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Short communication

Study of the Effect of Thermoultrasound on the Antioxidant Compounds and Fatty Acid Profile from Blackberry (*Rubus fruticosus spp*) Juice

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Abstract: Blackberry (*Rubus fruticosus spp*) is a fruit that has a significant antioxidant activity, due to its content in anthocyanins and antioxidant compounds. Emerging technologies are required as the thermoultrasound technique that ensures microbial reduction and release of compounds with antioxidant properties. The objective of this study was to evaluate changes in the antioxidant content and fatty acid profile of blackberry juice when it is subjected to conservation treatment (pasteurization and thermoultrasound). The blackberry juice and the extracted oil of blackberry juice with n-hexane (control, pasteurized and thermoultrasonicated) were evaluated in antioxidant activity, fatty acid profile and antioxidant content. The thermoultrasound juice had the highest ($p < 0.05$) amount of total phenols (1011 mg GAE/L), anthocyanins (118 mg Cy-3-GlE/L); antioxidant activity by ABTS (44 mg VCEAC/L) and DPPH (2665 μmol TE/L) compared to the control and pasteurized samples, as well as, as well as, oil extract of obtained of the thermoultrasound juice presented high antioxidant activity (177.54 mg VCEAC/L to ABTS and 1802.60 μmol TE/L to DPPH). The fatty acid composition of the oil extract showed the presence of myristic, linolenic, stearic, oleic, and linoleic acid, the fatty acid profile was similar in the different samples with the exception of stearic acid where this was higher in control sample. The thermoultrasound treatment can be an alternative to pasteurization treatments because keeps and releases antioxidant bioavailable compounds, preserving their fatty acids.

Keywords: Blackberry juice; Thermoultrasound; Antioxidant Compounds; Fatty Acids.

1. Introduction

The blackberry fruit is a crop with high commercial value due their sensory and chemical characteristics [1–4]. In Mexico the blackberry production usually has two destinations, a 30% as consumption in fresh and a 70% as raw material for the production of jams, candy, ice cream, yogurt, ates, wine and juice-beverage among others [5,6]. For the processing of blackberry juice typically the seed are removed; however, the seed of blackberry fruit has 6-7% of protein and 11-18% of oil, between the fatty acids that contains are 53-63% linoleic acid (C18:2), 15-31% linolenic acid (C18:3), 3-8% saturated fatty acids and others phytochemicals [7]. Besides, blackberries are of great interest due to their high content of polyphenols. All these compounds may benefit human health, can reduce the risk of coronary heart disease and cancer [8].

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On the other hand, to decrease or to kill bacteria in liquid food are used the conventional methods as the pasteurization, however, the application of heat is not suitable for most of nutrients that be found in the fruits and vegetables [9]. An alternative to preserve juice beverages are the emerging technologies, one of them is the ultrasound [10]. This technology has the potential to pasteurize several foods, as juice beverage through its exposure to high-intensity sound waves, effect that generates physical disruptions and induces chemical reactions on the material to which it is applied [11,12]. Ultrasound allows the preservation of quality parameters such as color, flavor and antioxidant properties and at the same time facilitates the release of bioactive compounds [13]. However, ultrasound alone is not very effective hence, so that combined application of ultrasound and mild temperatures between 50 and 60 °C (called thermoultrasound) to reduce the temperature and/or time of the sterilization process to prevent damage in the product have been used [14,15].

The objective of this study was to evaluate the impact of thermoultrasound compared with the pasteurization on antioxidant properties and the fatty acids profile of blackberry juice.

2. Results and Discussion

2.1. Antioxidant Activity of Juices

Different blackberry juices were developed: control (CTL), pasteurized (PAS) and thermoultrasonicated (TUS), which presented a yield of 57 ± 1% for the juice and 43 ± 1% in the residue. Subsequently juices were subjected to freeze drying obtaining a yield of extracts corresponding to each sample (about 1 g for every 10 mL of lyophilized juice). Table 1 shows the results of antioxidant properties of blackberry juice. The content of bioactive compounds and antioxidant activity were slightly maintained and even were higher ($p < 0.05$) in thermoultrasonicated sample for total phenols, anthocyanins and antioxidant activity compare with control and pasteurized juice.

2.1.1. Effect of the Thermoultrasound and Pasteurization on the Ascorbic Acid Content

The ascorbic acid content decreased in both treatments applied, where a drastic reduction of 24% in the thermoultrasonicated juice compared with pasteurized juice (9%) respect to control was observed (see Table 1). The slight decrease in pasteurized juice could be due to the oxidation reactions of ascorbic acid caused by the temperature below boiling point (72°C pasteurization). While the loss of ascorbic acid during sonication process might be by oxidative processes in aerobic and anaerobic environments associated with the production and use of hydroxyl radicals [16, 17].

