Vol. 17, No. 1 (2018) 13-28
Revista Mexicana de Ingeniería Química

BIOFUNCTIONALITY, COLORIMETRIC COEFFICIENTS AND MICROBIOLOGICAL STABILITY OF BLACKBERRY (*Rubus fructicosus* var. Himalaya) JUICE UNDER MICROWAVE/ULTRASOUND PROCESSING

BIOFUNCIONALIDAD, COEFICIENTES COLORIMÉTRICOS Y ESTABILIDAD MICROBIOLÓGICA DEL JUGO DE ZARZAMORA (*Rubus fructicosus* var. Himalaya) PROCESADO POR MICROONDAS/ULTRASONIDO

B. Pérez-Grijalva[1], J. C. García-Zebadúa[1], V. M. Ruíz-Pérez[1], D. I. Téllez-Medina[1], S. García-Pinilla[1], R.I. Guzmán-Gerónimo[2], R. Mora-Escobedo[1]*
[1]*Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Unidad Profesional "Adolfo López Mateos", Campus Zacatenco, Av. Wilfrido Massieu s/n, C.P. 0773. Ciudad de México, México.*
[2]*Instituto de Ciencias Básicas, Universidad Veracruzana. Col. Industrial Ánimas, C.P. 91190, Xalapa, Veracruz, México.*

Received May 13, 2017; Accepted June 24, 2017

Abstract

The bio-functionality of blackberry juice has acquired great relevance in the food industry, since it reduces the risk of chronic-degenerative diseases due to its high content of antioxidant compounds such as polyphenols/anthocyanins. However, conventional thermal processing decreases the content of these compounds. Hence, alternative technologies -as microwaves and ultrasound- are currently in research. The objective was to determine the effect of thermal and microwave/ultrasound processing on the physicochemical properties, polyphenols and anthocyanins, antioxidant capacity and microbiological stability of blackberry juice. Results showed that pH and °Brix increased in microwave/ultrasound processed juice (MUJ) compared to conventionally pasteurized juice (FPJ and SPJ) and raw juice (CJ). MUJ showed good microbiological stability after 30 days of storage at 4 °C as well as conventionally processed juices (FPJ and SPJ); nevertheless, in MUJ increased color parameters (L* (a* / b*), ΔE*, H*, C*), content of polyphenols and anthocyanins (2665.97 mg GAE L^{-1} and 427.49 mg C3G L^{-1} respectively), as well as antioxidant bioactivity DPPH and FRAP (11.63 and 14.06 mM TEAC L^{-1} respectively) were significant compared to other juices. Therefore, the microwave/ultrasound process might be used in order to obtain a functional beverage with high antioxidant capacity, safe for human consumption and preserving color characteristics after one month of storage.

Keywords: blackberry juice, microwaves/ultrasound, polyphenols/anthocyanins, antioxidant activity, microbial stability.

Resumen

La biofuncionalidad del jugo de moras ha adquirido gran relevancia en el campo de los alimentos, ya que puede reducir el riesgo de enfermedades crónico-degenerativas debido a su elevado contenido de compuestos antioxidantes como polifenoles/antocianinas. Sin embargo, el procesamiento térmico convencional disminuye el contenido de dichos compuestos, por lo que se están investigando tecnologías alternativas como microondas y ultrasonido. En este marco, se evaluó el efecto del procesamiento térmico y de microondas/ultrasonido sobre las propiedades fisicoquímicas, contenido de polifenoles y antocianinas, capacidad antioxidante y estabilidad microbiológica del jugo de zarzamora. Los resultados muestran que pH y ºBrix incrementaron su valor en jugo procesado por microondas/ultrasonido (MUJ) respecto al jugo pasteurizado convencionalmente (FPJ y SPJ) y al jugo sin procesar (CJ). MUJ mostró buena estabilidad microbiológica durante 30 días de almacenamiento a 4°C al igual que los jugos procesados convencionalmente (FPJ y SPJ); sin embargo, en MUJ incrementaron significativamente parámetros de color (L*(a*/b*), ΔE*, H*, C*), contenido de polifenoles y antocianinas (2665.97 mg GAE L^{-1} y 427.49 mg C3G L^{-1}), así como bioactividad antioxidante DPPH y FRAP (11.63 y 14.06 mM TEAC L^{-1}) respecto a los demás jugos. Por lo tanto, la combinación de microondas/ultrasonido se podría utilizar para obtener una bebida funcional con alto contenido de antioxidantes, segura para el consumo humano y manteniendo su color después de un mes de almacenamiento.

Palabras clave: jugo de zarzamora, microondas/ultrasonido, polifenoles/antocianinas, antioxidante, estabilidad microbiológica.

---

* *Corresponding author. E-mail*: `rosalmorae@gmail.com`

doi: 10.24275/10.24275/uam/izt/dcbi/revmexingquim/2018v17n1/Perez
issn-e:2395-8472

1 Introduction

Currently, a holistic view focusing on the relationship of bioactive molecules-food processing-dietary intake-health is necessary. In this context, investigations on promising technologies for industrial processing of fruit juices have acquired great importance, especially in the field of functional foods, particularly that of fruits like berries, cherries, grapes, black currant, red onion, red cabbage, and blue corn, which are attracting the attention of consumers due to their high content of antioxidant compounds (polyphenols). Among berries, blackberry (*Rubus* sp. Rosaceae) is a fruit with high content of polyphenols (Luna-Ramírez *et al*., 2017), especially anthocyanins (Hager *et al*., 2008; Wen *et al*., 2015). Anthocyanins have a wide range of biological properties, mainly to their antioxidant capacity and anti-inflammatory effect, which contributes in the reduction of risks in chronic-degenerative diseases like cancer (Dai *et al*., 2009), obesity, diabetes, (Sun *et al*., 2012; Tsuda, 2008), cardiopathies (Vauzour *et al*., 2010), and neurologic disorders (Krikorian, 2012; Shih *et al*., 2010).

However, heating process (pasteurization), the most commonly used method for fruit juice processing to ensure safety and storage stability, contributing to the degradation of thermosensitive components, such as polyphenols (anthocyanins) (Verbeyst *et al*., 2010). Therefore, as an alternative technology to traditional systems, microwave and ultrasound-assisted processing has emerged (Aybastier *et al*., 2013; Oancea *et al*., 2013). Ultrasound technology is effective to produce functional juices from berries, since it preserves essential phytochemicals (Engmann *et al*., 2015; Tiwari *et al*., 2009) and enhances anthocyanin stability (Cavalcanti *et al*., 2012). While microwave technology contributes to improve sensory properties such as color, inactivation of oxidative enzymes such as polyphenol oxidase, to increase the retention of anthocyanins and to improve bioavailability (Bo *et al*., 2012; Brambilla *et al*., 2011). Also, microwaves offer a rapid extraction of phenolic compounds, including anthocyanins, and prevents their degradation during processing. Because, it requires a less time allowing to extract greater concentration of those compounds (Teng and Lee, 2013).

Further, both technologies (ultrasound/microwaves) have been noticed that microorganism decline as *E. coli* and *S. cerevisae* when are combined (Hosseinzadeh-Samani *et al*., 2016) due to ultrasonic waves disruptions bacterial wall by high temperature and pressure created by cavitation phenomenon resulting in localized cleansing effect by microregions (Hosseinzadeh-Samani *et al*., 2016; Li and Farid, 2016; Santhirasegaram *et al*., 2013), while microwaves rapidly increases temperature and pressure in the whole medium and inside of the cell causing break down of bacterial cell wall (Hosseinzadeh-Samani *et al*., 2016; Li *et al*., 2013), and consequently microorganism are destroyed.

Therefore, one of the main challenges for industrial processing of anthocyanins is to preserve high quantity and quality of these compounds in juices, which is affected by physicochemical factors during thermal processing and storage. Thus, the aim of the present work was to evaluate the effect of conventional thermal and microwave/ultrasound processing on color parameters, polyphenols and monomeric anthocyanins content, antioxidant capacity, and microbial load of blackberry juice, due to the great potential of applications that polyphenols (anthocyanins) represent for the industry of functional foods.

2 Materials and methods

2.1 *Blackberry fruits*

Blackberries (*Rubus fructicosus* var. Himalaya) in mature stage were obtained from Jalancingo village, Veracruz, Mexico. Fruits were washed and frozen at -20 ºC.

2.2 *Blackberry mash and juice*

Frozen blackberries were thawed to room temperature and crushed using a mortar. Then, 50 g of mash were centrifuged at 1260 g and 4 ºC during 30 min, obtaining approximately 20 mL of juice.

2.3 *Conventional heating process*

Blackberry juice pasteurization was carried out by immersion in autoclave of 50 L capacity (steam heating). A first batch of blackberry juice (25 mL per bottle) was heated to 60 ºC during 30 min (slow pasteurization), while a second batch was pasteurized to 92 ºC for 10 s (fast pasteurization) (Azofeifa *et al*., 2015; Zhang *et al*., 2012). Temperature measurements

(Doric instrument, Mod. 400 A) were obtained using cupper T-type thermocouples placed at 1/3 high of bottle. Juices were stored into sterilized amber bottles of 25 mL at 4 ºC. Both pasteurization treatments were performed by triplicate.

