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Effect of ultrasound on microbiological load and antioxidant properties of blackberry juice

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Abstract

The objective of this study was to evaluate the effect of ultrasound (60 and 80% amplitude, 15 and 25 min) of blackberry juice on physicochemical (pH and total soluble solids), microbiological analysis (total plate count and *Enterobacteria*), and *in vitro* intestinal bioaccessibility of polyphenols and antioxidant activity (ABTS, DPPH). Ultrasound treatment caused a decrease of microbial count of blackberry juice and remained its physicochemical properties without change. The blackberry juice had a high antioxidant activity, however, after of the *in vitro* digestion process only 28% of total polyphenols were considered as intestinally bioaccessible, with the contribution of 30 to 60% of antioxidant activity in the bioaccesible fraction. The treatment at 80% amplitude for 15 min was the better treatment due it had an increase of bioaccessibility of polyphenols and antioxidant activity measured by ABTS. Our results showed that ultrasound treatment can preserve the blackberry juice without impairing to its antioxidant characteristics.

Practical applications

Blackberries are mostly consumed fresh but are also commercialized as individually quick frozen packs, bulk, frozen, seedless or seeded puree, freeze-dried, juice, or concentrate. During fruit juices processing, thermal treatment and other conditions such as oxidation, light exposure, sugar addition, changes in pH, and temperature readily reduce the content of antioxidant compounds and its nutritional properties. Ultrasound is a non-thermal processing technology with potential to replace the traditional thermal pasteurization, achieve microbial safety in fruit juices and remained its antioxidant properties of the blackberry juice.

1 | INTRODUCTION

Blackberries are an important crop in the United States and México, where they are commercially produced over wide geographic range (Reyes-Carmona, Yousef, Martínez-Peniche, & Lila, 2005). It is a highly valued fruit due to its high content of anthocyanins and ellagitannins, and other phenolic compounds of high antioxidant capacity (Cho, Howard, Prior, & Clark, 2004). Blackberries are mostly consumed fresh but are also commercialized as individually quick frozen packs, bulk frozen, seedless or seeded puree, freeze-dried, juice or concentrate. During fruit juices processing, thermal treatment and other conditions such as oxidation, light exposure, sugar addition, changes in pH, and temperature readily reduce the content of antioxidant compounds and its nutritional properties (Sadilova, Stintzing, Kammerer, & Carle, 2009).

Alternative processing methods that preserve nutritional characteristics and extend shelf life may prevent this loss. Several emerging technologies have been used for blackberries processing such as high-pressure, pulsed electric fields, ozonation, UV-B irradiance, ultrasound, and others (Barba et al., 2012; Basiouny, 1998; Patras, Brunton, Da Pieve, & Butler, 2009). Ultrasound is a non-thermal processing technology with potential to replace the traditional thermal pasteurization and achieve microbial safety in fruit juices (Cheng, Soh, Liew, & Teh, 2007; Raviyan, Zhang, & Feng, 2005; Valero et al., 2007; Zafra-Rojas et al., 2013). When the high-power ultrasound propagates in a liquid, cavitation bubbles are generated due to pressure changes. These micro-bubbles collapse violently in the succeeding compression cycles

Abbreviations: ABTS, 2,2′azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt; DPPH, 1,1-diphenyl-2-picrylhydrazyl; TC, total plate count; TPC, total phenolic content; TSS, total soluble solids.

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of a propagated sonic wave (Tiwari, Muthukumarappan, O'Donnell, & Cullen, 2008), reducing the microbial load. This reduction might be attributed to the combined physical and chemical mechanisms that occur during cavitation leading to thinning of microbial cell membranes as well as to the restricted mild heating that occurs during sonication (Oyane et al., 2009). Studies have reported the effect of ultrasound on the enhanced extractability of antioxidant compounds in fruit juices (Bhat, Kamaruddin, Min-Tze, & Karim, 2011) and their absorption at intestinal level (Anese, Mirolo, Beraldo, & Lippe, 2013). Antioxidant compounds in blackberry are an attractive target, so it is relevant to estimate their extent of release from the food matrix and intestinal bioaccessibility after the ultrasound treatment, which may be an indicator of the benefits of this technology. Then, the objective of this study was to evaluate the effect of ultrasound on pH, soluble solids, and microbiological parameters, and after of the *in vitro* intestinal bioaccessibility of polyphenols and antioxidant activity of blackberry juice.

