## Introduction

Nowadays consumers have a major awareness of the necessity for and importance of healthy food, which leads to a rise in the consumption of fresh fruit and fruit products. Sweet cherry (*Prunus avium* L.) is a fruit belonging to the genus *Prunus* in the Rosaceae family, native to Europe, being very attractive and distinguished by several characteristics, such as sweetness, colour, sourness and firmness. It is rich in nutrients and bioactive compounds, having relatively low caloric content while containing substantial amounts of anthocyanins, polyphenols and high antioxidant activity. The sweet cherry composition has been extensively studied due to its impact on human health, since it is claimed to have several potential health benefits in relation to cancer, diabetes, inflammatory and cardiovascular diseases. Since sweet cherry is a relatively seasonal and perishable fruit, is not always available to the consumer as a fresh fruit, but it can be processed into juice, meeting the consumer demand for drinks with potential health benefits.

To prevent fruit juices from spoiling and assure their microbial safety, they are frequently submitted to thermal pasteurization (TP), which usually results in the loss of important compounds during the processing treatment and subsequent storage. Many reactions such as anthocyanins degradation, Maillard reactions and ascorbic acid oxidation occur during thermal processing, which has negative effects on organoleptic and nutritional attributes.

As alternative methods, nonthermal technologies are emerging, with special emphasis to high pressure processing (HPP). Although there are several new applications for HPP, such as biotechnological processes *in vitro* and a novel possible food preservation process by microbial growth inhibition under moderate pressures with no temperature control, as a quasi-energy costless alternative to refrigeration, the main industrial application is still food cold pasteurization, which is an increasingly growing process due to the quality displayed by the processed products.

HPP uses water as a pressure-transmitting medium to instantaneously transmit isostatic pressure to foods, usually in the 400-600 MPa range at cold, room or mild temperatures ( $\ll 60^\circ\text{C}$ ), independent of size, shape and food composition. HPP inactivates pathogens and vegetative spoilage microorganisms, but it has limited impacts on covalent bond molecules. Thus, it does not significantly affect general physicochemical properties, colour, flavour and health-promoting bioactive compounds, being suitable to produce nutritious and fresh-like fruit juices.

The effect of HPP cold pasteurization has been widely applied in fruit products such as juices and purées. However, as far as the authors are aware, there are no studies on the HPP and TP effects on sweet cherry juice. Thus, this work was intended to evaluate HPP (400 MPa/ 5 min; 550 MPa/2 min) and TP (70°C/30 s) effects on sweet cherry juice microbiological stability (total aerobic mesophilic (TAM) and yeasts and moulds (YM)), physicochemical parameters (pH, titratable acidity (TA) and total soluble solids), bioactive compounds (total phenols, total anthocyanins) and antioxidant activity after processing and during four weeks of refrigerated storage, which is a common shelflife time for HPP juices.

## Materials and methods

### Chemicals

Folin-Ciocalteu reagent, gallic acid, sodium carbonate, ethanol and 2,2-diphenyl-1-picryl-hydrazil (DPPH) were purchased from Sigma-Aldrich (Seelze, Germany). Sodium hydroxide (> 98% purity) was obtained from Fluka (St. Louis, MO). Hydrochloric acid was obtained from Riedel-de Haen (Seelze, Germany). Plate count agar (PCA) and rose bengal chloramphenicol agar (RBCA) were purchased from Merck (Darmstadt, Germany).

### Sweet cherry juice preparation

Fresh sweet cherries (*Prunus avium* L.) grown in Cova da Beira, Portugal, were purchased at commercial maturity from a local supermarket and kept at 4°C for one day in the laboratory until use. The fruits were washed in running water and manually ginned, then the pieces were crushed at 4°C with a blender (Braun MR 6500/500, Kronberg, Germany), producing the juice. After filtration with a four-layer cheese cloth, the cleared juice was placed in low permeability polyamide-polyethylene bags (PA/PE-90, Albipack - Packaging Solutions, Águeda, Portugal) and manually heat sealed with caution to minimize the amount of air left inside and immediately processed. The packaging film was previously sterilized by irradiation with UV light for 15 min (BioSafety Cabinet Telstar Bio II Advance, Terrassa, Spain). The raw sweet cherry juice control samples (unprocessed) were also treated the same way.

