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Full Length Research Paper

Berries Harvested in Mexico and its Use to Prepare a Functional Drink Conserved By Ultrasonication

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Recently, the food industry has shown a great interest in berries because the consumption has increased due to their content of bioactive compounds. In the present work, a group of Mexican-harvested berries (blueberries, strawberries, raspberries, pomegranates and blackberries) was analyzed, and the physicochemical and antioxidant properties were determined. Via conventional pasteurization and ultrasonication as preservation methods, a drink was produced from a mixture of the five fruits. The results show that blueberry has the highest content of phenols and anthocyanins, while blackberry has the highest antioxidant capacity. Polyphenols such as catechin, cyanidin-3-glucoside and resveratrol were identified by HPLC. Regarding formulated and processed beverages, the ultrasonicated beverage had an increased content of bioactive compounds compared to the pasteurized beverage. Both treatments inhibited the growth of pathogenic microorganisms and inactivated enzymes that produce undesirable changes in mixed fruit drinks. The sensorial evaluation showed that the ultrasonicated beverage had the greatest sensory characteristics and had greater acceptance compared to the commercial and pasteurized beverages.

Keywords: berries, antioxidants, beverages, pasteurization, ultrasonication

1. INTRODUCCIÓN

In recent years, the global trends in nutrition indicate a great interest of consumers toward certain foods, which are generally known as functional foods; in addition to provide nutritional value, these functional foods contribute to

improving health and reducing the risk of cardiovascular diseases (Sedó, 2002, Langreo, 2008). Some vegetables and fruits contain a unique class of phytochemicals that are synthesized as a response to external stress caused by droughts, sunburn, extreme temperatures, insect damage and pathogen infections (Lobo *et al.*, 2010; Bigliardi & Galati, 2013). Other bioactive compounds such as anthocyanins have been determined to have various

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pharmacological and therapeutic properties, such as the reduction in coronary disease and anticancer, antitumor, anti-inflammatory and antidiabetic effects. In addition, to be effective in trapping reactive oxygen species, these bioactive compounds inhibit lipoprotein oxidation and platelet aggregation; such therapeutic effects are related to the antioxidant activity of these compounds (Ghiselli *et al.*, 1998; Chaovanalikit & Wrolstad, 2004; Garzón *et al.*, 2009; Routray & Orsat, 2011).

The antioxidant activity in functional beverages is directly related to their content of bioactive compounds; it has been reported that functional beverages that use a higher percentage of natural fruit have greater antioxidant activities than those made with artificial flavors and colors (Murillo, 2005). Currently, Mexico is the fifth producer of berries worldwide; the state of Jalisco is the highest producing state and produces more than 46 thousand tons (76% of the national total) of berries. Mexico exports berries mainly to the following countries: Chile, Canada, the United States, the Netherlands, Japan and the United Kingdom (SAGARPA, 2017).

A large amount of pasteurized liquid foods based on fruit drinks have emerged as innovative products and have experienced rapid growth in recent years, which indicates a new trend in the market (Bertsias *et al.*, 2005; Balasundram *et al.*, 2006; Chemat & Khan, 2011; Rajauria & Tiwari, 2017). Several investigations related to functional foods have shown great interest in the analysis of antioxidant properties, the determination of vitamins A and C, phenolic compounds and carotenenes content, and the study of the *in vitro* bioavailability of bioactive compounds. Likewise, the application of emerging technologies such as ultrasonication have been studied for the preservation of bioactive compounds, the improvement of formulations, and the use of ingredients to improve their nutritional value and microbiological stability (Zulueta *et al.*, 2010; Andres *et al.*, 2014; Rodríguez-Roque *et al.*, 2014).

Ultrasonication is an innovative technique that generates bubbles that cavitate and implode due to the pressure changes that are generated, and the collapse of the bubbles generates high pressure (1000 atmospheres) that results in the rupture of cells and the release of bioactive compounds; ultrasonication creates regions of high localized temperatures and pressures of up to 5000 K and 50000 kPa, respectively (Piyasena *et al.*, 2003; Pingret *et al.*, 2013). Ultrasonication is also an emerging nonthermal technique recently used in the processing of functional juices and beverages that improves the nutritional and sensory qualities, extends the shelf life, reduces the microbial load, reduces the production cost, increases the yield and decreases the energy consumption (Zenker *et al.*, 2003; Bhat *et al.*, 2011; Bevilacqua *et al.*, 2018; Roobab *et al.*, 2018).

Mexican-harvested berries have only been characterized by their chemical composition; the type of bioactive

compounds present in the juices and drinks made with these fruits has not been characterized via ultrasonication methods. In the present investigation, the study of five Mexican-harvested berries (blueberries, strawberries, raspberries, pomegranates and blackberries) was performed; the study investigated the physicochemical parameters, antioxidant capacity and the identification and quantification of bioactive compounds (polyphenols and anthocyanins) by HPLC.

A comparative analysis of the content of bioactive compounds between the processed beverages and commercial brands drinks was performed. In addition, the determination of the enzymatic content and microbiological analysis, the identification of the main bioactive compounds by HPLC and the sensory evaluation of the processed beverages were performed.

2. METHODOLOGY

2.1. Raw material: The fruits were obtained from one region of origin: blueberry from Jalisco, raspberry from Michoacán, strawberry from Guanajuato, pomegranate from Edo, and blackberry from Michoacán. These samples were purchased from the Central de Abasto of CDMX (CEDA). The samples were transferred to the laboratory and kept refrigerated (4 °C) until their use.

2.2. Sample preparation: The five berries (blueberry, raspberry, strawberry, pomegranate, and blackberry) were washed with running water, disinfected with a chlorinated water solution of 250 ppm, and rinsed with purified water. The shell was removed from the pomegranate, and in the other berries, the stem was removed. The clean berries were triturated and filtered with gauze fabric to obtain the juice for further analysis (NOM-173-SCFI-2009).

2.2.1. Mixed drink: The mixed drink was created by mixing the five berries using the volume corresponding to 9.6% juice from each berry to obtain a total of 48% pulp, and it was completed to 100% with potable water (NOM-173-SCFI-2009). The drink was adjusted to 12 °Brix with organic agave honey (low glycemic index) to maintain its functional properties. Samples of 60 ml of the mixed drink were stored in amber glass bottles previously sterilized with a capacity of 80 ml for the immediate processing by ultrasonication or pasteurization.

2.2.2. Processing of mixed drink: The drink obtained from the mixture of berries was subjected by two processes:

a) **Ultrasonication:** A homogenizing ultrasonicator (Branson S-250A) was used at a constant temperature of 40 °C and a frequency of 50-60 kHz for 60 minutes, following the methodology reported by Abid *et al.* (2013) and by Zafra *et al.* (2013). After ultrasonication, the product obtained was sealed with parafilm and stored at 4 °C. The

samples were analyzed in triplicate every seven days for twenty-one days.

b) *Conventional pasteurization*: The beverage was pasteurized in a hot water bath for 30 minutes at a constant temperature of 70 °C, according to the methodology reported by Odriozola, (2009). After pasteurization, the product was stored at 4 °C and analyzed in triplicate every seven days for fifty-six days.

The drinks, both ultrasonicated and pasteurized, were analyzed over 21 and 56 days, respectively, which are the respective maximum conservation times for the treatment to ensure that the product is safe and free of pathogenic microorganisms (Gancel *et al.*, 2011).

2.3. Physicochemical parameters: The parameters were determined with the methodologies of the Mexican standard norms: the percentage of humidity of each berry was determined with the gravimetric method using a Sartorius thermos-balance model MA45 (NMX-116-SSA1-1994); the relative density of the berry juices and the beverages (ultrasonicated, pasteurized and commercial samples) was determined with a pycnometer (NMX-SCFI-075-2012); the pH was determined using a Hanna Instruments potentiometer (NMX-F-317-S-1978); the titratable acidity was determined and reported as a percentage of citric acid (NMX-F-102-S-1978); and the total soluble solids (° Brix) were determined with an Abbe refractometer (NMX-F-103-1982).