2.4 *Microwaves processing*

Blackberry mash (50 g) was heated using a microwaves oven (Panasonic, 2450 MHz, and 453 W, as calculated according to Buffler method (Buffler, 1993) for 60 s. Briefly, the energy (E) for the microwaves was calculated using Eq. (1)

$$E = \frac{Wt}{m} \tag{1}$$

Where W is microwaves oven power in W; t is time of microwave exposure in s, and m, the sample amount in kg (Ortiz *et al.*, 2003). After heating, the blackberry mash was cooled in ice water until 20 °C. The juice was obtained by centrifugation at 1260 g and stored into sterilized amber bottles of 25 mL at 4 ºC. Treatment was done by triplicate.

2.5 *Ultrasound processing*

Ultrasound treatment was assessed employing an ultrasonic homogenizer (Cole-Palmer Instrumental Company, VCX-750, USA) at 20 kHz frequency and 750 W power, equipped with a tip (13 mm, diameter) operating to 32 microns of amplitude, 5 s pulse (on/off) during 10 min (Tiwari *et al.*, 2009). Blackberry juice samples of 80 mL were placed in a 100 mL vessel for the treatment with ultrasound and temperature was maintained at 10 °C. Juice was stored into sterilized amber bottles of 25 mL at 4 ºC. Treatment was done by triplicate.

2.6 *Microwaves/ultrasound processing*

The blackberry mash (50 g) was processed with microwaves and the juice was obtained by centrifugation and treated with ultrasound, then it was stored into sterilized amber bottles at 4 ºC. Treatment was done by triplicate.

2.7 *Physicochemical characterization*

At the end, six different juices were obtained: unprocessed juice (CJ), juice processed with fast conventional pasteurization (FPJ), juice processed with slow conventional pasteurization (SPJ), juice processed with ultrasound (UJ), juice obtained from mash heated with microwaves (MJ), juice obtained from mash heated with microwaves and then processed with ultrasound (MUJ). A bottle of each juice was stored for each day of sampling. These juices were characterized as follows.

2.7.1 *Measurement of pH, ºBrix and color index*

The pH values were determined (Conductronic, pH-120). Total soluble solids (ºBrix) content was obtained using a refractometer (Atago, HSR-500; Japan). Both analyses were performed by triplicate. Color levels were obtained using a color reader (CR-10, Konica Minolta Inc., Japan); thus, data were expressed as L* (whiteness or brightness/darkness), a* (redness/greenness), and b* (yellowness/blueness). Thus, the chromatic model based on CIELAB system was used to calculate four color coefficients (Acosta-Montoya *et al.*, 2010; Cesa *et al.*, 2017; Marszalek *et al.*, 2015; Rodrigo *et al.*, 2007): coloration, total color difference (ΔE*), hue angle (color angle, H*) and chroma (color intensity, C*) were acquired by:

$$\text{Coloration} = L^* \times \left(\frac{a^*}{b^*}\right) \tag{2}$$

$$\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \tag{3}$$

$$H^* = \tan^{-1}\left(\frac{b^*}{a^*}\right) \tag{4}$$

$$C^* = \sqrt{a^{*2} + b^{*2}} \tag{5}$$

Ten measurements were obtained from each different juice.

2.8 *Biofunctional properties*

2.8.1 *Total phenolics content*

Total phenolics content was determined according to Folin-Ciocalteu method (Singleton *et al.*, 1999). Absorbance measurement was done at 760 nm using a spectrophotometer (BOEGO, S-22; Germany). The amount of phenolics was expressed as mg of gallic acid equivalents per liter (mg GAE L^{-1}). A standard curve (20-120 mg L^{-1}) was obtained by linear regression ($y = 0.0024x - 0.0066$, $r^2 = 0.996$, $p < 0.05$). Dilutions were 1:25. Analysis was carried out by triplicate.

2.8.2 *Monomeric anthocyanins content*

Monomeric anthocyanins content was carried out by means of differential pH method (Giusti and Wrolstad, 2005). Absorbance screening was from 400 to 700 nm using a plate reader (Thermo-Scientific, Multiskan-GO spectrophotometer). Anthocyanins content was expressed as mg of cyanidin-3-O-glycoside per liter (mg C3G L^{-1}). Dilutions were 1:25. Analysis was performed by triplicate.

2.8.3 *Antioxidant capacity*

2.8.3.1. DPPH assay

Radical scavenging activity was carried out by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Brand-Williams *et al*., 1995). Absorbance was determined at 515 nm using a spectrophotometer (BOEGO, S-22; Germany). Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxilic acid) standard curve was used for quantification. Results were expressed as millimole of Trolox® equivalent antioxidant capacity per liter (mM TEAC L^{-1}). Standard curve (100-800 μM L^{-1}) was obtained by linear regression ($y = 0.0007x - 0.8437$, $r^2 = 0.997$, $p < 0.05$). Dilutions were 1:25. All determinations were carried out by triplicate.

2.8.3.2. FRAP assay

Fe^{3+}/Fe^{2+} reducing antioxidant power was performed according to the method described by Benzie and Strain (1996). Absorbance was read to 593 nm using a spectrophotometer (BOEGO, S-22; Germany). Trolox® standard curve was used for quantification. Results were expressed as mM TEAC L^{-1}. Standard curve (160-800 μM L^{-1}) was obtained by linear regression ($y = 0.0012x - 0.0841$, $r^2 = 0.998$, $p < 0.05$). Dilutions were 1:25. All determinations were done by triplicate.

2.9 *Microbiological test*

Counting of coliform, mould/yeast and aerobic mesophilic bacteria was carried out by using Mexican Official Standard methods (NOM-113-SSA1-1994, NOM-111-SSA1-1994 and NOM-092-SSA1-1994, respectively). Then, 1 mL of juice samples were diluted (10^{-1}, 10^{-2}) in peptone (0.1 %) sterilized water. Dilutions were placed by duplicate into plates of agar. Two assays were done per treatment. Results were expressed as colony-forming units per milliliter (CFU mL^{-1}). The inactivation effect and reduction of logarithmic cycles were estimated by $\log_{10} N$ and $\log_{10}(N_0/N)$, respectively; where N_0 and N are microbe levels at initial and end times.

2.10 *Statistical analysis*

Data were acquired at times 0, 7, 15 and 30 days under storage at 4 ºC. ANOVA Two-Way tests were carried out to evaluate significant differences. Tukey's test ($p < 0.05$) was used for mean multiple contrast between treatments and times. Equations of linear regression were obtained for all standard curves. Coefficient of Pearson's correlation was estimated ($p < 0.05$). Data analyses were supported by Prism 6 software (GraphPad, USA).

3 Results and discussion

3.1 *pH, ºBrix and color index*

Fig. 1a shows the values of pH. Respect to unprocessed juice (CJ, pH= 3.16 ±0.01), the conventional heating process caused a little decrease of 0.03 units (pH 3.13); but microwaves (MWJ) and ultrasound processing (USJ) showed a rise of 2.5 and 3.5 % respectively (pHs 3.21 and 3.24). While, the combination of microwaves/ultrasound (MUJ) generated only an increase of 0.06 units. After 30 days of storage under refrigeration conditions, pH significant changes ($p < 0.05$) between treatments were observed. A significant reduction of pH was reported by MUJ (3.7 %), MJ (3.7 %), UJ (5.2 %), FPJ (0.6 %), and CJ (1.9 %) respect to initial time, but not SPJ (0.03 %).

Although, there were pH variations between treatments by effect of storage time, pH values were kept under acidic conditions ($3 < pH < 3.5$) in the range of typical berry juices ($2 < pH > 4$) (Howard *et al*., 2012; Luna-Ramírez *et al*., 2017). Regarding this, in acidic medium, the flavylium cation is hydrated forming the carbinol pseudobase, which over time is converted into the chalcone pseudobase (Howard *et al*., 2012; Patras *et al*., 2010). Both juices processed by microwaves and ultrasound increased pH value. In the case of processing by ultrasound alone, this could promote chemical reactions, which cause changes in pH due to generation of new molecules in aqueous media (Martínez-Flores *et al*., 2015).