### Thermal pasteurization

Usual industrial TP conditions for premium juices are 71.1°C for 30s, 90°C/2 s or 84°C/20 s and the condition 70°C for 30 s was used in this work. TP was carried out by submersing the packed samples in a thermostatic bath (Selecta Frigiterm 6000382, Barcelona, Spain) at 70°C. The required time to reach the desired temperature (70 s) was previously estimated using a K type thermal couple connected to a digital thermometer. As soon as the desired temperature was reached, the samples were held in the water bath for 30 s and afterwards quickly cooled in ice.

### High pressure treatments

High pressure treatments were carried out using a hydrostatic press (High pressure system U33, Institute of High Pressure Physics, Warsaw, Poland). This equipment has a pressure vessel of 35 mm inner diameter and 100 mm height surrounded by an external jacket and equipped with a thermocouple, connected to a thermostatic bath (Huber Compatible Control CC1, New Jersey, USA) to control the temperature. A mixture of propylene glycol and water (40:60) was used as a pressurizing fluid and to control the temperature in the external jacket. Pressures ranging from 350 to 500 MPa with holding times between 1 and 5 min are sufficient to inactivate microorganisms in acidic juices, and 400 MPa for about 5 min significantly reduce microbial loads, although 500-600 Mpa with 1-2 min holding time are the usual industrial conditions for this type of juices. Taking this into consideration, two distinctive conditions were chosen: 400 MPa and 5 min holding time (P1) as a mild condition and 550 MPa and 2 min holding time (P2) as an industrial condition representative both at 10°C. The compression rate was approximately 6.0 MPa/s and the decompression rate was 18.0 MPa/s. All raw and processed samples were stored at 4°C, to study the juice parameters evolution with time.

### Microbiological analyses

Microbiological analyses were performed immediately after treatments (day 0) and after 7, 14, 21 and 28 days of storage at 4°C. To carry out the analyses, 1.0 mL of each sample was aseptically obtained and homogenized with 9.0 mL of Ringer's solution. Then, decimal dilutions were made and triplicates of dilutions plated on the proper media, according to the following procedures: TAM were enumerated in PCA, after incubation at 30 ± 1°C for 72 ± 3 h (ISO 4833:2003); YM were counted on RBCA after incubation at 25 ± 1°C for 5 days (ISO 7954:1987). The results were expressed as logarithmic of colony-forming units (CFU) per sweet cherry juice's mL (Log<sub>10</sub> CFU/mL).

### Standard chemical analyses

The samples were collected after 0, 7, 14, 21 and 28 days of storage at 4°C, frozen and kept at -80°C until the analyses were made. The samples pH value was measured at 25°C with a properly calibrated glass electrode (pH electrode 50 14, Crison Instruments, S. A., Barcelona, Spain) based on the Association of Official Agricultural Chemists (AOAC) Official Method 981.12. TA was determined by titrating 10 mL of diluted sweet cherry juice (5 mL of sweet cherry juice and 5 mL of distilled water) to pH = 8.1 with a standardized 0.1 M sodium hydroxide solution, using an automatic titrator (Titromatic 1S, Crison Instruments, S. A., Barcelona, Spain), based on the AOAC Official Method 962.12 and the results

expressed in mg malic acid/mL of sweet cherry juice. The total soluble solids (TSS) content was determined by measuring °Brix (Hand-held Refractometer Atago, ATC-1E, Tokyo, Japan) at 20°C, based on the AOAC Official Method 932.12.

#### Total phenolic content

The total phenolic content was measured using the Folin-Ciocalteu colorimetric method. The samples were diluted with distilled water (1:10) and 125 µL added to 125 µL of the Folin-Ciocalteu solution and 500 µL of distilled water, then the solution was allowed to stand in the dark for 6 min. Afterwards, 1.25 mL of 7% sodium carbonate solution was added and the solution mixed again. After 60 min at room temperature, the solution absorbance was read at 720 nm using a UV-VIS spectrophotometer (Microplate Spectrophotometer Multiskan Go, Thermo Scientific, USA). The total phenolic content was calculated, using a previously determined calibration curve, with gallic acid as the standard, and expressed as mg gallic acid (GAE)/mL of sweet cherry juice.