2.4. Polyphenolic extracts: The extract of each berry was made with four different solvents (80% ethanol, 70% acetone, 80% methanol and 80% methanol:1% HCl) to select an extract with the highest extraction since in the literature reports different amounts of extracted phenols (Howell *et al.*, 2001; Kajdzanoska *et al.*, 2011). A 10 g sample of each berry was crushed and mixed with 10 mL of solvent, sonicated for 10 minutes, shaken for two hours and then filtered with Whatman 40 paper. Once the best solvent was determined, the extracts for the pasteurized, ultrasonicated and commercial beverages were prepared.

2.5. Total phenolic compounds (TPC): Folin-Ciocalteu reagent was used according to the methodology reported by Huang *et al.* (2012). A 0.4 mL aliquot of the extract was diluted with 2 ml of the Folin-Ciocalteu reagent (0.5 mol/L) and sonicated for 10 minutes. Subsequently, the reaction was neutralized with 2 ml of 75 mg/ml saturated Na₂CO₃. The absorbance was measured at 765 nm after incubation for 2 hours at room temperature in the dark. The results were expressed as milligram equivalents of gallic acid (mg GAE/100 g wet base (wb)).

2.6. Antioxidant activity: The quantification of the antioxidant capacity was performed by preparing a standard curve using Trolox, and antioxidant capacity was measured based on the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) following the methodology reported by Kumaran & Karunakaran (2007), with some modifications. DPPH was prepared at a concentration of

0.1 mM in 80% methanol. A 0.1 mL aliquot of the extract, previously obtained for the determination of phenols, was mixed with 3.9 mL of the DPPH solution, vigorously shaken and sonicated for 10 minutes, and the absorbance of the sample was immediately measured at 515 nm. The percentage of reduced DPPH during the reaction was obtained from the data by applying Equation 1 (Soler-Rivas *et al.*, 2000) as follows:

$$\%DPPH = \frac{(abs_{DPPH0} - abs_t)}{abs_{DPPH0}} \quad \text{Eq. (1)}$$

where abs_{DPPH0} is the initial absorbance of the 0.1 mM DPPH solution and abs_t is the absorbance of the samples at different times. To calculate the IC₅₀, a linear regression of the calibration curve was performed (% of DPPH * vs. the concentration of the added extract). The IC₅₀ value is defined as the concentration of the evaluated sample required to inhibit 50% of the DPPH radical.

2.7. Anthocyanins by the differential pH method (Giusti & Wrolstad, 2001): The structures that anthocyanins can form when subjected to different pH values were taken into account. In this method, two dilutions of the extract (0.5 ml) were prepared; one dilution was prepared with 4.5 ml of a potassium chloride solution (0.025 M) at pH 1.0, and the other dilution was prepared with 4.5 mL of a sodium acetate solution (0.4 M) at pH 4.5. Subsequently, a spectroscopic scan was made from 510 nm to 700 nm. The anthocyanin concentration was calculated by Equation 2 as follows:

$$\text{Anthocyanins concentration} \left(\frac{\text{mg}}{\text{L}} \right) = \frac{(A_{\lambda_{\text{max}}} - A_{700 \text{ nm}})_{\text{pH } 1.0} - (A_{\lambda_{\text{max}}} - A_{700 \text{ nm}})_{\text{pH } 4.5}}{(\epsilon * 1)} \quad \text{Eq. (2)}$$

where $A = (A_{\lambda_{\text{max}}} - A_{700 \text{ nm}})_{\text{pH } 1.0} - (A_{\lambda_{\text{max}}} - A_{700 \text{ nm}})_{\text{pH } 4.5}$; $A_{\lambda_{\text{max}}}$ is the maximum absorbance of anthocyanin; $A_{700 \text{ nm}}$ is the correction reading due to interfering substances; ϵ is the molar absorptivity of the anthocyanin majority; PM is the molecular weight of the majority anthocyanin; and FD is the dilution factor.

2.8. Vitamin C. (AOAC 967.21, 2000): The standard solution of ascorbic acid (1 mg/ml) was prepared, and then a 2 mL aliquot was transferred to a flask containing 5 ml of an acetic acid-metaphosphoric acid solution. The vitamin C content was determined by titration with a solution of 2,6-dichlorophenolindophenol (DCPI), and at the same time, a blank was evaluated. The normalization of the indicator solution (DCPI) was carried out in the presence of a standard solution of ascorbic acid. The calculation was performed with Equation 3 as follows:

$$\text{mg ascorbic acid} = \frac{([\text{colorant}])(\text{mL consumed})(100 \text{ mL sample})}{(10 \text{ mL sample})} \quad \text{Eq. (3)}$$

2.9. Beverage shelf life: The analysis of physicochemical properties (pH, °Brix, titratable acidity and density) and the determination of bioactive compounds of the beverages were performed weekly during the shelf life experiments, in which the ultrasonicated beverages were measured for 21 days and the pasteurized beverages for 56 days. Each evaluation was performed in triplicate; the products were

stored in amber bottles under hermetic conditions and at 4 °C in a dark room.

2.10. Kinetics of bioactive compounds in beverages:

An analysis of the degradation kinetics of the bioactive compounds of the ultrasonicated and pasteurized drinks was carried out during 21 and 56 days of storage, respectively. With the obtained data, a first-order model was tested according to Turfan *et al.* 2012. The constant of the first-order reaction rate (k) and the half-life ($t_{1/2}$) were calculated by means of Equations 4 and 5 as follows:

$$\ln([C_t]/[C_0]) = -k \cdot t \quad \text{Eq. (4)} \quad t_{1/2} = -\ln 0.5 \cdot k^{-1} \quad \text{Eq. (5)}$$

where [Ct] is the content of the bioactive compounds (phenols or anthocyanins)/L at a time t (minutes) of heating at a given temperature, [C₀] is the content of each bioactive compound (phenols or anthocyanins)/L at the beginning of the kinetics, k is the reaction rate constant (days⁻¹), and t is the time in days. With the use of Equation 5, the half-life time ($t_{1/2}$) was determined, where k is the degradation constant of the bioactive compound. The half-life time is defined as the time when the concentration of the bioactive compound (C) is reduced by half (Morris, 1982).

2.11. Bioactive compounds by HPLC: The identification and quantification of phenolic compounds and anthocyanins were carried out by high-performance liquid chromatography (HPLC) according to the methodology reported by García *et al.* (2002) with some modifications. HPLC-grade phenol and anthocyanin standards were used by dissolving 1 mg of each standard in 5 mL of methanol (200 ppm). The juice obtained from each of the fresh berries and the processed drinks were mixed separately with 10 mL of methanol and formic acid (3%), sonicated for 10 minutes and stirred for two hours. Subsequently, the obtained extracts were centrifuged for 10 minutes and filtered with a Millipore nylon membrane (0.45 µm pore size). Then, 50 µL of each standard and each sample was injected into the HPLC (Agilent technologies, 1200 Infinity). A C18 column (250 x 4 mm, Licrochart RP-18 Merck-Hitachi, Darmstadt, Germany) was used. Phase A was HPLC water + formic acid (5%), and phase B was methanol HPLC 100% at an elution rate of 1 mL per minute with an injection volume of 50 µL. The running time was 67 minutes at 25 °C, and chromatograms were obtained at 520 and 280 nm.

A gradient elution was performed, starting with 2% phase B up to 32% phase B at 30 minutes, 40% of B at 40 minutes and 95% of B at 50 minutes. An isocratic flow was used at the end with 98% B for 5 minutes. The outstanding peaks were identified, and the phenol standards (280 nm) included ferulic acid, catechin, epicatechin, quercetin, resveratrol, and rutin; the anthocyanin standards (520 nm) included chlorinated cyanidin, cyanidin-3-glucoside, delphinidin, pelargonidin, and pelargonidin.