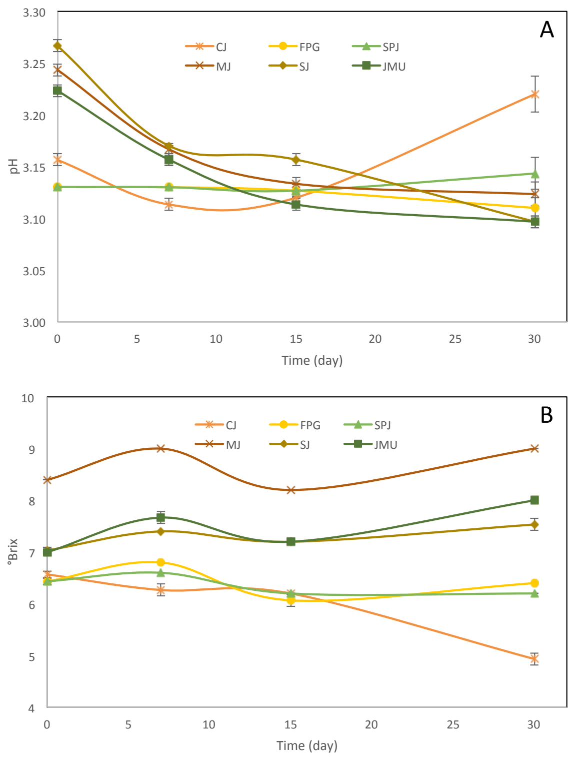

Fig. 1. pH (a) and °Brix (b) data of blackberry juice at day zero and after 30 days of storage at 4 ºC. Bars represent mean ±SD, $n = 3$; Tukey's test, $p \leq 0.05$ (* statistically significant difference). CJ= Unprocessed, FPJ= Fast pasteurization (92ºC, 10 s), SPJ= Slow pasteurization (60 ºC, 30 min), MJ= Microwaves (0.525 kJ/g, 60 s), UJ= Ultrasound (20 kHz, 10 min), MUJ= Microwaves/Ultrasound.

While, in microwaves, because of exposure to high energy, fast heating and temperature increase, association reactions (polymerization) could be occurring and generating pH modifications. On the other hand, the reduction of pH values after 30 days of storage can be explained by microbial and endogenous enzymatic activity (Martínez-Flores *et al.*, 2015). Thus, microwave/ultrasound processing improved anthocyanins stability of blackberry juice.

In the total soluble solids (ºBrix) measurements significant variations (p <0.05) were observed between all treatments at day zero (Fig. 1b), except between both pasteurization process (FPJ and SPJ= 6.43 ±0.06) and unprocessed juice (CJ= 6.57 ±0.06). In relation to CJ, the treatments UJ and MUJ enhanced this parameter on 7.0 and 6.5 % respectively; but MJ caused a drastic rise of 27.9 %. While, UJ, MUJ and MJ increased 9.1, 8.7 and 29.9 %, respectively, in relation to both thermal processes. After one month of storage, significant differences (p <0.05) between treatments were observed. A drastic reduction of total soluble solids content was observed in CJ (4.93 °Brix); while, SPJ decreased it significantly 0.23 units; but MJ (9.0 ºBrix), UJ (7.53 ºBrix), and MUJ (8.0 ºBrix) had a drastic increase respect to initial time. FPJ did not report significant changes.

This important content of solids could be the result of plant cell wall breakage (Li and Farid, 2016; Martinez-Flores *et al.*, 2015) and release of cellular components to medium, mainly when blackberry juice was treated by microwaves. Microwave heating promotes the rupture of plant cell structure releasing their content; but in this case, the energy applied causes *in situ* movement and rotation of water molecules of the sample leading a fast heating in short time, which increases the pressure inside cell and, subsequently, collapses the cell wall (Li *et al.*, 2012; 2013). Hence, microwaves cause drastic rise of total soluble solids content.

Colorimetric estimations were performed to evaluate color changes in blackberry juice due to different processing. The observed color of blackberry juice is presented as a combination of a (red/green) and b (yellow/blue) coordinates associated with L (lightness/darkness) value. Changes of juice's color were evaluated by the coefficient of coloration, color difference (Marszalek *et al.*, 2015; Rodrigo *et al.*, 2007), color angle and color intensity (Acosta-Montoya *et al.*, 2010; Cesa *et al.*, 2017). In Fig. 2, differences of color parameters due to treatment by heating, microwaves/ultrasound, as well as 30 days after refrigerated storage (4 ºC) are showed. All coefficients showed a dependence on L, a, and b; therefore, in blackberry juice an increase mainly in L (brightness) and a (redness) was interpreted as an improvement of the visual color, which is reflected in any of the determined colorimetric coefficients (Yousefi *et al.*, 2012).

At initial time, the coloration values, Eq. (2), were significantly different (p <0.05) among all treatments (Fig. 2a). A strong reduction of this value in juice processed by microwaves (MJ= 3.15 ±0.33), rapid thermic processing (FPJ= 2.51 ±0.31), and ultrasound (UJ= 3.56 ±0.56) was observed respect to unprocessed juice (CJ= 6.03 ±0.49) indicating that a ratio of FPJ:UJ:CJ of 1:1.48:2.4 was obtained. While, slow heating (SPJ= 6.03 ±0.49) did not show variation, having the same effect of CJ. However, a drastic rise of this coefficient in juice processed by microwaves/ultrasound combination (MUJ= 18.56

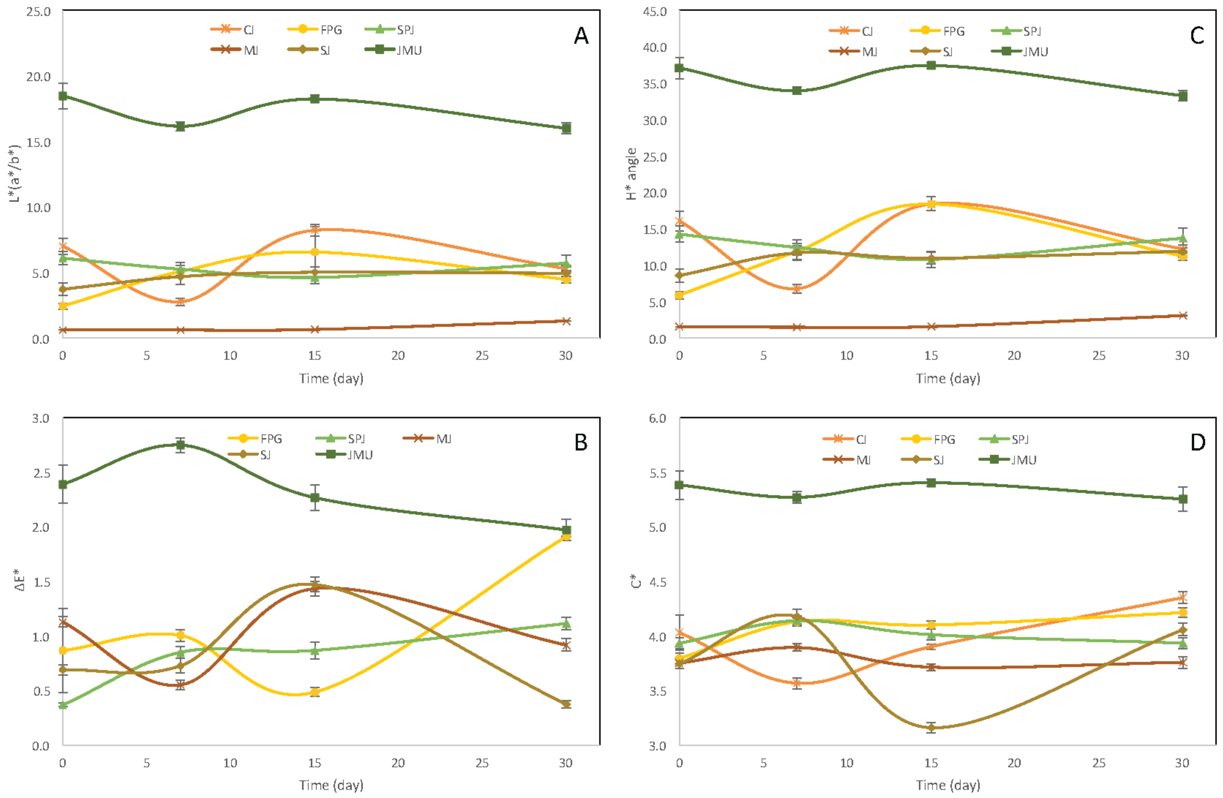

Fig. 2. Colorimetric index of blackberry juice, at day zero and after 30 days of storage at 4 ºC. Coefficients L* x (a*/b*) (a), Δ*E** (b), H*(c), and C*(d). Bars represent mean ±SD, n=3; Tukey's test, $p \leq 0.05$ (* statistically significant difference). CJ= Unprocessed, FPJ= Fast pasteurization (92 ºC, 10 s), SPJ= Slow pasteurization (60 ºC, 30 min), MJ= Microwaves (0.525 kJ/g, 60 s), UJ= Ultrasound (20 kHz, 10 min), MUJ= Microwaves/Ultrasound.

±0.98) was noted, been 7.4 times higher than FPJ providing more coloration which means that this process preserved the color better than the other methods. At day 30, SPJ (5.7 ±0.57) and UJ (4.93 ±0.5) were not significantly different ($p < 0.05$) to CJ (5.28 ±0.25), but FPJ (4.47 ±0.25) and MJ (1.41 ±0.26) showed significant decrease in their coloration. However, MUJ (15.99 ±0.42) maintained a higher coloration over the rest of the juices. Respect to time, all treatments showed significant changes after 30 days of storage at 4 °C, except SPJ. Nevertheless, CJ and SPJ was reduced in 1.57 and 0.33 units, respectively. While, FPJ, MJ and UJ had an increase of 1.96, 1.10 and 1.37 units, respectively. Although, MUJ overdue 13.84 %, continued being the best coloration value in relation to those processing and time, which is important for juice storage, showing that only 13.84 % of color is lost after 30 days of storage.