#### Total anthocyanin content

Total anthocyanin content was evaluated as described elsewhere with few modifications. One millilitre of sweet cherry juice was diluted to 10 mL using a 10.15:39.85 (v/v) mixture of 95% ethanol and 37% hydrochloric acid. Absorbance was measured at 720 nm using the spectrophotometer described above and cyanidin-3-glucoside (CG) was used as the standard. The total anthocyanin content in milligram equivalents of cyanidin-3-glucoside (CGE) per litre was calculated using the Beer-Lambert law, using the molar absorptivity coefficient of 60.45 mL-/mg-cm.

#### Antioxidant activity determination

The antioxidant activity was determined according to the modified method of Kelebek, Canbas using DPPH as a free radical. A set of five different solutions was obtained by diluting the samples (2.5%, 5%, 10%, 20% and 50%) in distilled water (v/v). A 75 µL aliquot of the diluted sweet cherry juice solution was added to 2.93 mL of a 60 µM DPPH solution in methanol, and left to react during 45 min in the dark at room temperature, then the absorbance at 515 nm was recorded to determine the remaining DPPH concentration. The DPPH remaining percentage for the five dilutions was plotted versus the ratio mg DPPH/mL sweet cherry juice. The half maximal effective concentration (EC<sub>50</sub>) parameter was then graphically calculated.

#### Statistical analyses

Statistical data analysis was carried out using analysis of variance (ANOVA) and Tukey's honestly significant difference test to determine the difference between the means with a 5% level of significance.

#### Results and discussion

##### Microbiological stability

TAM and YM loads were assessed as general spoilage/quality indicators. Unprocessed raw juice showed an initial load of  $3.50 \pm 0.04$  and  $4.70 \pm 0.02$  Log<sub>10</sub> CFU/mL for TAM and YM, respectively. After 14 storage days, both counts were already very high, the TAM concentration being  $4.51 \pm 0.20$  Log<sub>10</sub> CFU/mL and the YM counts being  $6.17 \pm 0.20$  Log<sub>10</sub> CFU/mL. These results reveal that at this point, the unprocessed juice presents unacceptable microbial conditions for consumption. After 28 storage days at 4°C, these values increased to  $5.82 \pm 0.16$  and  $7.22 \pm 0.24$  Log<sub>10</sub> CFU/mL, respectively. The YM higher levels are due to these microorganisms' higher tolerance to acid environments than TAM, being the major responsible for fruit juices spoilage. TP is known to successfully produce microbiological safe products and both HPP conditions (P1 and P2) considerably reduced the initial microbiological load. TAM and YM loads decreased to undetectable levels ( $< 1$  Log<sub>10</sub> CFU/mL), both after processing and during the storage time tested for all the processed samples. This confirms the data in the literature that reports that several HPP conditions in the range of those studied in the present study effectively reduce microbial load in acidic fruit juices, including red fruit (pomegranate and strawberry) juices where HPP proved to be extremely efficient in inactivating microorganisms.

##### pH, TA and total soluble solids

The initial pH value of raw sweet cherry juice was  $3.68 \pm 0.01$  (Table 1), which is in agreement with the literature data, and this value remained fairly stable during the evaluated storage period. However, all treatments had an impact on the samples pH value, since P1, P2 and TP increased the pH to  $3.83 \pm 0.04$ ,  $3.77 \pm 0.03$  and  $3.74 \pm 0.05$ , respectively. Although differing in relation to

most studies where there is no pH variation, this increase in the pH value is

Table 1. Raw and processed sweet cherry juice physicochemical parameters after processing and during storage.