2.12. Microbiological analysis: To guarantee and corroborate the harmlessness of the drinks, microbiological

analyses (mold and yeast count) were performed according to the methodology reported by Herrera (2007), with some modifications. The analysis was carried out during the storage time (at time 0, in the middle of each processing (10 days for the ultrasonicated drink and 30 days for the pasteurized drink) and at the end of the kinetics (21 days for ultrasonicated drink and 56 days for pasteurized drink). For each drink, samples were analyzed at time 0, 10 and 30 days and at the end of the kinetics (21 or 56 days). An aliquot of 100 µL of each beverage was diluted in 900 µL of sterile peptone water (5 g/200 mL) and then prepared dilutions with their respective repetition from 10⁻¹ to 10⁻⁷ were performed in Eppendorf tubes. Finally, deep sowing was performed in Petri dishes, which were incubated at 37 °C for 48 hours for bacteria and yeasts and at 32 °C for 120 hours for molds. Once the dilutions of each drink were made, sowing was carried out in Petri dishes with potato dextrose agar (PDA) media with streptomycin (100 µL of antibiotic per 100 mL of PDA media). In each plate, 50 µL of each dilution was placed and spread using a bacteriological loop. The total fungal and yeast counts were determined after five days of incubation at 28 °C.

2.13. Enzymatic content: The enzymatic content of the ultrasonicated and pasteurized beverages was determined to verify the effectiveness of the enzymatic inactivation. The activity of the polyphenol oxidase, peroxidase, and pectin methylesterase enzymes was performed in the enzymatic extract, which was preserved at 4 °C until used. The **enzyme extracts** were prepared by diluting 100 mL of ultrasonicated or pasteurized beverage and adding 100 mL of 5% sodium chloride to each sample. The samples were stirred and centrifuged for 15 minutes at 4,000 rpm, and the supernatant was separated and dried for each enzyme determination (Herrera, 2007).

2.13.1. Pectin methylesterase determination (Hultin, 1948; Elez *et al.*, 2007): A 5 mL sample of 1% pectin solution, 4 mL of distilled water and 0.2 mL of 0.4 N NaOH were added to a flask. The mixture was placed on a magnetic stirrer, the agitator and the electrode were immersed, and the pH was adjusted to 8. A 0.8 mL aliquot of the enzyme extract was added; time zero was recorded at time of the addition. The pH of 8.0 was kept constant by the addition of 0.01 N NaOH. After 5 minutes, the NaOH volume was recorded, and the reaction was terminated. With the data obtained, calculations were made to report the activity of pectin methylesterase using Equation 6:

$$UPME = \frac{N \times V \times 1000}{A \times t} \quad \text{Eq. (6)}$$

where UPME is the units of pectin methylesterase (M sus /mL min), M sus is the moles of transformed substrate, N is the normality of NaOH (meq/mL), V is the volume of NaOH (mL), A is the volume of enzymatic extract used (mL), and t is the reaction time (minutes). One unit of pectin methylesterase (UPME) is defined as the amount of enzyme that releases a microequivalent (µeq) of carboxyl

groups, at pH 8.0, per minute, at room temperature (25 °C).

2.13.2. Peroxidase determination(Maehly, 1954; Kader *et al.*, 2002):The following reagents were prepared: 1) Stock solution: 1 mL of 30% H₂O₂ diluted in 100 mL of distilled water; 2) Substrate: 1 mL of stock solution adjusted to 100 mL with phosphate buffer; and 3) Dye: O-dianisidine 1% in methanol.

The assays were performed using 0.880 mL of buffer at pH 3.5 (0.1 M citric acid adjusted to the correct pH by adding 0.2 M dibasic potassium phosphate), 0.010 mL of 0.5% o-dianisidine (w/v in methanol), and 0.010 mL of the enzyme extract. The reaction was initiated by adding 0.100 mL of 50 mM H₂O₂. The increase in the absorbance at 460 nm was measured on a UV-spectrophotometer at 25 °C, and the absorbance was recorded at the beginning and every minute for 5 minutes. The rate of change per minute was determined with Equation 7 as follows:

$$\frac{\text{Units}}{\text{mg}} = \frac{A_{460 \text{ nm/minute}}}{11.3 \text{ mM} \times \frac{\text{enzyme}}{\text{mL}} \times \text{reaction mix}} \text{Eq. (7)}$$

The ratio between the oxidized dye and the moles of catabolized hydrogen peroxide was determined by measuring the absorbance of the oxidized dye in the presence of excess enzyme.

2.13.3. Polyphenol oxidase(Kimberly & Lee, 1981; Meza *et al.*, 2007): To measure the polyphenol oxidase enzyme, 0.2 mL of extract plus 2.4 mL of phosphate buffer were placed in a cell (10 mM at pH 6.5 and 0.4 mL of 0.5 M catechol) and a blank was made in parallel to adjust the spectrophotometer to zero. The increase in absorbance was measured every minute for 5 minutes, and the specific activity was determined with Equations 8 and 9:

$$\text{AU} = \frac{\Delta A_{420 \text{ nm}}}{T} \text{Eq. (8)} \quad \text{EA} = \frac{\text{UA}}{\text{mg}} \text{Eq. (9)}$$

Where AU is the absorbance unites, EA is the specific activity; T is the time, and ΔA is the change in absorbance.

2.14. Sensory evaluation: The sensory evaluation was performed with 50 untrained panelists, and the ultrasonicated and pasteurized mixed drinks were evaluated. The sensory analyses applied were the degree of acceptance, the preference and the level of pleasure; a comparative analysis was made with commercial mixed drinks made with these fruits (Rodas & Nancy 2011; Rojas *et al.*, 2012).The amount of sample from each beverage that was used for the sensory evaluation was 30 mL at 10 °C.

2.15. Statistical analysis: The statistical analysis of the results was carried out with Minitab version 15. All the data are reported as the mean ± standard deviation from the average of three repetitions. A single factor analysis of variance (ANOVA) was utilized to determine if there was a significant difference (p <0.05) between each fruit studied and between each variable using the Tukey method.

3. RESULTS AND DISCUSSION

3.1. Physico-chemical parameters: Table 1 shows the result of the physicochemical parameters in the juice of the fresh berries evaluated, with an average value of three repetitions ± standard deviation. The **soluble solids** are in the range of 7.9 to 17.2 °Bx and are significantly different (p <0.05). There was no significant difference between the values of blackberry and pomegranate juices. Some of the values obtained are different from those reported by different authors; Godoy (2004) reported 8 °Brix for the blueberry and reported that values of 11 °Bx or lower correlated to fruits that are immature and do not present an adequate sweetness.

The **acidity** value of all fruits ranged from 0.73 to 1.65%, presenting a significant difference (p <0.05); the pomegranate had the highest value. Strawberry samples showed a higher value than the value of 0.8% reported by Kader (1997), which is recommended for an acceptable flavor.

The **pH** values of the berries were similar and ranged from 2.9 to 3.5, which were similar to those previously reported: for blueberry, the pH was 2.9 according to Godoy (2004), and for raspberry, the pH was 2.87 according to Konic-Ristic *et al.* (2011) and 3.18 according to Dujmovic *et al.* (2012). Dujmovic *et al.* (2012) and Szajdek *et al.* (2008) reported pH values of 3.45 and 5.6, respectively, for blackberry. The results obtained from the **relative density** of the samples varied between 1.14 and 1.17 g/mL, and there was no significant difference between blueberry, raspberry and strawberry. There was also no significant difference between pomegranate and blackberry; no report of this value was found. The **moisture** values obtained showed a significant difference (p <0.05); however, the values reported by the food composition tables of Muñoz *et al.* (2010) (89.7% for strawberry, 76.6% for pomegranate, and 81.7% for blackberry) are similar to those obtained in the present study, while the values for blueberry and raspberry were not previously reported.