2 | MATERIALS AND METHODS

2.1 | Samples and treatments

Blackberries (*Rubus fruticosus)* were obtained locally of Atotonilco, Hidalgo, México, during winter 2012, and only fruits without external injuries were selected. The fruits were introduced in a commercial extractor (Hamilton Beach HealthSmart model 67900-MX) to obtain the blackberry juice. Ultrasound (ultrasonic processor VCX-1500, Sonics & Materials, Inc. Newtown, CT, USA) treatment was set up at 1500 W and constant frequency of 20 kHz; two amplitudes and times were evaluated: 60 and 80% for 15 and 25 min with pulse durations of 2 s on and 4 s off. Untreated juice was used as control. After ultrasound treatment aliquots of the samples were lyophilized and stored at −86 °C for further analysis.

2.2 | pH and total soluble solids

The pH was measured using a potentiometer (pH 210, Hanna instruments, Microprocessor pH-meter, USA) and total soluble solids (TSS) (expressed as °Brix) were measured using a refractometer (Brix/ATC FG-113, Hangzhou Chincan Trading Co., Ltd., China).

2.3 | Microbiological analysis

Serial dilutions of blackberry juice were performed in peptone water solution for microbial count. Total plate count (TC) was determined in plate count agar incubated at 30 °C for 48 hr. *Enterobacteria* were determined in violet red bile glucose (VRBG) incubated at 37 °C for 24 hr. Results were expressed as log colony forming units per milliliter (log CFU/ml) of juice according to Cruz-Cansino et al. (2007).

2.4 | Simulation of *in vitro* gastrointestinal digestion

It was estimated using an *in vitro* digestion model followed by dialysis (Trinidad, Wolever, & Thompson, 1996), based on the method described by Miller, Schricker, Rasmussen, and Van-Campen (1981) with some modifications. In brief, 500 mg of lyophilized sample were homogenized in 20 ml of water, and adjusted to pH 2.0 with 6 mol/L of HCl. The samples were successively incubated in a shaking water bath with 120 μl of pepsin solution (40 mg pepsin—Sigma Aldrich P-7000—per ml 0.1 mol/L HCl) at 37 °C for 2 hr. After incubation, 1.5 ml pancreatin-bile solution (5 mg pancreatin enzyme—P-1750 Sigma Aldrich plus 25 mg porcine bile-B-8631 Sigma Aldrich—per ml 0.1 mol/L $NaHCO_3$) was added. Digestion products were placed in a dialysis bag (12,000–14,000 molecular weight cut-off; Sigma Aldrich) and dialyzed in 250 ml of sodium bicarbonate solution (pH 7.5) for 12 hr. Dialysis aliquots were removed and dialyzed antioxidant compounds and antioxidant activity were determined. The relative value of polyphenols (or antioxidant capacity) in the dialyzed fraction was related to the sample without digestion and it was used as an indicator of bioaccessibility in the small intestine.

2.5 | Extraction and quantification of total phenolic content

Lyophilized sample was extracted by shaking at room temperature with methanol-water (50:50 vol/vol, 50 ml/g sample, 60 min, room temperature; constant shaking) and acetone-water (70:30 vol/vol, 50 ml/g sample, 60 min, room temperature; constant shaking). After centrifugation (15 min, 25 °C, 3000 *g*) supernatants were combined. Both methanol and acetone extracts were combined to reach a final volume of 25 ml. The extracts obtained from the lyophilized and dialyzed samples after the *in vitro* digestion were used for the determination of phenolic content and antioxidant activity.

Total phenolic content was determined following the Folin-Ciocalteau procedure (Montreau, 1972). Briefly, 100 μl of extract was mixed with 100 μl of Folin–Ciocalteau reagent. After 3 min, 2 ml of sodium carbonate solution (75 g/L) were added and mixed. Additional distilled water was added (2.8 ml) and mixed thoroughly by inverting the tubes several times. After 1 h, the absorbance at 750 nm was measured using a microplate reader (Power Wave XS UV-Biotek, software KC Junior, USA). The results were expressed as mg gallic acid equivalents per 100 g of dried basis (mg GAE/100 g db).