Parameter	Treatment	Days of storage				
		0	7	14	21	28
pH	Raw	3.68 ± 0.01 aB	3.67 ± 0.01 abcC	3.65 ± 0.00 cD	3.66 ± 0.00 bcC	3.68 ± 0.00 aB
	TP	3.74 ± 0.05 aAB	3.71 ± 0.01 aB	3.68 ± 0.00 aC	3.68 ± 0.01 aBC	3.67 ± 0.00 aC
	P1	3.83 ± 0.04 aA	3.79 ± 0.01 abA	3.74 ± 0.00 bcA	3.75 ± 0.00 bcA	3.71 ± 0.00 cA
	P2	3.77 ± 0.03 aAB	3.72 ± 0.01 abB	3.70 ± 0.00 bB	3.70 ± 0.01 bB	3.68 ± 0.00 bB
Total soluble solids (°Brix)	Raw	16.5 ± 0.4 aA	16.4 ± 0.3 aA	16.2 ± 0.0 aA	16.0 ± 0.0 abAB	15.2 ± 0.0 bC
	TP	16.2 ± 0.0 aA	15.8 ± 0.3 abA	16.1 ± 0.1 abAB	15.6 ± 0.0 bC	16.0 ± 0.0 abAB
	P1	16.6 ± 0.0 aA	16.0 ± 0.0 abA	15.7 ± 0.1 cB	16.1 ± 0.1 bA	16.2 ± 0.0 bA
	P2	16.4 ± 0.1 aA	16.2 ± 0.0 abA	15.9 ± 0.1 bcAB	15.8 ± 0.0 cBC	15.9 ± 0.1 bcB
TA (mg malic acid/mL)	Raw	6.07 ± 0.01 bA	6.14 ± 0.03 bA	6.44 ± 0.06 aA	6.48 ± 0.09 aA	6.50 ± 0.03 aA
	TP	6.18 ± 0.07 aA	6.29 ± 0.03 aA	6.27 ± 0.02 aA	6.35 ± 0.17 aA	6.32 ± 0.29 aA
	P1	6.17 ± 0.10 aA	6.18 ± 0.05 aA	6.30 ± 0.10 aA	6.25 ± 0.02 aA	6.37 ± 0.09 aA
	P2	6.03 ± 0.27 aA	6.26 ± 0.06 aA	6.51 ± 0.03 aA	6.40 ± 0.07 aA	6.17 ± 0.11 aA

Notes: TP, thermal pasteurization; P1, 400 MPa/5 min; P2, 550 MPa/2 min. Different letters (a, b, c) indicate significant differences ( $p < .05$ ) for the same treatment at different storage times. Different letters (A, B, C, D) indicate significant differences ( $p < .05$ ) between treatments at the same storage time.

not new and has already been verified in other vegetable products, both after TP and pressure processing. A constant pH decline in the subsequent storage for all processed samples was observed until reaching approximately the initial pH value, and at the end of this period, the pH values of all samples were similar to the raw sweet cherry juice. This decline during storage may result from a probable gradual organic acids leakage from the vegetable cell organelles to the juice matrix as previously suggested.

The initial TSS was  $16.5 \pm 0.4$  °Brix (Table 1), similar to the values reported by Ballistreri et al. All treatments (P1, P2 and TP) did not affect significantly ( $p > .05$ ) TSS, which is in accordance with the data in the literature for other types of red fruit juices and juices in general. During storage, there were slight fluctuations among the processed samples, while the raw juice presented a decline, reaching a final value of  $15.2 \pm 0.1$  °Brix. The same was verified in HPP pomegranate juice.

The TA results (Table 1) are consistent with the above stated for pH, although there were no significant ( $p > .05$ ) variations among the processed samples during storage, the average values suggest a trend, being verified by a rise in the organic acids concentration, which may be responsible for the increased acidity.

#### Bioactive compounds

The initial total phenolic content in the raw juice was  $1.42 \pm 0.13$  mg GAE/mL (Table 2), which is in the range of previously reported data. The different treatments caused different effects on these compounds concentration: TP increased them to  $1.51 \pm 0.15$ , P1 did not significantly ( $p > .05$ ) affect the initial value and P2 decreased them to  $1.26 \pm 0.19$  (all values are mg GAE/mL). During the 28 days of storage, the raw juice had a decline in the total phenolic content that was more accentuated at the 7th and 14th days, reaching a total decrease of about 26% in the end of the evaluated period. The same tendency was verified in the TP samples which showed a 20% decrease. A similar behaviour was already reported in TP pomegranate juice. P1 samples maintained the initial total phenol content until the 14th day and decreasing afterwards,



Table 2. Raw and processed sweet cherry juice bioactive compounds content after processing and during storage.