Difference in color, Eq. (3), among treatments respect to unprocessed juice were recorded (Fig. 2b). At day zero, microwaves/ultrasound combination showed the highest ΔE* value (2.38 ±0.18), being significantly different ($p < 0.05$) to juices processed by heating (FPJ= 0.86 ±0.38, SPJ= 0.50 ±0.36), ultrasound (USJ= 0.78 ±0.29), and microwaves (MJ= 1.25 ±0.4). The microwave treatment reported significant difference with respect to both pasteurization process and ultrasonication. When data were collected after 30 days of storage at 4 ºC, MUJ (1.97 ±0.1) this parameter was kept significantly better ($p < 0.05$) respect to both pasteurization processes (FPJ= 1.91 ±0.01, SPJ= 1.11 ±0.06), and juices treated only by microwaves (MJ= 0.91 ±0.06) or ultrasound (UJ= 0.36 ±0.05). Nonetheless, only FPJ and SPJ reported a significant rise respect to the initial value, while MJ, UJ and MUJ presented a decline, although MUJ continued being the best ΔE* value in relation of those process and time.

Hue angle (color angle), Eq. (4), displayed significant difference ($p < 0.05$) between all treatments at initial time (Fig. 2c). *H angle* expresses color appearance according to similarity of red/yellow/blue/green combination (Cesa *et al*., 2017). The highest value was obtained by combination of microwaves/ultrasound (MUJ= 37.03 ±1.44). However, juice processed only by microwaves (MJ= 0.774 ±0.82) or ultrasound (UJ= 8.3 ±1.5) reported lower values than MUJ. Also, thermic processing (FPJ= 6.06 ±0.75, SPJ= 13.2 ±3.44) displayed low scores of *H* angle* respect to MUJ and CJ. MUJ showed an increase of 20.98 degrees in relation to unprocessed juice (CJ= 16.05 ±1.41). When *H* angle* values were recorded after 30 days of refrigerated storage, significant difference (p <0.05) were maintained in MUJ respect to all treatments. However, *H* angle* value of MUJ (33.26 ±0.72) had a little but significant decrease in relation to day zero, while juice treated by microwaves (MJ= 3.35 ±0.61), ultrasound (UJ= 11.48 ±1.15), and rapid thermal processing (FPJ= 11.23 ±0.62) significantly increased this parameter, but not slow heating treatment (SPJ= 13.69 ±1.34). Also, unprocessed juice (CJ= 12.22 ±0.56) showed a significant reduction. Therefore, MUJ continued being the best process by treatment and time.

Chroma (color intensity) values, Eq. (5), presented significant changes (p <0.05) between all treatments at day zero (Fig. 2d). Chroma expresses the color intensity defined by red/green/yellow/blue saturation (Cesa *et al*., 2017). The highest value was found for microwaves/ultrasound combination (MUJ= 5.38 ±0.13), but juices treated only by microwaves (MJ= 3.75 ±0.05) or ultrasound (UJ= 3.74 ±0.05) showed lower value than MUJ. Also, both juices processed by heat (FPJ= 3.79 ±0.05, SPJ= 3.93 ±0.05) had low values. Only MUJ was significantly better than unprocessed juice (CJ= 4.03 ±0.16). After of 30 days of storage significant variations (p <0.05) were maintenance between all treatments. Respect to time, slow heating process (SPJ= 3.93 ±0.05) not change. But, microwaves (MJ= 3.76 ±0.05), ultrasound (UJ= 4.05 ±0.06), rapid pasteurization (FPJ= 4.21 ±0.05), and unprocessed juice (CJ= 4.35 ±0.05) had a light increase. While, microwaves/ultrasound treatment (MUJ= 8.37 ±0.19) displayed a decline; but despite of them, MUJ continued being the best process in relation to treatment and time.

The color is a sensory attribute very important in the visual appreciation of foods by consumers (Espinoza-Velazquez *et al*., 2016). In berry juices, the attractive purple-red color is mainly given by anthocyanins (Cuevas-Rodríguez *et al*., 2010; Cesa *et al*., 2017). Regarding this, it is well known that in foods processed by heat, the amount of monomeric anthocyanins decline (Hager *et al*., 2008; Patras *et al*., 2010; Zhang *et al*., 2012), which strongly impacts nutritional and color quality (Patras *et al*., 2010). Previously, Hager *et al*. (2008) reported that the percent of monomeric anthocyanins falls while polymeric color increases in blackberry juices and purees thermally processed. This correlation was also observed after long-term storage. In the case of microwaves, it is known that blanched effect causes color changes, which can be explained by cellular disruption as consequence of internal heating and pressure; causing important release of anthocyanins (Li *et al*., 2013; Wen *et al*., 2015; Zou *et al*., 2012). Wrolstad (1980) reported than blanching by microwaves improves color stability and has a protective effect on total phenols and anthocyanins content in strawberry concentrate. However, this blanching stage also cause anthocyanins degradation as observed in strawberry puree (De Ancos *et al*., 1999) depending on time and temperature exposure. While, in non-thermal processing such as ultrasound, polymerization of anthocyanins is low due to mechanical stress, which causes the breakdown of macromolecules (as polymeric anthocyanins) in the juice (Santhirasegaram *et al*., 2013; Tiwari *et al*., 2009). On the other hand, a microwaves/ultrasound combination results in better color parameters. Thus, the differences of color indexes among treatments and by storage time found in this work could be explained by degradation of monomeric anthocyanins and formation of polymeric anthocyanins.

Although, the four colors coefficients of the blackberry juice evaluated here are the most commonly used and they can be good estimators for this sensory feature, other coefficients based on tri-stimulus relations of L, a, b such as Lab, L/ab, La/b (Tiwari *et al*., 2009), L/L_0, a/a_0, b/b_0 (Yousefi *et al*., 2012; Cesa *et al*., 2017) and browning could be considered also to enhance the capacity of resolution of colorimetric analysis.

3.2 *Biofunctional quality: polyphenols and anthocyanins content, and antioxidant capacity*

Polyphenols content expressed as mg GAE L^{-1} (Fig. 3a) did not significantly increase in juices after conventional thermal processing (FPJ=1495.83 ±20.83 and SPJ=1454.17 ±18.04) respect to unprocessed juice (CJ= 1433.33 ±20.83) at initial time (day zero). Fast or slow heating conditions did not show significant differences, but a decrease of 10.21 % and 7.6 % in juice ultrasonicated (UJ= 1343.06 ±49.23) was observed respect to FPJ and SPJ, respectively. The treatment of juice with microwaves (MJ= 1933.33 ±65.05) displayed a significant increase respect to those treatments. However, a drastic rise in juice treated with microwaves/ultrasound combination (MUJ= 2665.97 ±21.68) was observed, being 1.86 fold the content of polyphenolics of the control (CJ) and ranged 1.78-1.83 fold respect to fast and slow heating conditions, respectively. One month later, all samples stored at 4 ºC displayed a significant rise in the content of polyphenolics being 94 % in CJ, 76 and 86 % in FPJ and SPJ, respectively, 74 % for MJ, 95 % in UJ, and 78 % for MUJ respect to initial conditions.

Monomeric anthocyanins content expressed as mg C3G L^{-1} (Fig. 3b) displayed a behavior comparable to that regarding phenolic content. At initial time (day zero), CJ (150.43 ±27.43), FPJ (168.66 ±3.28), SPJ (148.55 ±12.9), UJ (216.11 ±31.71), and MJ (278.48 ±35.3) were not significantly different among them, but a strong significant increase was observed in MUJ (427.49 ±25.75). This non-significant relation was maintained after 30 days of storage at 4 ºC between CJ (196.42 ±50.78) and both conventional heating (FPJ= 138.32 ±49.24, SPJ= 153 .98 ±49.12), UJ (206.58 ±63.78), and MJ (319.44 ±26.47); however, MJ displayed a significant value respect to conventional thermal process but not respect to UJ. While, MUJ (922.41 ±94.11) experienced a reduction but was maintained over other treatments. Therefore, this continued to be the most convenient processing.