2.6 | Antioxidant capacity

2.6.1 | Antiradical capacity (ABTS)

Antiradical capacity was measured according to Kuskoski, Asuero, Troncoso, Mancini-Filho, and Fett (2005). Briefly, the radical cation ($ABTS^{\bullet+}$) was produced by reacting 7 mmol/L ABTS (2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt) stock solution with 2.45 mmol/L potassium persulfate under dark conditions and room temperature for 16 hr before use. The ABTS solution was diluted with deionized water to an absorbance of 0.70 ± 0.10 at 754 nm. After the addition of 20 μl of extract to 980 μl of diluted ABTS solution, absorbance readings (754 nm) were taken after incubation for 7 min at room temperature in a microplate reader (Power Wave XS UV-Biotek, software KC Junior, USA). The antioxidant capacity was expressed as μmol Trolox equivalents per 100 g of dried basis (μmol TE/100 g db).

2.6.2 | Free radical scavenging activity

Antiradical activity was measured with DPPH (1,1-diphenyl-2-picrylhydrazyl) radical as described by Morales and Jimenez-Perez (2001). An ethanolic solution (7.4 mg/100 ml) of the stable DPPH radical was prepared. Then 100 μl of extract were taken into vials and 500 μl of DPPH solution were added, and the mixture was left to stand 1 hr at room temperature. The solution was stirred and centrifuged at 3,000 rpm during 10 min to finally measure the absorbance at 520 nm using a microplate reader. Antiradical activity was expressed as μmol Trolox equivalents per 100 g of dried basis (μmol TE/100 g db).

2.7 | Statistical analysis

Results were expressed by mean ± standard deviation (SD) ($n = 3$). Data were analyzed performing a one-way analysis of variance (ANOVA) and differences among means were determined using a Tukey test with a level of significance of $p < 0.05$. The statistical package SPSS System for WIN version 15 was used.

3 | RESULTS AND DISCUSSION

3.1 | Effect of ultrasound on physicochemical parameters and microbial count of blackberry juice

Blackberry is a fruit characterized by a low pH (between 3.27 and 3.83) and high acidity (about 1.70%), making it unsuitable for microorganisms, and in addition with an attractive red color which is its main sensory attribute. TSS and pH play an important role in fruit quality and consumer acceptability (Aday, Temizkan Büyükcan, & Caner, 2013). Therefore, it is important to keep these characteristics during processing of fruit. Table 1 shows the effect of ultrasound treatment on the pH, TSS, and microbial count (total plate count and *Enterobacteria*) of the blackberry juice. Ultrasound did not have significant effect on pH and soluble solids, while microbiological parameters were reduced significantly. Total plate count (TC) was reduced significantly by ultrasound treatment in all the treatments with a higher reduction in 80% 25 min (2.89 log CFU/ml) in comparison with the sample without treatment, while *Enterobacteria* remained without changes. The results showed that high amplitude and time could disrupted the bacteria cell wall as it was showed in other studies (Bhat, 2011; Chouliara, Georgogianni, Kanellopoulou, & Kontominas, 2010; Zafra-Rojas et al., 2013). Cell disruption is due to several factors such as the combined physical and chemical mechanisms that occur during cavitation, the formation of free radicals and hydrogen peroxide, and this effect may affect the microbial cell membranes, and the restricted mild heating that occurs during sonication (Bhat, 2011). The ultrasound reduced microbial count until established recommendations for the sanitary regulations (2 log UFC/ml to TC and *Enterobacteria*) for pasteurized juices (CEC, Brussels, 2005; NOM-130-SSA1–1995, 1995).

3.2 | Effect of ultrasound on *in vitro* intestinal bioaccessibility of antioxidant properties on blackberry juice

Figure 1 shows the effect of ultrasound treatment on total phenolic content (TPC) and antioxidant activity in the blackberry juice and dialyzed fraction. We found in this study a high phenolic content (1990.99 ± 131.01 mg GAE/100 g db) in blackberry juice according with other studies of this fruit (Cho et al., 2004), and, in comparison with other fruits (oranges, banana, melon, and apple) (Bouayed, Hoffmann, & Bohn, 2011; Saura-Calixto & Goñi, 2006). After the *in vitro* digestion process only 28% (560.53 ± 26.51 mg GAE/100 g db) of phenolic compounds were quantified in the dialyzed fraction (Figure 1a), suggesting that an incomplete release of these compounds occurred during digestion. According with different authors, the digestion process affects the food matrix (solubility, water availability); polyphenol structure (molecular weight, glycosylation and esterification level) or gastrointestinal conditions (pH, intestinal transit, biliary excretion or intestinal fermentation) (Saura-Calixto, Serrano, & Goñi, 2007; Scalbert, Morand, Manach, & Rémésy, 2002), among others with a strong influence in the release of polyphenol compounds with a high availability to be absorbed and contributing with health benefits.