Parameter	Treatment	Days of storage					%
		0	7	14	21	28	
Total phenolic content (mg GAE/mL)	Raw	1.42 ± 0.13 aB	1.22 ± 0.47 Cb	1.24 ± 0.16 bcA	1.30 ± 0.56 bA	1.05 ± 0.13 dC	26
	TP	1.51 ± 0.15 aA	1.21 ± 1.00 bB	1.28 ± 0.72 bA	1.20 ± 0.17 bB	1.21 ± 0.21 bB	20
	P1	1.38 ± 0.46 aB	1.33 ± 0.18 aA	1.32 ± 0.21 aA	1.19 ± 0.68 bB	1.23 ± 0.22 bAB	11
	P2	1.26 ± 0.19 bC	1.34 ± 0.23 aA	1.29 ± 0.25 abA	1.27 ± 0.22 bAB	1.25 ± 0.14 bA	1
Total anthocyanin content (mg CGE/L)	Raw	9.37 ± 0.59 aB	7.81 ± 0.18 bB	8.00 ± 0.22 dD	6.11 ± 0.05 eD	5.47 ± 0.14 cB	42
	TP	9.19 ± 0.03 aAB	8.70 ± 0.14 bA	6.42 ± 0.11 dD	5.74 ± 0.07 eD	6.95 ± 0.08 cB	24
	P1	10.13 ± 0.28 aA	7.41 ± 0.25 cB	8.54 ± 0.19 bA	6.53 ± 0.11 dB	7.89 ± 0.01 cA	22
	P2	10.21 ± 0.03 aA	9.17 ± 0.17 bA	7.34 ± 0.12 cC	7.23 ± 0.07 cA	6.03 ± 0.11 dC	41

Notes: TP, thermal pasteurization; P1, 400 MPa/5 min; P2, 550 MPa/2 min. Values in the last column show the percentage reduction at the day 28. Different letters (a, b, c, d, e) indicate significant differences ( $p < .05$ ) for the same condition at different storage times. Different letters (A, B, C, D) indicate significant differences ( $p < .05$ ) between conditions at the same storage time.

reaching a decrement of 11% at day 28. Other authors also verified this decrease in other HPP red fruit juices, namely in strawberry and pomegranate juices. The observed decrease trend can be the result of enzyme activity, mainly polyphenol oxidase and peroxidase that usually are not inactivated by HPP, and/or due to the phenolic compounds oxidation. P2 samples seemed to be fairly stable during storage maintaining the same total phenolic content, however minor variations were observed. Although presenting different behaviours during the storage period, the final total phenolic content (day 28) was similar in all pasteurized samples, tending to be higher (about 20%) in the HPP samples compared with the raw juice.

The sweet cherry raw juice had an initial total anthocyanin content of  $9.37 \pm 0.59$  mg CGE/L (Table 2), which is in agreement with values reported in the literature. TP had no significant ( $p > .05$ ) effect on the initial value, while both HPP treatments significantly ( $p < .05$ ) increased the content of these compounds to  $10.13 \pm 0.28$  (P1) and  $10.21 \pm 0.03$  mg CGE/L (P2). These compounds are described as being stable during HPP and there are some studies in which pressure treatments even increased the extractability of coloured pigments in food components. All the samples presented a steady decline during storage, where the raw juice showed the major decrease reaching  $5.47 \pm 0.14$  mg CGE/L (a 42% decrease), and P1 the smallest decrease with a final content of  $7.89 \pm 0.01$  mg CGE/L (a 22% decrease) at the end of the studied period. This decrease was expected, as similar behaviours were verified in other red fruit juices, both with HPP and TP. The observed decline may be justified by condensation between anthocyanins, and/or due to residual activity of several enzymes such as p - glucosidase, polyphenol oxidase and peroxidase which have been associated with anthocyanins degradation.