It was found that there is a significant difference (p <0.05) between the **ratio of soluble solids and the titratable acidity** (SS/TA) of each fruit; the blackberry was the one that presented the highest value, which indicates a better correlation in the balance between the sweetness and the acidity (Barra, 2011). The lowest value was the strawberry (6.42). These results vary depending on the crop, climatic conditions, fruit maturity and variety. The SS/TA ratio of 20 or less at harvest, which was present for all the fruits used in the present study, is related to a better aptitude for conservation (Galleta *et al.*, 1971, Moggia, 1991).

3.2. Antioxidant compounds: Table 1 shows the average of the results obtained from TPC, AA, vitamin C and anthocyanins. The concentration of the extract in 100 g of sample was determined.

Table 1. Physicochemical parameters, total phenols, mono meric anthocyanins, vitamin C and antioxidant activity of fresh fruits.

	Blueberry	Raspberry	Strawberry	Pomegranate	Blackberry
°Brix	13.8±0.29 ^d	12.5±0.50 ^c	7.9±0.10 ^b	16.8±0.30 ^a	17.2±0.29 ^a
Titrateable acidity (%)	1.03±0.032 ^b	1.48±0.034 ^a	1.23±0.007 ^c	1.65±0.013 ^a	0.73±0.143 ^d
pH	3.1±0.09 ^c	2.9±0.07 ^b	3.5±0.19 ^a	3.5±0.19 ^a	3.4±0.21 ^a
Relative density	1.142±0.0010 ^a	1.143±0.0003 ^a	1.144±0.0002 ^a	1.170±0.0004 ^b	1.158±0.0003 ^b
Humidity (%)	81.50±0.7 ^b	76.86±1.0 ^a	90.07±0.7 ^c	77.91±0.3 ^a	81.77±0.3 ^d
SS/Acidity Ratio	13.39 ^a	8.44 ^{a,b}	6.42 ^{b,c}	10.18 ^{a,b,c}	23.56 ^d
Total phenols (mg GAE/100 g wb)	449.3±2.6 ^b	297.2±2.0 ^c	205.4±2.1 ^d	328.7±2.5 ^a	448.0±2.0 ^a
Monomeric anthocyanins (mg cyanidin-3- glucoside/100 g wb)	138.4±0.05 ^a	28.8±0.8 ^b	26.4±0.2 ^b	130.0±1.14 ^a	60.4±0.07 ^c
Vitamin C (mg ascorbic acid/ 100 g wb)	18.33 ^a	43.42 ^b	37.28 ^c	15.74 ^e	11.13 ^d
Antioxidant activity (IC50 µL de extract/ml DPPH)	21.45 ^a	21.85 ^{a,b}	25.99 ^c	17.85 ^b	19.01 ^{a,b}

Mean ± standard deviation (n=3). Different lowercase letters in each column indicate significant differences between edible berries (P < 0.05). SS: Soluble solids-; wb: wet base

3.3. Total phenolic compounds (TPC): A multilevel factorial design was carried out following a general linear model for phenols according to each solvent to determine the best extraction and quantification conditions of the phenolic compounds of the fruits studied. There was no significant difference in the extraction of phenolic acid with respect to the type of solvent (Fig. 1a), so 80% methanol acidified with hydrochloric acid 1% HCl was chosen. There was a significant difference (p < 0.05) in the TPC among the 5 berries (Fig. 1b), which is attributed to the type and variety of fruit (Sellappan *et al.*, 2002; Anttonen & Karjalainen, 2005; Szajdek & Borowska, 2008; Tzanakis *et al.*, 2006).

The fruit with the highest phenol content was blueberry, with 449.3 mg GAE/100 g, and the lowest value was associated with strawberry— 205.4 mg GAE/100 g. In all cases, the berries evaluated were within the ranges reported by different authors (Sellappan *et al.*, 2002; Anttonen & Karjalainen, 2005; Szajdek & Borowska, 2008; Tzanakis *et al.*, 2006), with the exception of blackberry, whose value was below the value range of 361-555 mg GAE/100 g reported by Szajdek & Borowska (2008).

3.4. Antioxidant activity: According to the results of the main effect chart (Fig. 1c), there was a significant difference (p < 0.05) in relation to the solvent used in the extraction; the largest was associated with 70% acetone. The antioxidant capacity was quantified based on

extraction in acetone and expressed as IC₅₀ (Table 1). The juice of the fruit with the highest antioxidant capacity was the blackberry. This value coincides with that reported by Konic-Ristic *et al.* (2011), who analyzed the juice of blackberries, raspberries and blueberries and reported IC₅₀ values of 41.0 ± 0.3, 66.7 ± 2.1, and 70.5 ± 3.9, respectively. The sample with the lowest antioxidant capacity was the strawberry, which also had the lowest content of phenols.

The relationship between the content of total phenols (mg GAE/100 g wb) and the antioxidant capacity reported as a percentage of inhibition was obtained by means of a scatter plot (Fig. 1d) and a regression analysis. The relationship was positive, presenting an adjustment of 70.4%. Nielsen *et al.* (2003) noted that the antioxidant capacity is not always due to polyphenols, but these contributed to a great extent, and the rest were attributed to compounds, such as anthocyanins and vitamins, contained in each food.

3.5. Monomeric anthocyanins: Table 1 shows the results obtained from the quantification of anthocyanins expressing these results as mg of cyanidin-3-glucoside/100 g wb. A significant difference (p < 0.05) was found between the anthocyanin content of each fruit. The values obtained are within the ranges reported by different authors as follows: blueberry, 89-331 mg cyanidin-3-glucoside/100 g wb; strawberry, 20.7 mg cyanidin-3-glucoside/100 g wb;