The ultrasound treatment in blackberry juices did not affect the TPC due they showed values (between 1920 and 2159 mg GAE/100 g db), similar to the control. However, the ultrasound caused the increase of polyphenols in the dialyzed fraction of the samples, until 15% in the treatment at 80% amplitude 15 min (645.39 ± 26.74 mg GAE/100 g db) in comparison with the control. This behavior agrees with other ultrasound studies (Bouayed et al., 2011; Rodríguez-Roque, Rojas-

TABLE 1 Effect of ultrasound on pH, soluble solids, and microbial load of blackberry juice*

			Microbial load	
Treatment**	pH	TSS (°Brix)	TC (log CFU/ml)	*Enterobacteria* (log CFU/ml)
Control	3.21 ± 0.005[a]	10.93 ± 0.11[a]	3.89 ± 0.01[d]	2.11 ± 0.16[a]
60% 15 min	3.21 ± 0.015[a]	11.26 ± 0.11[a]	3.38 ± 0.05[c]	1.91 ± 0.07[a]
60% 25 min	3.20 ± 0.000[a]	11.20 ± 0.20[a]	2.13 ± 0.23[b]	2.09 ± 0.18[a]
80% 15 min	3.20 ± 0.005[a]	11.13 ± 0.11[a]	2.14 ± 0.33[b]	1.89 ± 0.11[a]
80% 25 min	3.20 ± 0.011[a]	11.20 ± 0.00[a]	1.00 ± 0.57[a]	1.99 ± 0.09[a]

*Values are mean ± standard deviation, $n = 3$.
**Conditions of treatment by ultrasound: amplitude (%), time (min).
Different letters (a, b, c, d) indicate a significant difference ($p < 0.05$) between treatments.