#### Antioxidant activity

The obtained EC50 is inversely correlated to the sample's antioxidant capacity, since it expresses the antioxidant quantity needed to decrease the radical concentration by 50%, so the lower the EC50 value, the greater is the sample antioxidant activity. All the treatments did not significantly ( $p > .05$ ) affect the sweet cherry juice antioxidant activity, which remained stable until the 14th day (Figure 1). This is in accordance with the data available in the literature for other fruit and vegetable products. At the 21st day, raw and TP samples increased their EC50 value (up to 20%), remaining above the other samples EC50 value until the end of storage, indicating that these samples had the lowest antioxidant activity during this period. This increase was also

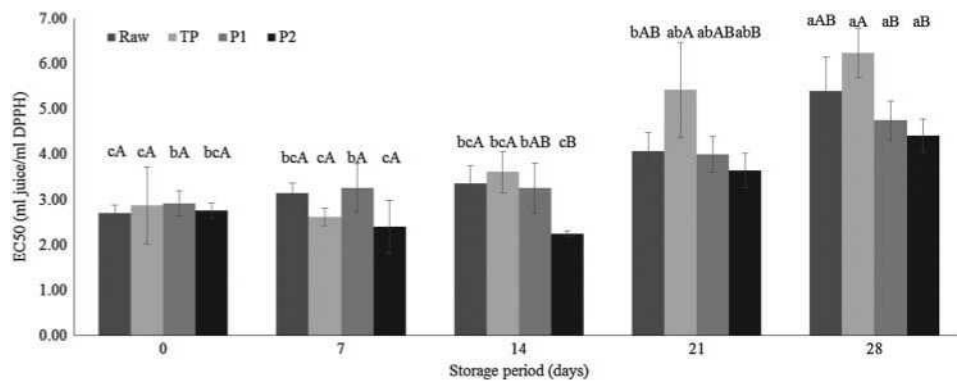


Figure 1. Raw and processed sweet cherry juice antioxidant activity after processing and during storage. Different letters (a, b) indicate significant differences ( $p < .05$ ) for the same treatment at different storage times. Different letters (A, B) indicate significant differences ( $p < .05$ ) between treatments at the same storage time.

verified in the other samples, but with no statistical differences between them ( $p > .05$ ). For the two last sampling days (21 and 28), HPP samples showed tendentially the lowest EC50, demonstrating that these samples had the highest antioxidant activity at the final stage of storage. This may be justified by the reported HPP capability to preserve ascorbic acid, which presents a relative substantial antioxidant capacity contribution to the total antioxidant capacity, and is degraded by TP.

## Conclusion

In conclusion, all the tested treatments studied in this work (TP for 30 s at 70°C and pressure processing at 400 MPa and 550 MPa for 5 and 2 min, respectively) reduced the initial micro- biological load to levels below the method detection limit ( $< 1 \text{ Log}_{10} \text{ CFU/mL}$ ), which were maintained throughout storage, whereas the raw juice spoiled rapidly. These results demonstrate the HPP efficiency producing microbiological safe juices. Both HPP and TP caused minimal changes in the raw juice physicochemical quality parameters and bioactive compounds. Although all treatments produced similar results in most parameters, HPP samples exhibited the highest antioxidant activity at the final stage of storage. Further studies should focus on studying longer storage periods and other quality parameters.

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## References

- [1] McCune LM, Kubota C, Stendell-Hollis NR, Thomson CA. Cherries and health: a review. *Cr Rev Food Sci.* 2011;51:1-12.
- [2] Kelebek H, Selli S.: Evaluation of chemical constituents and antioxidant activity of sweet cherry (*Prunus avium* L.) cultivars. *Int J Food Sci Technol.* 2011;46:2530-253007.
- [3] Serrano M, Guillén F, Martínez-Romero D, Castillo S, Valero D. Chemical constituents and antioxidant activity of sweet cherry at different ripening stages. *J Agr Food Chem.* 2005;53:2741-2745.
- [4] Usenik V, Fabcic J, Stampar F. Sugars, organic acids, phenolic composition and antioxidant activity of sweet cherry (*Prunus avium* L.). *Food Chem.* 2008;107:185-192.
- [5] Serra AT, Duarte RO, Bronze MR, Duarte CMM. Identification of bioactive response in traditional cherries from Portugal. *Food Chem.* 2011;125:318-25.
- [6] Ballistreri G, Continella A, Gentile A, Amenta M, Fabroni S, Rapisarda P. Fruit quality and bioactive compounds relevant to human health of sweet cherry (*Prunus avium* L.) cultivars grown in Italy. *Food Chem.* 2013;140:630- 638.
- [7] Gonçalves B, Silva AP, Moutinho-Pereira J, Bacelar E, Rosa E, Meyer AS. Effect of ripeness and postharvest storage on the evolution of colour and anthocyanins in cherries (*Prunus avium* L.). *Food Chem.* 2007;103:976-84.
- [8] Heckman M, Sherry K, Gonzalez de Meija E. Energy drinks: an assessment of their market size, consumer demo- graphics, ingredient profile, functionality, and regulations in the United States. *Compr Rev Food Sci Food Safety.* 2010;9:303-317.
- [9] Keenan DF, RoBle C, Gormley R, Butler F, Brunton NP. Effect of high hydrostatic pressure and thermal processing on the nutritional quality and enzyme activity of fruit smoothies. *LWT Food Sci Technol.* 2012;45:50-7.
- [10] Patras A, Brunton NP, Da Pieve S, Butler F. Impact of high pressure processing on total antioxidant

- activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. *Innov Food Sci Emerg Technol.* 2009;10:308-313.
- [11] Mota MJ, Lopes RP, Delgadillo I, Saraiva JA. Microorganisms under high pressure - adaptation, growth and biotechnological potential. *Biotechnol Adv.* 2013;31:1426-1434.
- [12] Fidalgo LG, Santos MD, Queirós RP, Inácio RS, Mota MJ, Lopes RP, Gonçalves MS, Neto RF, Saraiva JA. Hyperbaric storage at and above room temperature of a highly perishable food. *Food Bioproc Technol.* 2014;7:2028-2037.
- [13] Queiros RP, Santos MD, Fidalgo LG, Mota MJ, Lopes RP, Inacio RS, Delgadillo I, Saraiva JA. Hyperbaric storage of melon juice at and above room temperature and comparison with storage at atmospheric pressure and refrigeration. *Food Chem.* 2014;147:209-214.
- [14] Rastogi NK, Raghavarao KS, Balasubramaniam VM, Niranjana K, Knorr D. Opportunities and challenges in high pressure processing of foods. *CrRevFoodSci.* 2007;47:69-112.
- [15] Norton T, Sun D-W. Recent advances in the use of high pressure as an effective processing technique in the food industry. *Food Bioproc Technol.* 2008;1:2-34.
- [16] Mujica-Paz H, Valdez-Fragoso A, Samson CT, Welte-Chanes J, Torres JA. High-pressure processing technologies for the pasteurization and sterilization of foods. *Food Bioproc Technol.* 2011;4:969-985.
- [17] Balasubramaniam V, Farkas D, Turek E. Preserving foods through high-pressure processing. *Food Technol.* 2008;62:32-38.
- [18] Oey I, Lille M, Van Loey A, Hendrickx M. Effect of high-pressure processing on colour, texture and flavour of fruit- and vegetable-based food products: a review. *Trends Food Sci Technol.* 2008;19:320-328.
- [19] Butz P, Fernández García A, Lindauer R, Dieterich S, Bognár A, Tauscher B. Influence of ultra high pressure processing on fruit and vegetable products. *J Food Eng.* 2003;56:233-236.
- [20] Barba FJ, Esteve MJ, Frígola A. High pressure treatment effect on physicochemical and nutritional properties of fluid foods during storage: a review. *Compre Rev Food Sci Food Safety.* 2012;11:307-322.
- [21] Patterson MF. Microbiology of pressure-treated foods. *J Appl Microbiol.* 2005;98:1400-1409.
- [22] Timmermans RAH, Mastwijk HC, Knol JJ, Quataert MCJ, Vervoort L, der Plancken IV, Hendrickx ME, Matser AM. Comparing equivalent thermal, high pressure and pulsed electric field processes for mild pasteurization of orange juice. Part I: impact on overall quality attributes. *Innov Food Sci Emerg Technol.* 2011;12:235-243.
- [23] Singleton V, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Viticul.* 1965;16:144-158.
- [24] Rapisarda P, Fanella F, Maccarone E. Reliability of analytical methods for determining anthocyanins in blood orange juices. *J Agr Food Chem.* 2000;48:2249-2252.
- [25] Kelebek H, Canbas A, Selli S. Determination of phenolic composition and antioxidant capacity of blood orange juices obtained from cvs. Moro and Sanguinello (*Citrus sinensis* (L.) Osbeck) grown in Turkey. *Food Chem.* 2008;107:1710-1716.
- [26] Varela-Santos E, Ochoa-Martinez A, Tabilo-Munizaga G, Reyes JE, Pérez-Won M, Briones-Labarca V, Morales-Castro J. Effect of high hydrostatic pressure (HHP) processing on physicochemical properties, bioactive compounds and shelf-life of pomegranate juice. *InnovFoodSci Emerg Technol.* 2012;13:13-22.
- [27] Barba FJ, Esteve MJ, Frígola A. Physicochemical and nutritional characteristics of blueberry juice after high pressure processing. *Food Res Int.* 2013;50:545-549.
- [28] Chen D, Xi H, Guo X, Qin Z, Pang X, Hu X, Liao X, Wu J. Comparative study of quality of cloudy pomegranate juice treated by high hydrostatic pressure and high temperature short time. *Innov Food Sci Emerg Technol.* 2013;19:85-94.
- [29] Ferrari G, Maresca P, Ciccarone R. The application of high hydrostatic pressure for the stabilization of functional foods: pomegranate juice. *J Food Eng.* 2010;100:245-253.
- [30] Gonçalves B, Landbo A-K, Knudsen D, Silva AP, Moutinho-Pereira J, Rosa E, Meyer AS. Effect of ripeness and postharvest storage on the phenolic profiles of cherries (*Prunus avium* L.). *J Agr Food Chem.* 2004;52:523-530.
- [31] Jacobo-Velazquez DA, Hernandez-Brenes C. Biochemical changes during the storage of high hydrostatic pressure processed avocado paste. *J Food Sci.* 2010;75:S264-S270.
- [32] Wang R, Wang T, Zheng Q, Hu X, Zhang Y, Liao X. Effects of high hydrostatic pressure on color of spinach puree and related properties. *J Sci Food Agri.* 2012;92:1417-1423.
- [33] Pérez-Vicente A, Serrano P, Abellán P, García-Viguera C. Influence of packaging material on pomegranate juice colour and bioactive compounds, during storage. *J Sci Food Agri.* 2004;84:639-644.
- [34] Cao X, Bi X, Huang W, Wu J, Hu X, Liao X. Changes of quality of high hydrostatic pressure processed cloudy and clear strawberry juices during storage. *Innov Food Sci Emerg Technol.* 2012;16:181-90.