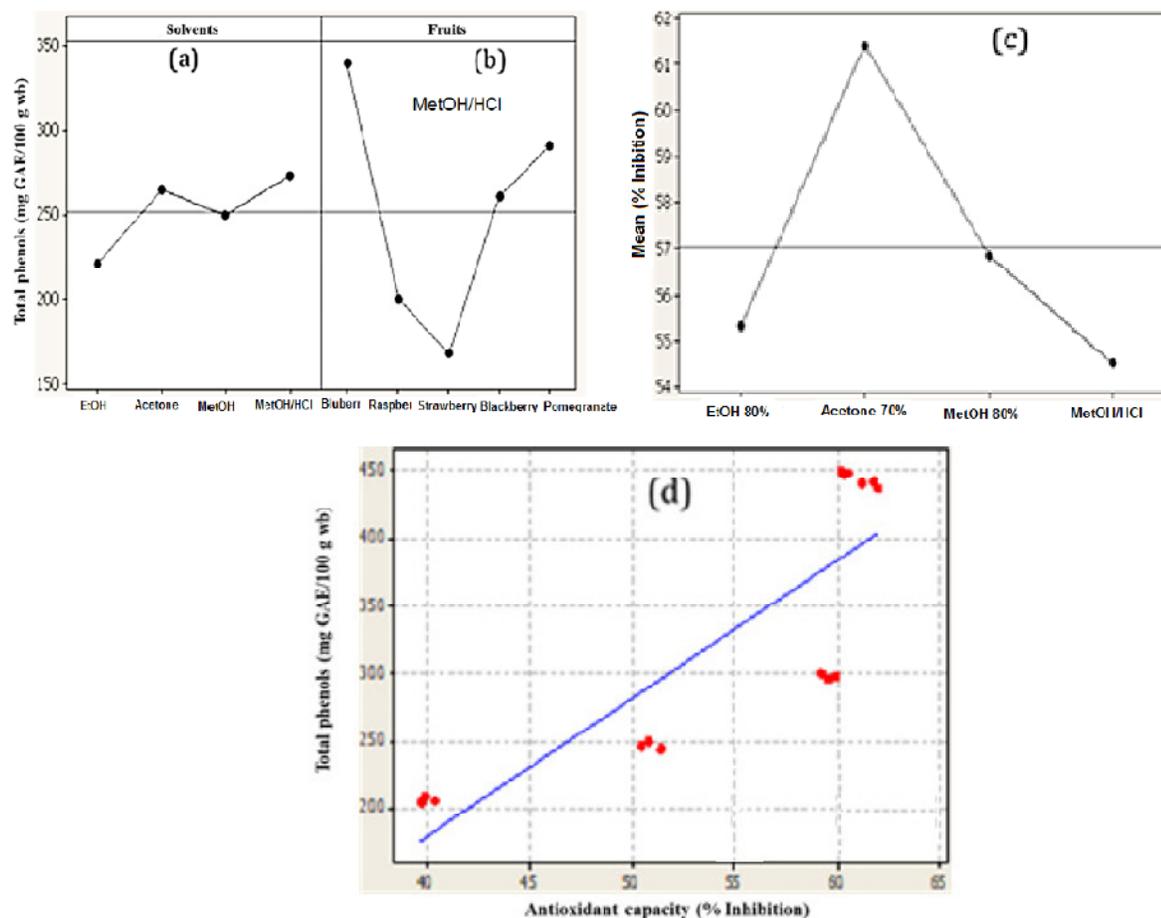


Figure 1. a) Main effects of TPC in relation to the solvent used b) TPC for each fruit (MetOH 80%:HCl 1%) c) Main effects of antioxidant activity (% inhibition) and solvent (MetOH 80%:HCl 1%) d) TPC vs antioxidant capacity dispersion graph of five fruits ($R^2=70.4\%$). Mean \pm standard deviation $n=3$, with $p > 0.05$ for the solvent and $p < 0.05$ for the fruits. BB: Blackberry, R: Raspberry, S: Strawberry, BB: Blackberry, P: Pomegranate, MeOH: Metanol 80%

blackberry, 134.6-152.2 mg cyanidin-3-glucoside/100 g wb; and pomegranate, 18.9-109.1 mg cyanidin-3-glucoside/100 g wb (Kalt, 2001; Pantelidis *et al.*, 2007; Zheng *et al.*, 2007; Turfan *et al.*, 2012). The exception to this was for raspberry, as its value was lower than the value range of 35.1-45.1 mg of cyanidin-3-glucoside/100 g wb reported by Pantelidis *et al.* (2007). The differences found are due to the fruit, variety, origin and growing conditions (Welch *et al.*, 2008; He *et al.*, 2010; Pervaiz *et al.*, 2017). The fruit with the highest content of monomeric anthocyanins was blueberry with 138.4 mg/100 g wb, and the fruit with the lowest value was strawberry with 26.4 mg/100 g wb.

3.6. Vitamin C: The values obtained for vitamin C showed a significant difference between the five fruits (Table 1); raspberry had the highest value, followed by strawberry, blueberry, blackberry and pomegranate, with values ranging between 43.42 and 11.13 mg of ascorbic

acid/100 g wb. Muñoz *et al.* (2010) reported in the food composition table a vitamin C contents of 57.0, 6.0 and 21.0 mg ascorbic acid/100 g wb for strawberry, pomegranate and blackberry, respectively, which differ from those obtained in this study. These values that report this reference were obtained from various sources from other countries, and the origin was not specified. Likewise, values for blueberry and raspberry were not reported in this reference. The data may differ due to various factors, such as the variety, state of maturation, climatic conditions and geographical region of the crop (Pérez *et al.*, 1997; Fredes *et al.*, 2014; Di Vittori *et al.*, 2018).

Benvenuti *et al.* (2004) obtained similar values for blueberry, ranging between 12.4 and 13.1 mg ascorbic acid/100 g wb. De Ancos *et al.* (2000), reported raspberry data between 22.7 and 31.09 mg/100 g wb, which are similar values to those reported by Haffner (2002), which

ranged from 15.4 to 32.0 mg/100 g wb. For strawberry, pomegranate and blackberry, the values reported by Hakala (2003), Amararatne *et al.* (2012) and Benvenuti *et al.* (2004) were in the ranges of 32.4-84.7, 6.3-11.62 and 15.5-16.3 mg ascorbic acid/100 g wb, respectively.

3.7. Analysis of ultrasonicated, pasteurized and commercial beverages: 2 shows the physicochemical parameters, phenols, antioxidant capacity, anthocyanins and vitamin C values obtained from the processed beverages compared with commercial drinks. There was a significant difference ($p < 0.05$) in the pH values, ranging from 2.71 to 3.43; these values are similar to those reported by Konić-Ristić *et al.* (2011), who reported pH values between 2.62 and 3.61 in juices of red raspberry, blackberry, blueberry and currants. For the titratable acidity, this study reported an interval between 0.74-1.97% for the same fruits; for the titratable acidity obtained from the 5 berries in the present work, values ranged from 0.41 to 0.95%. In general, there is no norm that specifies the physicochemical parameters in processed fruit drinks. According to the NOM-173-SCFI-2009 and the CODEX STAN 247 (2005), the main characteristics of the beverages made from multiple fruits or a mixture of several fruits are the range of soluble solids that have been reported between 7.5 and 16 °Bx with a low acidity index (Ocampo, 2000; Hellín *et al.*, 2003; CODEX STAN 247-2005). The analyzed beverages are within the stipulated parameters, such as the percentage of natural fruit juice used, which must be between 16 and 50% (v/v). The relationship between soluble solids and acidity in fruit juices is of interest to ensure good quality in the final product (Masithoh *et al.*, 2016; Suszek *et al.*, 2017).

All the bioactive compounds in the beverages presented a significant difference. Similar data from TPC were found to those reported by Medina, (2011) (70-238 mg GAE/100 mL). The data obtained on drinks made from similar fruits, with the exception of drink number three, had a very low content of bioactive compounds. The IC₅₀ obtained in the analyzed beverages of the present work was found between 14.9 and 58.5. The values obtained for anthocyanins ranged from 0.7 to 61 mg/100 mL. Mullen *et al.* (2007) reported an anthocyanins range of 2.8-7.5 mg of cyanidin-3-glucoside/100 mL, which were obtained from 13 commercial drinks of a similar fruit mix. The processed beverages in this work have a higher content of anthocyanins because they were made with natural fruit juice.

Regarding the content of vitamin C, the NOM-086-SSA1-1994 stipulates a value of 60 mg/100 mL in fruit beverages. Table 2 shows that all commercial beverages, except drinks number one and number four, are below the stipulated value in the standard norm; the other beverages meet this specification since most have added vitamin C to be within the established intervals. It should be noted that the processed drinks of the present work did not have

added vitamins or preservatives. In general, it is observed that for both pasteurized and ultrasonicated drinks, the highest values were obtained in the content of phenols, anthocyanins and antioxidant capacities, with the exception of the commercial beverage number five that presented the highest antioxidant capacity, which might be related to the addition of multiple vitamins and antioxidant extracts as indicated on the label and not precisely to the antioxidant capacity of the fruit itself.

Table 3 also shows that the percentage of natural fruit juice contained in commercial beverages is very low with the exception of commercial beverage number four; drinks made by ultrasonication and pasteurization contain 48% natural fruit juice, a value within the interval of 16 to 50% as reported by CODEX STAN 247 (2005).

For comparative purposes, the effect of pasteurization on the bioactive compounds content of each beverage of individual fruit was analyzed. The individual drink of each fruit was prepared with 9.6% volumetric juice to 100 ml and adjusted to 12 °Bx as the mixed drink was prepared. The results showed that the fruit drink made of blueberry had the highest content of phenols (26.7±0.7 mg GAE/100 g) and anthocyanins (6.92±0.03 mg cianidina-3-glucósido/100 g) and the fruit drink made with strawberry had the lowest content of phenols (21.07±0.5 mg GAE/100 g) and anthocyanins (1.31±0.02 mg cianidina-3-glucósido/100 g). The highest antioxidant capacity was the pomegranate drink (IC₅₀=1.85±0.06), and the lowest value was the strawberry drink (IC₅₀ = 5.77±0.45). The percentage of decrease in the content of phenols was between the values of 6% (blackberry) and 42% (pomegranate). The loss of the antioxidant capacity was so variable in the individual beverages of the five fruits, and the percentage of decrease was 3.24% for pomegranate and up to 68.7% for strawberry. The loss of anthocyanins was close to 50% in all processed beverages.