In relation to above mentioned, it has been observed that thermal processing of blackberry juice reduces anthocyanins content (Azofeifa *et al*., 2015; Gancel *et al*., 2011; Hager *et al*., 2008). By contrast, it is expected that microwave- and ultrasound- assisted processing increases the amount of total polyphenolics and anthocyanins. In this work microwave treatment was very efficient for extraction of phenols while ultrasound treatment was less efficient in relation to conventional heating processing; however, combination of both processes resulted in higher recovering of phenolic compounds These rises in phenols-extraction occurs due to ultrasound waves create microbubbles that collapse violently (cavitation bubbles) increasing local temperature and pressure and consequently disrupts plant-cell wall and delivering compounds as phenols/anthocyanins (Tiwari *et al*., 2009); while, microwaves generates a rapid increase of temperature and pressure inside the cell collapsing the plant-cell wall and releasing phenols/anthocyanins molecules (Li *et al*., 2013). For them, ultrasound/microwaves combination improved the polyphenols extraction. Earlier researches have documented that ultrasound- and microwaves-processing can improve the extraction of phenols. Regarding this, Oancea *et al*. (2013) observed that anthocyanins content of ultrasonicated raspberry juice was similar to conventional extraction in shorter time. But, Ivanovic *et al*. (2014) reported an increase of anthocyanins in blackberry juice processed by ultrasound dependent of time and temperature. Whereas, Marzsalek *et al*. (2015) found less degradation of anthocyanins in strawberry puree, when this was processed by microwaves than by conventional heating.

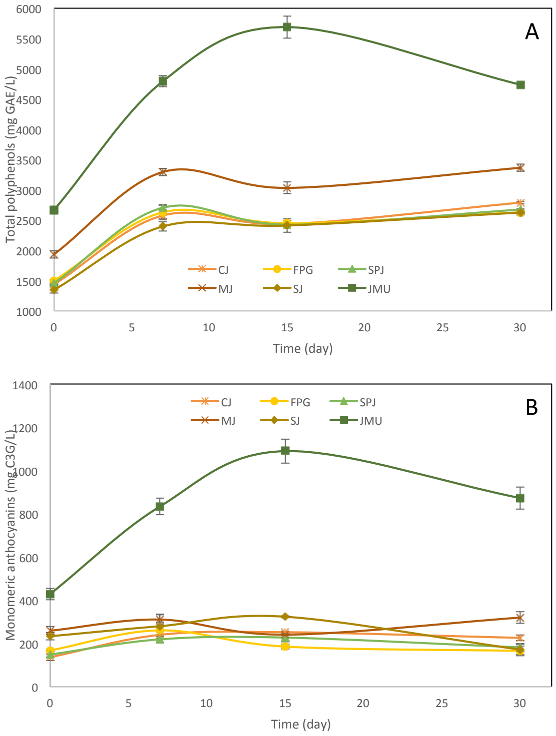

Fig. 3. Polyphenols (a) and monomeric anthocyanins (b) content of blackberry juice at day zero and after 30 days of storage (4 ºC). Bars represent mean ±SD, n=3; Tukey's test, $p \leq 0.05$ (* statistically significant difference); GAE= Gallic acid equivalent, C3G= Cyanidin-3-O-glicoside; CJ= Unprocessed, FPJ= Fast pasteurization (92 ºC, 10 s), SPJ= Slow pasteurization (60 ºC, 30 min), MJ= Microwaves (0.525 kJ/g, 60 s), UJ= Ultrasound (20 kHz, 10 min), MUJ= Microwaves/Ultrasound.

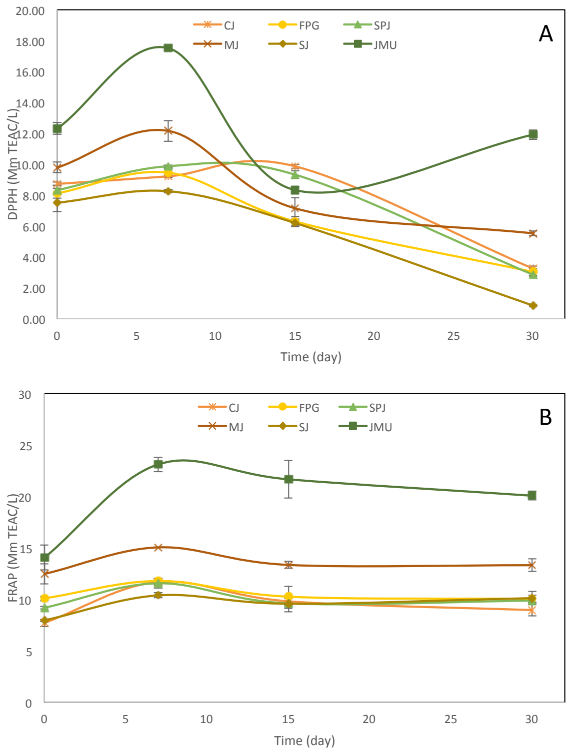

Fig. 4. Antioxidant capacity of blackberry juice at day zero and after 30 days of storage at 4ºC expressed as mM TEAC L^{-1} by DPPH (a) and FRAP (b) assay. Bars represent mean ±SD, n=3; Test's Tukey, $p \leq 0.05$ (* statistically significant difference). TEAC= Trolox® equivalent antioxidant capacity; CJ= Unprocessed, FPJ= Fast pasteurization (92 ºC, 10 s), SPJ= Slow pasteurization (60 ºC, 30 min), MJ= Microwaves (0.525 kJ/g, 60 s), UJ= Ultrasound (20 kHz, 10 min), MUJ= Microwaves/Ultrasound.

This enhances of phenols has also been reported for carrot juice thermos-sonicated (Martínez-Flores *et al.*, 2015), apple juice sonicated (Abid *et al.*, 2014) and apple mash treated by microwaves heating (Gerard and Roberts, 2004). Additionally, variations in the content of phenolic acids, monomeric anthocyanins and antioxidant activity have also been observed during long-time storage at low temperatures in berry fruits and juices (Hellström *et al.*, 2013; Reque *et al.*, 2014; Türkben, 2010), which could be due to degradation of polymeric anthocyanins and reactions of copolymerization among monomeric anthocyanins and other polyphenols as acid-phenols or flavonoids.

Phenolic compounds mainly identified in blackberries are anthocyanins such as cyanidin-3-O-glicoside, cyanidin-e-O-rutinodside, cyanidin-3-O-arabinoside, pelargonidin3-O-glicoside (Cuevas-Rodríguez *et al.*, 2010; Verberic *et al.*, 2014; Zhang *et al.*, 2012) and polyphenols such as quercetin, catechin, kaempherol, hydroxyxinamic acids, and ellagic derivatives (Verberic *et al.*, 2014; Wen *et al.*, 2015). Investigations on the structure-activity relationship of polyphenols and anthocyanins indicate that these molecules act as reducing agents, hydrogen donating, "single oxygen quencher" and metal chelating (Leopoldini *et al.*, 2011; Rice-Evans *et al.*, 1996) agents. The action mechanism depends on the hydroxyl groups and their position in the molecule. Additionally, substitutions on hydroxyl groups can enhance or reduce the antioxidant capacity (Rice-Evans *et al.*, 1996). In the case of phenolic acids, the relative position of OH (-*ortho*, -*metha*, -*para*) respect to carboxylate group have strong influence on the antioxidant power, being *m*-OH > *o*-OH > *p*-OH. Moreover, greater number of OH groups on the ring can improve the antioxidant ability, but esterification on carboxylate group can diminish it (Rice-Evans *et al.*, 1996). In flavonoids, the antioxidant efficacy depends on hydroxyl groups, mainly on the 3-OH group in the C-ring, substitutions of hydroxyls, unsaturation of C-ring, and positions of OH-groups in B-ring (Dragan *et al.*, 2003; Farkas *et al.*, 2004; Rice-Evans *et al.*, 1996).

Nonetheless, like Madrigal-Santillán *et al.* (2013) point out each of these compounds in a fruit causes a synergistic effect, which can increase their antioxidant capabilities. Therefore, the antioxidant activity of blackberry juice can be attributed to accumulative effects of their polyphenolic compounds. Consequently, an increase of the total content of polyphenolic compounds and monomeric anthocyanins in blackberry juices represents better biofunctional quality of this food, since antioxidant capacity is associated directly to polyphenols (anthocyanins) quantity and quality.

Antioxidant activity (mM TEAC L^{-1}) estimated by DPPH assay (Fig. 4a) did not display significant differences between CJ (8.28 ±0.11), heating process (FPJ= 8.12 ±0.32, SPJ= 8.33 ±0.3), microwave (MJ= 9.79 ±0.34) and ultrasound treatment (UJ= 7.49 ±0.55) at day zero; however, a significant increase was observed with MUJ (11.63 ±1.21) respect to those treatments. After 30 days of storage at 4 ºC no significant difference was found between CJ (3.24 ±0.16) and conventional thermal (FPJ= 3.03 ±0.22, SPJ= 2.85 ±0.09); but there was a significant difference with relation to MJ (5.89 ±0.65), UJ (7.99 ±0.09) and MUJ (11.92 ±0.31) with the latter treatment being the best one.