- [35]Cao X, Zhang Y, Zhang F, Wang Y, Yi J, Liao X. Effects of high hydrostatic pressure on enzymes, phenolic compounds, anthocyanins, polymeric color and color of strawberry pulps. *J Sci Food Agri*. 2011;91:877-885.
- [36]Sanchez-Moreno C, Plaza L, Elez-Martinez P, De Ancos B, Martin-Belloso O, Cano MP. Impact of high pressure and pulsed electric fields on bioactive compounds and antioxidant activity of orange juice in comparison with traditional thermal processing. *J Agri Food Chem*. 2005;53:4403-4409.
- [37]Wang W-D, Xu S-Y. Degradation kinetics of anthocyanins in blackberry juice and concentrate. *J Food Eng*. 2007;82:271-5.
- [38]Torres B, Tiwari BK, Patras A, Cullen PJ, Brunton N, O'Donnell CP. Stability of anthocyanins and ascorbic acid of high pressure processed blood orange juice during storage. *Innov Food Sci Emerg Technol*. 2011;12:93-97.
- [39]Patras A, Brunton N, Da Pieve S, Butler F, Downey G. Effect of thermal and high pressure processing on antioxidant activity and instrumental colour of tomato and carrot purées. *Innov Food Sci Emerg Technol*. 2009;10:16-22.
- [40]Barba FJ, Esteve MJ, Frigola A. Ascorbic acid is the only bioactive that is better preserved by high hydrostatic pressure than by thermal treatment of a vegetable beverage. *J Agri Food Chem*. 2010;58:10070-100705.

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