3.8. Shelf life of the ultrasonicated and pasteurized drink: The physicochemical parameters of the ultrasonicated and pasteurized beverages during storage for 21 and 56 days, respectively, showed slight variations with respect to time. The pH and density did not vary during the days of storage by either process. For the beverage prepared by the pasteurization process, the acidity increased from 0.96 ± 0.00 to 1.60 ± 0.00 g/100 mL during the storage time, while with the ultrasonication, there was no significant difference in the acidity between the initial and final storage times. The °Bx decreased from 13 (initial time) to 12 (final time) in the pasteurized beverage, and the ultrasonicated beverage did not present variation in the °Bx value.

The main characteristics of commercial drinks made with multiple fruits are the following: the °Brix interval must vary between 10 and 16, the pH at 20 °C must be greater than 2.5, and the titratable acidity expressed as % citric acid must be greater than 0.2 g/100 mL (CODEX STAN 247,

Table 2. A) Physicochemical characteristics, B) phenols, anthocyanins and vitamin C of processed and commercial beverages

A)						
Sample	*Ingredients and specifications of each juice	pH	° Brix	Titulable acidity (%)	Relative density	
Ultrasonic beverage	Cranberry, raspberry, strawberry, pomegranate and blackberry: 48% natural fruit juice	3.26±0.015 ^{c,d}	12.6±0.115 ^{b,d}	0.80±0.030 ^a	1.14	
Pasteurized beverage	Cranberry, raspberry, strawberry, pomegranate and blackberry: 48% natural fruit juice	3.29±0.025 ^d	12.8±0.289 ^{b,d}	0.95±0.030 ^e	1.05	
Commercial beverage	Concentrated apple juice: 26%. Pomegranate extract: 4%					
1	Concentrated rehydrated juices: % NE Grape, pomegranate and cranberry	2.74±0.006 ^a	14.0±0.000 ^a	0.74±0.007 ^f	1.18	
2	Concentrated "Capri sun": % NE Apple, pomegranate and cranberry	2.74±0.006 ^b	13.1±0.115 ^b	0.82±0.013 ^a	1.15	
3	Blackberry and raspberry: 48% natural fruit juice	2.71±0.006 ^{a,b}	11.3±0.289 ^c	0.41±0.007 ^c	1.16	
4	Concentrated cranberry and pomegranate: % NE	3.43±0.006 ^e	13.9±0.115 ^a	0.66±0.007 ^d	1.17	
5	Grape, blueberry, apple, pear, cherry, pomegranate, antioxidant seeds, green and white tea, and aloe	3.16±0.010 ^f	11.6±0.115 ^c	0.51±0.007 ^b	1.04	
6		3.22±0.025 ^c	9.2±0.289 ^e	0.49±0.007 ^b	1.03	
B)						
	Phenols mg GAE/100 mL	Antioxidant capacity (IC ₅₀)	Anthocyanins mg/100 mL	Vitamin C mg/100 mL		
No treatment	312.32±6.48	23.9±0.17	57.934±0.62	75.32±6.66		
Ultrasonic beverage	329.356±7.29 ^h	21.7±0.08 ^b	58.3±0.71^h	65.09±6.6 ^e		
Pasteurized beverage	292.7±7.34 ^g	25.8±0.11 ^b	56.59±0.87^g	61.29±6.6 ^{a,b,c,d}		
Commercial beverage						
1	84.3±1.4 ^a	34.5±0.36 ^a	2.80±0.22 ^a	54.85±11 ^a		
2	57.2±0.9 ^b	44.0±0.54 ^c	6.31±0.39 ^b	182.92±12 ^f		
3	9.5±0.7 ^c	58.5±0.66 ^d	0.70±0.17 ^c	62.77±0 ^b		
4	151.9±2.1 ^d	34.0±0.23 ^a	43.58±0.52 ^d	27.66±11 ^a		
5	260.4±3.0 ^e	14.9±0.03 ^e	3.50±0.34 ^e	77.79±13 ^c		

Mean ± standard deviation (n=3). Different lowercase letters in each column indicate significant differences between edible berries (P< 0,05), according to Tukey's analysis. *Percentage of fruit and ingredients reported in the nutritional information table on the label of each beverage. NE: Not specified

Table 3. Average life of each bioactive analyzed.

Bioactive analyzed	Temperature	t ½ days pasteurized beverage	t ½ days ultrasonic beverage
Total phenols	4 °C	165	365
Antioxidant capacity	4 °C	51	257
Anthocyanins	4 °C	28	71
Vitamin C	4 °C	75	77

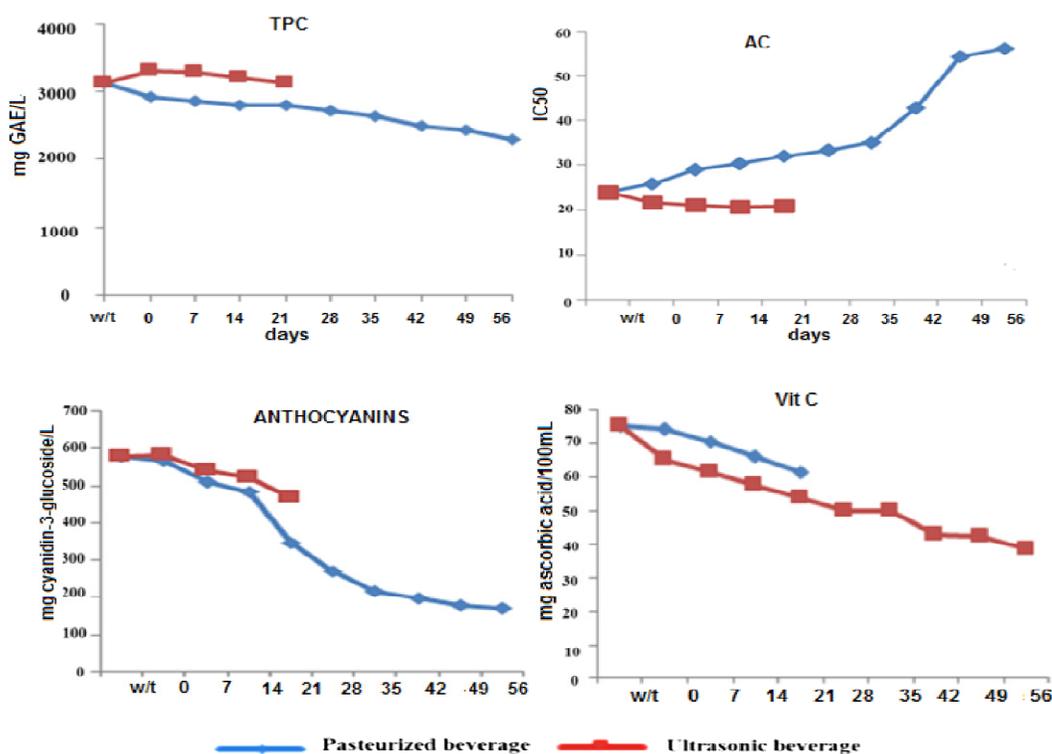


Figure 2. Kinetics of the bioactive compounds of the pasteurized and ultrasonic beverage. TPC:total phenolics compounds, AC: antioxidant capacity.

2005; Fernández et al., 2006). The results obtained for the prepared drinks are in the range of these values; the °Bx ranged from 13-12.07 with a slight decrease with storage time. The values were similar to those obtained by Vázquez et al. (2010), who analyzed 15 mixtures of beverages made with berries. For titratable acidity, Konić-Ristić et al. (2011) reported an interval between 0.74 and 1.97 for drinks made with berries. No reports were found for the relative density, and the results obtained in the present work ranged from 0.96 to 1.60.