While FRAP assay (Fig. 4b) showed that the juices treated by slow heating process (SPJ= 9.19 ±0.13) and ultrasonication (USJ= 7.94 ±0.1) had similar antioxidant capacity respect to unprocessed juice (CJ= 7.706 ±0.36). Nonetheless, with the fast thermal process (FPJ= 10.08 ±0.23) and when the mash was treated with microwaves (MJ= 12.46 ±0.98) a significant increase of antioxidant effect was observed. The microwaves/ultrasound combination (MUJ= 14.06 ±1.2) displayed the best antioxidant capacity. However, one month later, no significant changes between CJ (8.96 ±0.58), FPJ (10.05 ±0.34) and SPJ (9.88 ±0.19) and UJ (10.12 ±0.63) were found. In MJ (13.32 ±0.61), it was observed a significant increase though the most drastic rise of antioxidant capacity was obtained in MUJ (20.07 ±0.43) in relation to treatment and time. These phenomena occur because coloration is preserved better with MUJ than the other treatments, providing more reduction of the electron-donating antioxidants presented in the juice, with a higher antioxidant capacity determined by an efficiency of 142.74 %.

An increase in the content of polyphenols, mainly anthocyanins, is very important due to their antioxidant effect. The coefficient of Pearson‘s correlation (p <0.05) was estimated between the content of polyphenols, monomeric anthocyanins, and antioxidant capacity (DPPH and FRAP) at initial time. Results showed a strong positive correlation for all treatments with r^2 ranging 0.88-0.973 (p <0.05). As mentioned above, high antioxidant capacity is directly associated to higher content of polyphenols (phenolic acids and flavanols) (Cardenas-Sandoval *et al*., 2012; Quiroz-Reyes *et al*., 2013), and particularly to the anthocyanins group in the blackberry juice. Previously, Wang and Jiao (2000) evaluated the scavenging capacity on reactive oxygen species ($O\cdot^{-}$, $'\mathrm{O}_2$, $\cdot\mathrm{OH}$, $\mathrm{H_2O_2}$,) of different berry species, showing blackberry the highest antioxidant capacity. In the same year, Jiao and Wang found a positive correlation regarding antioxidant activity between oxygen radical absorbance capacity (ORAC) and oxygen radical scavenging enzyme activities of six blackberry cultivars. However, when blackberry juice was pasteurized, a decrease of DPPH scavenging capacity was observed (Azofeifa *et al*., 2015). Moreover, Gancel *et al*. (2011) noticed that antioxidant capacity by ORAC and DPPH assays of blackberry juice industrially processed dropped. Processing of blackberry juice by ultrasound, dependent on time and temperature, showed a positive effect on antioxidant properties by FRAP and DPPH assays (Ivanovic *et al*., 2014). Furthermore, it has been demonstrated that storage at 25 ºC of processed blackberry products results in losses up to 75 % of monomeric anthocyanins and, consequently, loss of antioxidant capacity.

3.3 Microbiological quality: Coliforms, mould/yeast and aerobic mesophilic bacteria

Microbial load at day zero of unprocessed juice was 1.3 $\log_{10}$ and 1.7 $\log_{10}$ for mould/yeast and aerobic mesophilic bacteria, respectively; and were not found coliforms. The juice processed by heating, ultrasound and microwaves/ultrasound combination reduced microorganisms to undetectable; while, microwaves treatment decreased to undetectable mould/yeast and aerobic bacteria from 1.4 $\log_{10}$ (Table 1). This represented a significant reduction of 1.3 $\log_{10}$ cycles for mould/yeast. Regarding aerobic bacteria, this generated a decline of 1.75 $\log_{10}$ cycles by heating, ultrasound and microwaves/ultrasound, but microwaves only reduced 0.37 cycles After 30 days, only in juices processed by heating and microwaves/ultrasound no microbial growth was observed. Nevertheless, in juices treated separately by microwaves or ultrasound, it was registered a mould/yeast growth of 3.42 and 2.03 $\log_{10}$, respectively, while for unprocessed juice it was 4.76 $\log_{10}$. Thus, FPJ, SPJ and MUJ had a reduction of 4.76 $\log_{10}$ cycles, but for MJ and UJ it was 1.35 and 2.73 $\log_{10}$ cycles. Both thermal processes and microwaves/ultrasound treatment were more effective on reducing microbial load.

The heating process is used in beverage industry for obtaining safe products to humans, but thermosensitive bioactive compounds such as polyphenols/anthocyanins as well as nutritional components are degraded through such treatment and, as consequence, biofunctionality is diminished. To minimize these changes of thermally sensitive compounds and reducing microbial load, it has been suggested ultrasonication as a good alternative (Aadil *et al*., 2013; Alighourchi *et al*., 2014). On the other hand, microwave processing is better for improving the amount of bioactive compounds in juice and also contributes to microbiological stabilization (Marzsalek *et al*., 2015). In the present work, at initial time the inactivation of microorganisms in the juice processed only by ultrasonication or microwaves, as well as the combination of both technologies reported comparable results to those from thermal processing; besides, over time only microwaves/ultrasound combination kept higher levels of inactivation.

Table 1. Effects of thermal treatment and microwave/ultrasound on microbial inactivation analysis of blackberry juice.

Storage day	Juice type	(log CFU/mL)		
		Coliform count	Aerobic plate count	*Yeast and Mould count*
0	CJ	ND	1.7±0.12d	1.3±0.01d
	FPJ	ND	ND	ND
	SPJ	ND	ND	ND
	MJ	ND	1.4±0.12e	ND
	UJ	ND	ND	ND
	MUJ	ND	ND	ND
30	CJ	ND	5.0±0.24a	4.76±0.12a
	FPJ	ND	ND	ND
	SPJ	ND	ND	ND
	MJ	ND	2.48±0.09b	3.42±0.10b
	UJ	ND	1.82±0.21c	2.03±0.12c
	MUJ	ND	ND	ND

Mean ±SD, n=4; Tukey's test, p <0.05 (letter different in-column represents significate difference). CFU=Colony-forming unit, ND=not detected, CJ= Unprocessed, FPJ= Fast pasteurization (92 ºC, 10 s), SPJ= Slow pasteurization (60 ºC, 30 min), MJ= Microwaves (0.525 kJ/g, 60 s), UJ= Ultrasound (20 kHz, 10 min), MUJ= Microwaves/Ultrasound.

This microbial decrease is possible due to mechanical damage on cellular membrane and wall of microbes by high pressure and temperature caused by ultrasound (Earnshaw *et al*., 1995; Li and Farid, 2016; Tiwari *et al*., 2009) and microwaves (Li *et al*., 2012; 2013) effects. Previously, some authors have noticed that application of ultrasound (Martínez Flores *et al*., 2015) or microwaves (Marzsalek *et al*., 2015) to fruit juice is effective for inactivating microbes and oxidative enzymes. Hence, as was demonstrated in the present work, microwaves/ultrasound combination improves microbiological stability of blackberry juice for one month of storage.

Conclusions

Blackberry juice processed by combination of microwave for 60 s and 210 W, followed by ultrasound for 10 min at 20 kHz and 750 W improved the amount of available polyphenols/anthocyanins by about 100%, when compared with the raw juice. Microwave /ultrasound combination improved microbiological stability of blackberry juice for one month of storage. In summary, microwaves in combination with ultrasound technologies present an alternative in the processing of fruit juices with high content of antioxidant compounds and good microbial quality, as demanded by consumers and industry.

Nomenclature

CJ	unprocessed juice
FPJ	Fast heating juice/rapid Pasteurization Juice
SPJ	Slow heating juice/slow Pasteurization Juice
MJ	Microwaves Juice
UJ	Ultrasound Juice
MUSJ	Microwaves/Ultrasound Juice
TEAC	Trolox® Equivalent Antioxidant Capacity
GAE	Gallic Acid Equivalent
C3G	cyanidin-3-O-glycoside equivalent
DPPH	2,2-diphenyl-1-picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
Symbols	
L*	Lightness(+)/Darkness (-)
a*	Redness(+)/Greenness(-)
b*	Yellowness (+)/Blueness (-)
ΔE*	difference of color
H*	Hue angle
C*	chroma

Acknowledgements

D.I. Téllez-Medina and R. Mora-Escobedo appreciate the economic support from Instituto Politécnico

Nacional, COFAA, EDI, and SNI. This project was financed by SIP-IPN 490 (20161789) and CONACYT (INFR-2014-01).

References

Aadil R. M., Zeng X. A., Han Z., and Sun, D. W. (2013). Effect of ultrasound treatments on quality of grapefruit juice. *Food Chemistry 14*, 3201-3206. https://doi.org/10.1016/j.foodchem.2013.06.008

Abid, M., Jabbar, S., Wu, T., Hashim, M. M., Hu, B., Lei, S., and Zeng, X. (2014). Sonication enhances polyphenolic compounds, sugars, carotenoids and mineral elements of apple juice. *Ultrasonics Sonochemistry 21*, 93-97. http://dx.doi.org/10.1016/j.ultsonch.2013.06.002

Acosta-Montoya, O., Vaillant, F., Conazzo, S., Mertz, C., Pérez, A. M., and Castro, M. V. (2010). Phenolic content and antioxidant capacity of tropical highland blackberry (*Rubus adenotrichus* Schltdl.) during three edible maturity stages. *Food Chemistry 119*, 1497-1501. https://doi.org/10.1016/j.foodchem.2009.09.032

Alighourchi H., Barzegar M., Sahari M. A., and Abbasi, S. (2014). The effects of sonication and gamma irradiation on the inactivation of *Escherichia coli* and *Saccharomyces cerevisiae* in pomegranate juice. *Iranian Journal of Microbiology 6*, 51-58.