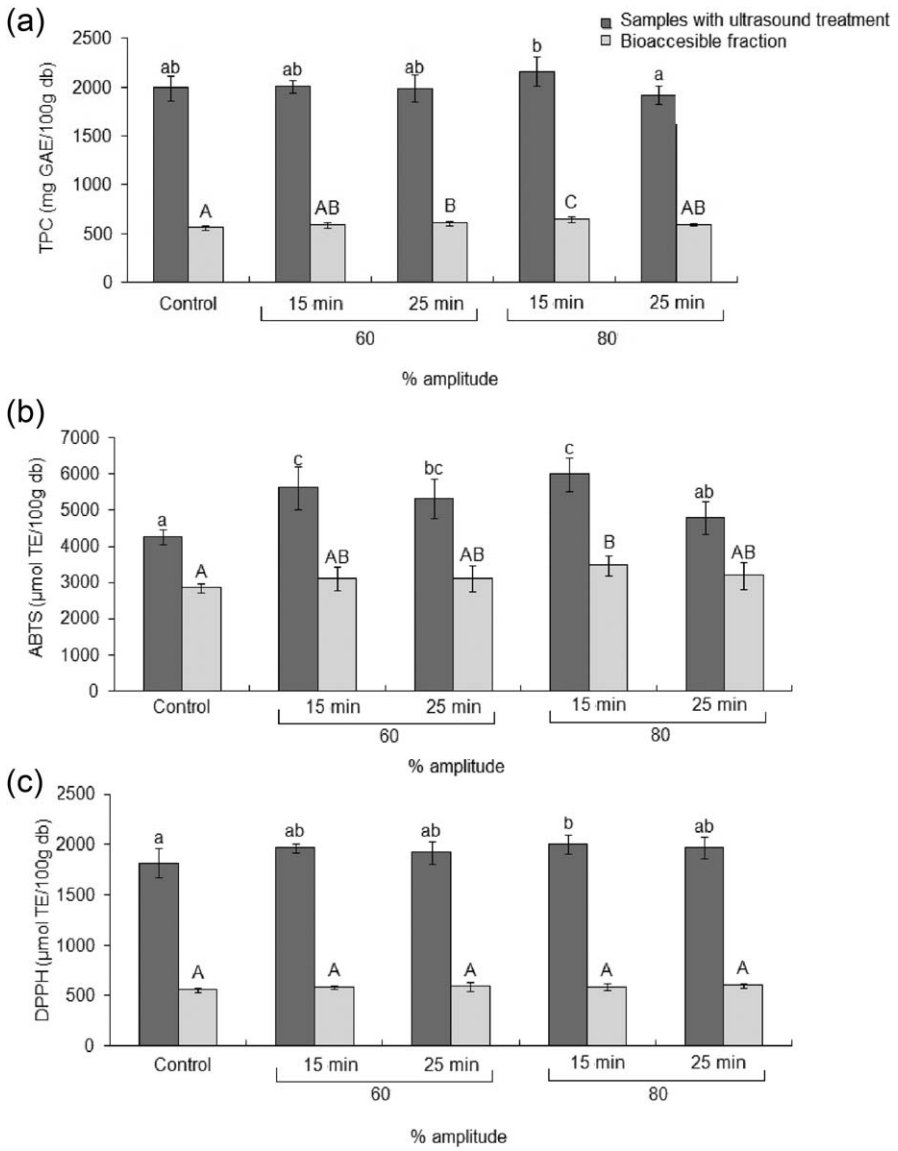

FIGURE 1 Effect of ultrasound on antioxidant activity and its *in vitro* bioaccessibility. (a) Total phenolic compounds (TPC) mg gallic acid equivalents per 100 g of dried basis (mg GAE/100 g db), (b) ABTS, (c) DPPH antioxidant activity µmol Trolox equivalents per 100 g of dried basis (µmol TE/100 g db) in blackberry juice. Values are mean ± standard deviation, $n = 3$. Different letters (a, b, c) indicate significant differences ($p < 0.05$) between treatments of the original sample. Different letters (A, B, C) indicate significant differences ($p < 0.05$) between treatments

Graü, & Elez-Martínez, 2013; Zafra-Rojas et al., 2013). The ultrasound treatment increased the release of phenolic compounds and it could contribute with the increase of its antioxidant activity.

In the blackberry juice control the antioxidant activity measured by ABTS was of 4271.60 ± 197 µmol TE/100 g db (Figure 1b), while the ultrasonicated samples had a higher antioxidant activity. The control sample remained 66% of the antioxidant activity (2847.17 ± 126.17 µmol TE/100 g db) after of the digestion process. This activity measured after digestion process was higher with the ultrasound treatment, at 80% of amplitude by 15 min, causing an increase of 21% (3470.7 ± 289.89 µmol TE/100 g db). Presumably due to the concentration of polyphenols found in this fraction, in agreement with other studies (Bouayed et al., 2011).

The antioxidant activity measured by DPPH had the same behavior than ABTS with the ultrasound treatment, however, the ultrasound no cause changes in the antioxidant activity measured by DPPH after the digestion process (Figure 1c). Although the working mechanism of the ABTS method for the evaluation of the antioxidant activity is the same as that of the DPPH, the evaluated results were no similar. According with different studies (Iqbal, Younas, Chan, Sarfraz, & Uddin, 2012; Teow et al., 2007) the difference in the response of both methodologies could be due to the selectivity of antioxidants of each methodology, the ABTS method is more reliable than the DPPH method due to the solubility of the ABTS reagent in both aqueous and organics solvents and rapid reaction with lipophilic as well as hydrophilic antioxidant species as compared to DPPH, in addition, color with

samples that contain anthocyanins, could be an interference of the DPPH assay that leads to under-estimation of antioxidant activity.

The higher amount of compounds presumably absorbed in the gastrointestinal tract from samples treated with ultrasound may be partially explained by the disruption of biological cell walls attributable to the cavitation produced by ultrasound, which facilitated the release of antioxidant molecules (Gawlik-Dziki, Dziki, Baraniak, & Lin, 2009; Mason, Paniwnyk, & Lorimer, 1996).

4 | CONCLUSIONS

Blackberry juice had a high content of polyphenols and ultrasound treatment of blackberry juice increased the release of these compounds. Our results confirmed that ultrasound treatment may enhance the antioxidant properties of polyphenol compounds and could contribute their absorption at intestinal level without compromising physicochemical and microbiological quality.

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