3.9. Bioactive compounds in ultrasonicated and pasteurized beverages: Fig. 2 shows a decrease in the values of TPC, anthocyanins and vitamin C for both pasteurized and ultrasonicated drinks.

During storage, 99% of the TPC were preserved in the ultrasonicated drink, while 73.1% of the TPC were preserved in the pasteurized drink; the antioxidant capacity was 87% and preserved in the ultrasonicated beverage, and the antioxidant capacity in pasteurized drink increased more than twice the initial value during storage. The ultrasonicated beverage conserved 81% of the anthocyanins, while the pasteurized drink retained only 29.1%; the ultrasonicated drink conserved 87% of the vitamin C, and the pasteurized drink preserved only 51%. The initial value of phenols was similar to that obtained by

Mitic et al. (2011), which was a value that ranged from 2698.63 to 2813.05 mg GAE/L. The same authors reported anthocyanin content that varied between 148 and 920 mg of cyanidin-3-glucoside/L in nine commercial juices, and these values were similar to those of the present work.

The total phenols, anthocyanins and antioxidant capacities were not as affected by the process of ultrasonication since this treatment favors the extraction of these substances and has been reported to have a minimal detrimental effect in sensory parameters, such as taste, color and smell, in juices and nectars made with these fruits (Tiwari et al., 2009; Alighourchi et al., 2014). The pasteurization temperature could help to release antioxidant compounds or produce changes or isomerization processes that form compounds with greater antioxidant activity.

Regarding the content of vitamin C, the Mexican standard NOM-086-SSA1-1994 stipulates a value of 60 mg/100 g for nectar, and this value was met in the processed beverages during the first seven days of storage. Both processed drinks suffered a decrease in vitamin C because it is very susceptible to degradation due to high temperatures and other factors, such as oxidation and interaction with metals (Ajibola et al., 2009).

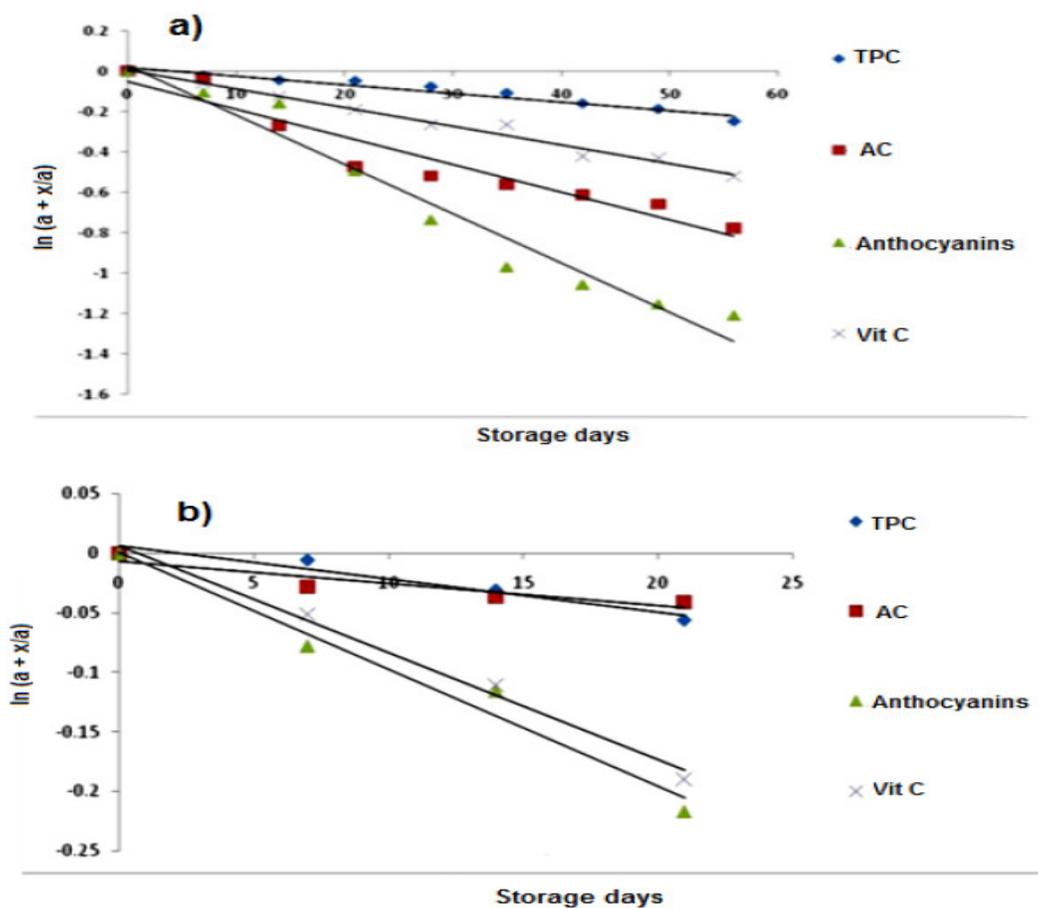


Figure 3.a) Kinetics of degradation of total phenols, antioxidant capacity and anthocyanins: a) pasteurized beverage, for 56 days in refrigeration (4 °C). **b)** ultrasonic drink, for 21 days in refrigeration (4 °C)

3.10. Kinetics of bioactive compounds in processed beverages. Fig. 3a and 3b show the first-order kinetic models that were applied to each of the data obtained from the bioactive compounds. The decrease during the storage days was adjusted to this model since a linear behavior was obtained with an $R^2 \geq 90\%$ in total phenols, antioxidant capacity, anthocyanins and vitamin C. Table 3 shows the average life of each bioactive compound analyzed. The compounds most susceptible to degradation in both processed beverages were anthocyanins followed by vitamin C; anthocyanins are unstable and produce a dark brown color during processing and storage (Turfan et al., 2012; Moura et al., 2017).

The most stable compounds were the total phenols; as mentioned by Barba et al. (2012), heat treatment does not have a significant effect on phenols because the enzymes that degrade phenols are inactivated after pasteurization or heat treatment. The values obtained correspond to the

days it takes to degrade 50% of the total content of the bioactive compounds.

3.11. HPLC analysis: Fig. 4 shows the chromatogram obtained by HPLC for the standards used. Polyphenols were identified at 280 nm, and the anthocyanins were identified at 520 nm. The retention times obtained for the phenols were as follows: ferulic acid, 36.97 minutes; catechin, 14.50 minutes; epicatechin, 30.05 minutes; quercetin, 48.87 minutes; resveratrol, 38.86 minutes; and rutin, 46.37 minutes. The retention times obtained for anthocyanins were as follows: cyanidin-3-glucoside, 32.67 minutes; chlorinated cyanidin, 49.88 minutes; delphinidin, 41.50 minutes; pelargonin, 26.63 minutes; and chlorinated pelargonidin, 61.05 minutes.