Aybastier, O., Isik, E., Sahin, S., and Demir, C. (2013). Optimization of ultrasonic-assisted extraction of antioxidant compounds from blackberry leaves using response surface methodology. *Industrial Crops and Products 44*, 558-565. https://doi.org/10.1016/j.indcrop.2012.09.022

Azofeifa, G., Quesada, S., Pérez, A. M., Vaillant, F., and Michel, A. (2015). Pasteurization of blackberry juice preserves polyphenol-dependent inhibition for lipid peroxidation and intracellular radicals. *Journal of Food Composition and Analysis 42*, 56-62. https://doi.org/10.1016/j.jfca.2015.01.015

Benzie, I. F. F., and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Analytic Biochemistry 239*, 70-76. https://doi.org/10.1006/abio.1996.0292

Bo, C., Del Riso, P., Brambilla, A., Gardana, C., Rizzolo, A., Simonetti, P., and Porrini, M. (2012). Blanching improves anthocyanins absorption from Highbush. *Journal of Agricultural and Food Chemistry 60*, 9298-9304.

Brambilla, A., Maffi, D., and Rizzolo, A. (2011). Study of the influence of berry-blanching on syneresis in blueberry pureés. *Procedia Food Science 1*, 1502-1508. https://doi.org/10.1016/j.profoo.2011.09.222

Brand-Williams, W., Cuvelier, M. E., and Berset, C. (1995). Use of free a radical method to evaluate antioxidant activity. *Lebensmittel - Wissenschaft und - Tecnologie/Food Science and Technology 28*, 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

Buffler, C. (1993). *Microwave Cooking and Processing*. (pp. 157-159). New York, USA: Van Nostrand Reinhold.

Cardenas-Sandoval B.A., López-Laredo B.P., Martínez-Bonfil B.P., Bermúdez-Torres K. and Trejo-Tapia G. (2012). Advances in the phytochemistry of *Cuphea aequipetala*, C. var. *hispida* and *C. lanceolate*: Extraction and quantification of phenolic compounds and antioxidant activity. *Revista Méxicana de Ingeniería Química 11*, 401-413.

Cavalcanti, R. N., Santos, D. T., and Meireles, M. A. A. (2012). Non-thermal stabilization mechanisms of anthocyanins in model and food systems-An overview. *Food Research International 44*, 499-509. https://doi.org/10.1016/j.foodres.2010.12.007

Cesa, S., Carradori, S., Bellagamba, G., Locatelli, M., Casadei, M. A., Masci, A., and Paolicelli, P. (2017). Evaluation of processing effects on anthocyanin content and colour modifications of blueberry (*Vaccinium* spp.) extracts: Comparison between HPLC-DAD and CIELAB analyses. *Food Chemistry 232*, 114-123. https://doi.org/10.1016/j.foodchem.2017.03.153

Cuevas-Rodríguez, E. O., Yousef, G. G., García-Saucedo, P. A., López-Medina, J., Paredes-López, O., and Lila, M. A. (2010). Characterization of anthocyanins and proanthocyanidins in wild and domesticated mexican blackberries (*Rubus* spp.). *Journal of Agricultural and Food Chemistry 58*, 7458-7464. https://doi.org/10.1021/jf101485r

Dai, J., Gupte, L., and Mumper, R. J. (2009). A comprehensive study of anthocyanins-containing extracts from selected blackberry cultivars: Extraction methods, stability, anticancer properties and mechanisms. *Food and Chemical Toxicology 47*, 837-847. https://doi.org/10.1016/j.fct.2009.01.016

De Ancos, B., Cano, M. P., Herandez, A., and Monreal, M. (1999). Effects of microwave heating on pigment composition and colour of fruit purees. *Journal of the Science of Food and Agriculture 79*, 663-670. https://doi.org/10.1002/(SICI)1097-0010(199904)79:5<663::AID-JSFA232>3.0.CO;2-L

Dragan Amic, Dusanka Davidovic-Amic, D. B. and N. T. (2003). Structure-radical scavenging activity relationships of flavonoids. *Croatica Chemica Acta 76*, 55- 61.

Earnshaw, R. G., Appleyard, J., and Hurst, R. M. (1995). Understanding physical inactivation processes: Combined preservation opportunities using heat, ultrasound and pressure. *Food Microbiology 28*, 197-219.

Engmann, F. N., Ma, Y., Tchabo, W., and Ma, H. (2015). Ultrasonication treatment effect on anthocyanins, color, microorganism and enzyme inactivation of mulberry (*Moraceae nigra*) juice. *Journal of Food Processing and Preservation 39*, 854-862. https://doi.org/10.1111/jfpp.12296

Espinosa-Velazquez., R., Dorantes-Alvarez, L., Gutiérrez-López, G.F., García-Armenta, E., Sánchez-Segura, L., Perea-Flores, M.J., Ceballos-Reyes, G.M., Ortíz Moreno., A. (2016). Morpho-structural description of unripe and ripe avocado pericarp (*Persea americana mill* var. drymifolia) description. *Revista Mexicana de Ingeniería Química 15*, 469-480. http://www.redalyc.org/articulo.ao?id=62046829014

Farkas, O., Jakus, J., and Héberger, K. (2004). Quantitative structure - antioxidant activity relationships of flavonoid compounds. *Molecules 9*, 1079-1088. https://doi.org/10.3390/91201079

Gancel, A. L., Feneuil, A., Acosta, O., Pérez, A. M., and Vaillant, F. (2011). Impact of industrial processing and storage on major polyphenols and the antioxidant capacity of tropical highland blackberry (*Rubus adenotrichus*). *Food Research International 44*, 2243-2251. https://doi.org/10.1016/j.foodres.2010.06.013

Gerard, K. A., and Roberts, J. S. (2004). Microwave heating of apple mash to improve juice yield and quality. *LWT-Food Science and Technology 37*, 551-557. https://doi:10.1016/j.lwt.2003.12.006

Giusti, M., and Wrolstad, R. E. (2005). Characterization and measurement of anthocyanins by UV-visible spectroscopy. *Handbook of Food Analytical Chemistry*, 2-2 (February), 19-31.

Hager, T. J., Howard, L. R., and Prior, R. L. (2008). Processing and storage effects on monomeric anthocyanins, percent polymeric color, and antioxidant capacity of processed blackberry products. *Journal of Agricultural and Food Chemistry 56*, 689-695. https://doi.org/10.1021/jf071994g

Hellström, J., Mattila, P., and Karjalainen, R. (2013). Stability of anthocyanins in berry juices stored at different temperatures. *Journal of Food Composition and Analysis 31*, 12-19. http://dx.doi.org/10.1016/j.jfca.2013.02.010

Hosseinzadeh-Samani, B., Hadi-Khoshtaghaza, M., Minaei, S., Zareifourosh, H., Naghi-Eshtiaghi, M., and Rostami, S. (2016). Design, development and evaluation of an automatic fruit-juice pasteurization system using microwave-ultrasonic waves. *Journal Food Science and Technology 53*, 88-103. https://doi.org/10.1007/s13197-015-2026-6

Howard, L. R., Prior, R. L., Liyanage, R., and Lay, J. O. (2012). Processing and storage effect on berry polyphenols: Challenges and implications for bioactive properties. *Journal of Agricultural and Food Chemistry 60*, 6678-6693. https://doi.org/10.1021/jf2046575

Ivanovic J., Tadic V., Dimitrijevic S., Stamenic M., Slobodan Petrovic S., and Zizovic I. (2014). Antioxidant properties of the anthocyanin-containing ultrasonic extract from blackberry cultivar "Čačanska Bestrna". *Industrial Crops and Products 53*, 274- 281. https://doi.org/10.1016/j.indcrop.2013.12.048

Jiao H., and Wang S. Y. (2000). Correlation of antioxidant capacities to oxygen radical scavenging enzyme activities in blackberry. *Journal of Agricultural Food and Chemistry 48*, 5672-5676.

Krikorian, R., Shidler, M. D., Nash, T. A., Kalt, W., Vinqvist-Tymchuk, M. R., ShukittHale, B., and Joseph, J. A. (2012). Blueberry supplementation improves memory in older adults. *Journal of Agricultural and Food Chemistry 58*, 3996-4000. https://doi.org/10.1021/jf9029332

Leopoldini, M., Russo, N. and Toscano, M. (2011). The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chemistry 125*, 288-306. https://doi.org/10.1016/j.foodchem.2010.08.012

Li, X., and Farid, M. (2016). A review on recent development in non-conventional food sterilization technologies. *Journal of Food Engineering 182*, 33-45. https://doi.org/10.1016/j.jfoodeng.2016.02.026

Li, Y., Fabiano-Tixier, A. S., Vian, M. A., and Chemat, F. (2013). Solvent-free microwave extraction of bioactive compounds provides a tool for green analytical chemistry. *TrAC - Trends in Analytical Chemistry 47*, 1-11. https://doi.org/10.1016/j.trac.2013.02.007

Li, Y. Han, L., Ma, R., Xu, X., Zhao, C., Wang, Z., *et al*. (2012). Effect of energy density and citric acid concentration on anthocyanins yield and solution temperature of grape peel in microwave-assisted extraction process. *Journal of Food Engineering 109*, 274-280.