Table 4 shows that catechin was identified in the individual juice of each fruit, with the exception of blackberry, and in the pasteurized and ultrasonicated beverages; the fruit with the highest contents of catechin

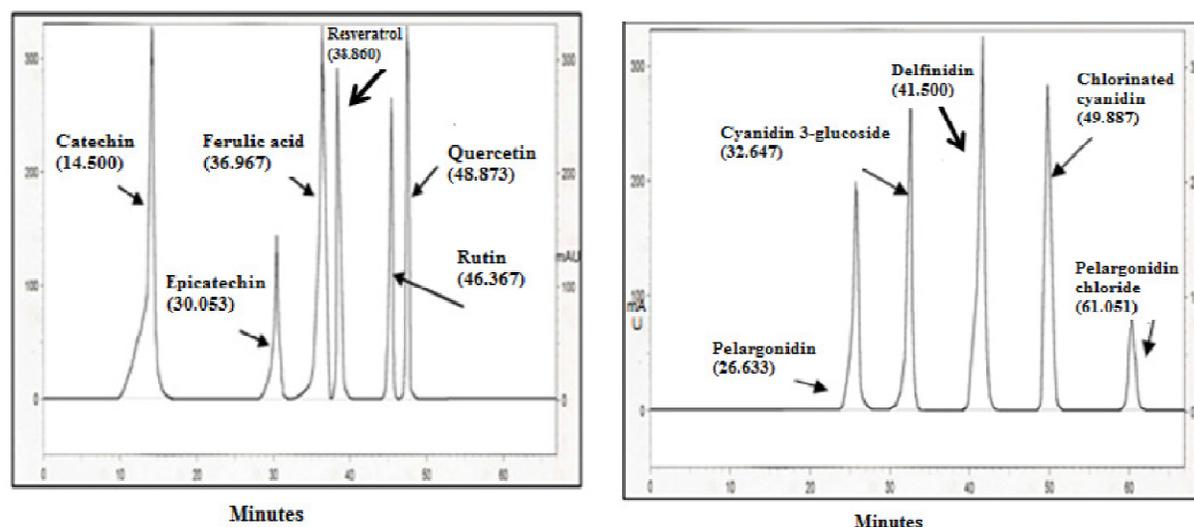


Figure 4. Standards of bioactive compounds identified by HPLC a) phenolic at 280 nm and b) anthocyanins at 520 nm

Table 4. HPLC quantification of phenolic compounds and anthocyanins ND: Not Detected, PB: Pasteurized beverage, UB: Ultrasonic beverage

SAMPLE	Catechin mg/100 g	Epicatechin mg/100 g	Ferulic acid mg/100 g	Resveratrol mg/100 g	Rutin mg/100 g	Pelargonin mg/kg	cyanidin-3- glucoside mg/100 g	Delphinidin mg/100 g
Blueberry	1.24	ND	ND	0.589	39.27	0.801	95.301	29.88
Raspberry	3.31	163.53	6.18	ND	37.14	ND	35.04	ND
Strawberry	2.26	86.10	37.5	0.760	ND	ND	ND	ND
Pomegranate	0.47	31.08	ND	1.570	ND	1.45	8.98	ND
Blackberry	ND	ND	3.21	ND	ND	ND	162.16	8.93
PB (mg/mL)	7.29	2.654	0.014	0.642	22.77	ND	0.358	0.28
UB (mg/mL)	8.53	8.99	0.021	1.312	28.95	ND	0.580	3.53

(3.31 mg/100 g) and epicatechin (163,536 mg/100 g) was raspberry. Strawberry was the fruit with the highest content of ferulic acid (37.5 mg/100 g), and pomegranate had the highest resveratrol content (1.57 mg/100 g); routine was identified only in blueberry and raspberry. All the bioactive compounds decreased both in the pasteurized drink and in the ultrasonicated drink compared with fresh fruit; this decrease is attributed to the preservation process but a dilution was used in the processed beverages (52% water).

The anthocyanins, pelargonin, cyanidin-3-glucoside and delphinidin were identified in the samples of the five fruits and in the pasteurized and ultrasonicated beverages. Cyanidin was found in all the samples analyzed except for the strawberry. Blueberry showed the highest content of delphinidin (29.88 mg/100 g).

Berries and their products are a good source of bioactive compounds and contribute significantly to the recommended intake of these compounds (Konić-Ristić *et al.*, 2011).

3.12. Microbiological stability: The inactivation of molds and yeasts in the processed beverages was achieved with pasteurization and ultrasonication processes. Thermal processing by pasteurization is the most effective method for the inactivation of microorganisms; however, the process of ultrasonication has also been shown in several studies to be effective in microbial inactivation due to the weakening of the bacterial membrane and in destruction of molds and yeasts by the effect of cavitation (Sala *et al.*, 1995; Villamiel & De Jong, 2000; Patist & Bates, 2008). The microbiological count was

negative in both treatments at 21 and 56 days; this count was determined until 90 days for the pasteurized juice and 30 days for the ultrasonicated juice to determine the moment of the growth of colonies. The result of the count was very low due to the efficient processing treatments, the acceptable sanitary state of the raw material, and the good hygienic conditions in the processing of the juices. In contrast, the pH of the products were close to 3.5, which positively influences low bacterial development (Parish *et al.*, 1990; Torres *et al.*, 2008).

3.13. Enzymatic activity

3.13.1. Pectin methylesterase (PME): The initial activity value of PME of the untreated beverage was 110.25%, while in the ultrasonicated and pasteurized beverages, no enzymatic activity was detected during storage (21 and 56 days, respectively). Awad (1980) reported that the PME is thermolabile at temperatures close to 74 °C, a temperature similar to that used in the pasteurized drink. The ultrasonication process also inhibited enzymatic activity. Terefe *et al.* (2009) reported the inactivation of the PME and the reduction in the particle size in tomato juices treated by ultrasonication.

3.13.2. Polyphenol oxidase (PPO): The initial value of the PPO in the juice without treatment was 299 AU/mg protein, and after the processing treatment, the PPO values were 16.13 AU/mg protein in the pasteurized drink and 27.52 AU/mg protein for the ultrasonicated drink, with inhibition percentages of 83.87% and 72.46%, respectively. Giner *et al.* (2001) reported that polyphenol oxidase has a maximum catalytic activity at a pH between 5 and 8, and the pH of processed beverages ranged from 3.36 to 3.56. In addition, the authors mentioned that at pH levels of 3.15, 3.6 and 4, less suitable conditions are generated for the enzymatic activity of polyphenol oxidase.

3.13.3. Peroxidase. The inactivation of peroxidase was achieved in beverages processed by pasteurization and ultrasonication. Previous studies concluded that treatment by emerging technologies, such as ultrasonication, reduced peroxidase (POD) in tomato juice by 97% and inhibited POD by 45-100% in orange juice and grape juice (Aguiló-Aguayo *et al.*, 2008; Elez-Martínez *et al.*, 2006; Marsellés-Fontanet & Martín-Belloso, 2007).

3.14. Sensory evaluation: In the acceptance grade test, the ultrasonicated beverage obtained 86% acceptance, the pasteurized beverage obtained with 68% acceptance, and the commercial drink obtained 66% acceptance and was the least accepted beverage. For the evaluation of the degree of preference, 60% of the panelists preferred the ultrasonicated drink, 52% preferred the commercial drink and 56% preferred the pasteurized drink. In the evaluation of the level of taste, the ultrasonicated drink had the highest percentage (79.8%), followed by the commercial drink (53.4%) and the pasteurized beverage (48.73%). In

general, the ultrasonicated beverage obtained the highest percentages in the three tests since the panelists mentioned that it was the drink with the best color and flavor.

CONCLUSIONS

Blueberry presented the highest contents of phenols and anthocyanins, while the fruit with the highest antioxidant capacity was blackberry; strawberry presented the lowest values of phenols, antioxidant activity and anthocyanins.

The ultrasonication process was the best method to preserve the highest value of TPC, anthocyanins and vitamin C. Catechin was identified in the juice of each fruit, with the exception of blackberry, as well as in pasteurized and ultrasonicated beverages. Epicatechin was identified in all samples except in blueberry and blackberry juices. Strawberry was the fruit with the highest content of ferulic acid, and pomegranate presented the highest resveratrol content; rutin was identified only in blueberry and raspberry. All the compounds decreased in both pasteurized and ultrasonicated drinks. Of the anthocyanins, only pelargonin, cyanidin-3-glucoside and delphinidin were identified in the samples of the five berries and in the pasteurized and ultrasonicated beverages. The mixed drink of the berries presented bioactive compounds, which signifies that it has the properties of a functional drink.

The processes of pasteurization and ultrasonication were effective both in microbial inactivation and in the inactivation of enzymes that deteriorate the sensory and nutritional quality of mixed drinks composed of berries. In the analysis of the sensory evaluation, the ultrasonicated beverages, followed by the commercial drinks, had the highest percentages in acceptance, preference and liking. In general, it is important to consider a conservation treatment different from the thermal treatment to guarantee a lower loss of bioactive compounds and favor the sensory attributes of this type of product.

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