Luna-Ramírez K.Y., Arellano-Cárdenas S., García Pinilla S. and Cornejo-Mazón M. (2017). Kinetic analysis of the stability of antioxidants in blackberry (*Rubus fruticosus* L.) liquor. *Revista Mexicana de Ingeniería Química 16*, 121-130.

Madrigal-Santillán, E., García-Melo, F., Morales-González, J. A., Vázquez-Alvarado P., Muñoz-Juárez S., Zuñiga-Pérez C., Sumaya-Martínez, M. T., Madrigal-Bujaidar E., and Hernández-Ceruelos A. Antioxidant and anticlastogenic capacity of prickly pear juice. *Nutrients 5*, 4145-4158. https://doi.org/10.3390/nu5104145

Marszalek, K., Mitek, M., and Skapska, S. (2015). Effect of continuous flow microwave and conventional heating on the bioactive compounds, colour, enzymes activity, microbial and sensory quality of strawberry purée. *Food and Bioprocess Technology 8*, 1864-1876. https://doi.org/10.1007/s11947-015-1543-7

Martínez-Flores, H., Garnica-Romo, M. G., Bermúdez-Aguirre, D., Pokhrel, P., and Barbosa-Cánovas, G. V. (2015). Physico-chemical parameters, bioactive compounds and microbial quality of thermo-sonicated carrot juice during storage. *Food Chemistry 172*, 650-656. https://doi.org/10.1016/j.foodchem.2014.09.072

Oancea, S., Grosu, C., Coman, D., and Stoia, M. (2013). Conventional and non-conventional extraction of anthocyanins from *Rubus* idaeus L. grown in Romania. *Advances in Environment Technologies, Agriculture, Food and Animal Science*, 247-251.

Official Mexican Norm (1995). Counting mould and yeast method in food. (Official Journal of the Federation NOM-111-SSA1-1994). México, D.F.

Official Mexican Norm (1995). Counting aerobic bacteria method in tray. (Official Journal of the Federation NOM-092-SSA1-1994). México, D.F.

Ortiz, M. A., Dorantes, A. L., Galindez, M. J., and Guzmán, R. I. (2003). Effect of different extraction methods on fatty acids, volatile compounds, physical and chemical properties of avocado (*Persea Americana* Mill) oil. *Journal of Agricultural and Food Chemistry 51*, 2216-2221.

Patras, A., Brunton, N. P., O'Donnell, C., and Tiwari, B. K. (2010). Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends* *in Food Science and Technology 21*, 3-11. https://doi.org/10.1016/j.tifs.2009.07.004

Quiroz-Reyes, C.N., Aguilar-Méndez M.A., Ramírez-Ortiz M.E., and Ronquillo-De Jesús E. (2013). Comparative study of ultrasound and maceration techniques for the extraction of polyphenols from cocoa beans (*Theobroma cacao* L.). *Revista Mexicana de Ingeniería Química 12*, 11-18.

Reque, P. M., Steffens, R. S., Jablonski, A., Flôres, S. H., Rios, A. O., and de Jong, E. V. (2014). Cold storage of blueberry (*Vaccinium* spp.) fruits and juice: Anthocyanin stability and antioxidant activity. *Journal of Food Composition and Analysis 33*, 11-116. http://dx.doi.org/10.1016/j.jfca.2013.11.007

Rice-Evans, C. A., Miller, N. J., and Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine 20*, 933-956. https://doi.org/10.1016/0891-5849(95)02227-9

Rodrigo, D., Loey, A., and Hendrickx, M. (2007). Combined thermal and high pressure colour degradation of tomato puree and strawberry juice. *Journal of Food Engineering 79*, 553-560.

Santhirasegaram, V., Razali, Z., and Somasundram, C. (2013). Effects of thermal treatment and sonication on quality attributes of Chokanan mango (*Mangifera indica* L.) juice. *Ultrasonic Sonochemistry 20*,1276-1282. https://doi.org/10.1016/j.ultsonch.2013.02.005

Shih, P. H., Chan, Y. C., Liao, J. W., Wang, M. F., and Yen, G. C. (2010). Antioxidant and cognitive promotion effects of anthocyanin-rich mulberry (*Morus atropurpurea* L.) on senescence-accelerated mice and prevention of Alzheimer's disease. *Journal of Nutritional Biochemistry 21*, 598-605. https://doi.org/10.1016/j.jnutbio.2009.03.008

Singleton, V. L., Orthofer, R., and Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology 299*, 152-178. https://doi.org/10.1016/S00766879(99)99017-1

Sun, C.-D., Zhang, B., Zhang, J.-K., Xu, C.-J., Wu, Y.-L., Li, X., and Chen, K.-S. (2012). Cyanidin-3-glucoside-rich extract from Chinese bayberry fruit protects pancreatic $\beta-$ cells and ameliorates hyperglycemia in streptozotocin-induced diabetic mice. *Journal of Medicinal Food 15*, 288-298. https://doi.org/10.1089/jmf.2011.1806

Teng, H., and Lee W. Y. (2013). Optimization of microwaves-assisted extraction of polyphenols from mulberry fruits (*Morus alba* L.) using response surface methodology. *Journal of the Korean Society for Applied Biological Chemistry 56*, 317-324.

Tiwari, B. K., O'Donnell, C. P., and Cullen, P. J. (2009). Effect of sonication on retention of anthocyanins in blackberry juice. *Journal of Food Engineering 93*, 166-171. https://doi.org/10.1016/j.jfoodeng.2009.01.027

Tsuda, T. (2008). Regulation of adipocyte function by anthocyanins; Possibility of preventing the metabolic syndrome. *Journal of Agricultural and Food Chemistry 56*, 642-646. https://doi.org/10.1021/jf073113b

Türkben, C., Sariburun, E., Demir, C., and Uylaser, V. (2010). Effect of freezing and frozen storage on phenolic compounds of raspberry and blackberry cultivars. *Food Analytical Methods 3*, 642-646. https://doi.org/10.1007/s12161-009-9102-3

Vauzour, D., Rodriguez-Mateos, A., Corona, G., Oruna-Concha, M. J., and Spencer, J. P. E. (2010). Polyphenols and human health: Prevention of disease and mechanisms of action. *Nutrients 2*, 1106-1131. https://doi.org/10.3390/nu2111106

Verbeyst, L., Oey, I., Plancken, V. I., Hendrickx, M., and Loey, A. (2010). Kinetic study on the thermal and pressure degradation of anthocyanins in strawberries. *Food Chemistry 123*, 269-274.

Verberic, R., Stampar, F., Schmitzer, V., Cunja, V., Zupan, A., Koron, D., and MiculicPetkovsek, M. (2014). Changes in the contents of anthocyanins and other compounds in blackberry fruits due to freezing and long-term frozen storage. *Journal of*

Agricultural and Food Chemistry 62, 6926-6935. https://doi.org/10.1021/jf405143w

Wang S. Y., and Jiao H. (2000). Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. *Journal of Agricultural Food and Chemistry 48*, 5677-5684. DOI: 10.1021/jf000766i.

Wen, Y., Chen, H., Zhou, X., Deng, Q., Zhao, Y., Zhao, C., *et al*. (2015). Optimization of the microwave-assisted extraction and antioxidant activities of anthocyanins from blackberry using a response surface methodology. *RSC Advanced 5*, 19686- 19695. https://doi.org/10.1039/C4RA16396F

Worlstad, R. E., Lee, D., and Poei, S. (1980). Effect of microwaves blanching on the color and composition of strawberry concentrate. *Journal of Food Science 45*, 1573-1577.

Yousefi, S., Emam-Djomeh, Z., Ali-Mousavi, S. M., and Askari, G. R. (2012). Comparing the effects of microwave and conventional heating methods on the evaporation rate and quality attributes of pomegranate (*Punica granatum* L.) juice concentrate. *Food Bioprocess and Technology 5*, 1328-1339. https://doi.org/10.1007/s11947-011-0603x

Zhang, L., Zhou, J., Liu, H., Khan, M. A., Huang, K., and Gu, Z. (2012). Compositions of anthocyanins in blackberry juice and their thermal degradation in relation to antioxidant activity. *European Food Research and Technology 235*, 637-645. https://doi.org/10.1007/s00217-012-1796-6

Zou , T., Wang, D., Gou, H., Zhu, Y., Luo, X., Liu, F., and Ling, W. (2012). Optimization of microwaves-assisted extraction of anthocyanins from mulberry and identification of anthocyanins in extract using HPLC-ESI-MS. *Journal of Food Science 77*, 46-50. https://doi.org/10.1111/j.1750-3841.2011.02447.x