2.1.2. Effect of thermoultrasound and pasteurization on Total Phenolic and Anthocyanins content of the juices

The thermoultrasonicated juice had a higher release of total phenols (1,011 mg GAE /L), which could be due to a synergistic effect between the temperature reached (see Table 1), processing time and exerted by the cavitation treatment [18]. Our results were similar to another study in watermelon juice [18], in this study a lower temperature and longer processing time were used. The juice subjected to pasteurization had a reduction of phenolic compounds, as well as, this result agree with studies with apple juice [19], where it describes that the polyphenolic compounds are degraded when the temperature of 80 °C for 15 min is reached.

Respect to anthocyanins content, the values obtained were also higher ($p < 0.05$) in thermoultrasonicated juice (118.69 mg Cy-3-GlE/L) compare with control and pasteurized juice (see Table 1), which coincides with that reported by other authors where the highest yield of extraction and release of 20% of anthocyanins in blackberry thermoultrasonicated juice was found [20]. An increase of 11.64% was obtained in thermoultrasonicated juice while pasteurized juice had a reduction of 11.23% respect to control sample.

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2.1.3. Effect of the thermoultrasound and pasteurization on antioxidant activity of the juices

Similar behaviour was observed in antioxidant activity by ABTS (Table 1), where the pasteurized juice presented a lower antioxidant activity (11.47 mg VCEAC/L) compared to the control (13.55 mg VCEAC/L) and thermoultrasonicated juice (44.69 mg VCEAC/L), the antioxidant activity by ABTS in control juice was similar to that reported in previous studies [21]. Both treatments increased the antioxidant activity by DPPH, the pasteurized juice showed values of 1320 µmol TE/L, while thermoultrasonicated juice the value was of 2656 µmol ET/L, being more than doubled in comparison to the value obtained for control juice (1147 µmol TE/L). Similar observations were reported by Zafra-Rojas et al. [13] who state that amplitude and time during treatment of ultrasound release antioxidants, and as in other fruits, the total antiradical activity in blackberry juice is the sum of the antiradical activities of all antioxidant compounds in the fruit, such as phenolic compounds, taurine, vitamins, betalains, anthocyanins content, ascorbic acid among others [13].

Table 1. Antioxidant compounds and antioxidant activity of control, pasteurized and thermoultrasonicated blackberry juice.

	CTL	PAS	TUS
Ascorbic acid mg AA/L	27.75 ± 0.83 [c]	25.23 ± 0.83 [b]	21.26 ± 0.62 [a]
Total phenols mg GAE/L	726.20 ± 4.77 [a]	789.59 ± 3.87 [b]	1011.56 ± 3.93 [c]
Anthocyanins mg Cy-3-GlE/L	106.31 ± 1.27 [b]	94.38 ± 4.23 [a]	118.69 ± 1.44 [c]
ABTS mg VCEAC/L	13.55 ± 0.17 [b]	11.47 ± 0.36 [a]	44.69 ± 1.20 [c]
DPPH µmol TE/L	1146.48 ± 1.86 [a]	1319.76 ± 1.04 [b]	2655.89 ± 14.00 [c]

[a-c] Different letters in the same line indicate significant difference (p < 0.05) between the juices in the same determination. AA= Ascorbic acid; GAE= Gallic acid equivalents; TE= Trolox equivalent; VCEAC= vitamin C equivalent antioxidant capacity; Cy-3-Gl= cyanidin-3-glucoside equivalent; CTL= Control; PAS= Pasteurized; TUS= thermoultrasound.

2.2. Antioxidant Activity and Fatty Acid Profile from the Extraccion with Hexane of Blackberry Juice

Extractions were performed subsequently with n-hexane in the control, pasteurized and thermoultrasound juices subjected to lyophilization. The yields of these extractions were of 0.12, 0.17 and 0.10 % to control, pasteurized and thermoultrasonicated juice, respectively.

2.2.1. Effect of the thermoultrasound and pasteurization on the antioxidant activity of the extracts

The fractions obtained with n-hexane were analyzed by ABTS and DPPH to find which treatment remains with the best antioxidant properties, the results of ABTS were of 63.69, 12.13 and 177.54 mg VCEAC/L to control, pasteurized and thermoultrasonicated juices respectively, the same way in the results of DPPH were obtained with 445.21, 99.45 and 1802.60 µmol TE/L. The higher values of antioxidant activity by ABTS and DPPH were in thermoultrasound treatment, this due to the disruption of biological cell walls facilitating the release of their antioxidant compounds [13] (Table 2).

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Table 2. Antioxidant activity of control, pasteurized and thermoultrasonicated extracts with n-hexane of blackberry juice.

	CTL	PAS	TUS
ABTS mg VCEAC/L	63.69 ± 1.12 [b]	12.13 ± 0.98 [a]	177.54 ± 0.69[c]
DPPH μmol TE/L	445.21 ± 1.56 [b]	99.45 ± 2.98 [a]	1802.60 ± 1.08 [c]

[a-c] Different letters in the same line indicate significant difference ($p < 0.05$) between the juices in the same determination. TE= Trolox equivalent; VCEAC= vitamin C equivalent antioxidant capacity; CTL= Control; PAS= Pasteurized; TUS= thermoultrasound.

2.2.2. Fatty acid profile from blackberry juice

Figure 1 and Table 3 shows the results of the fatty acids contained in the blackberry juice. The fatty acids identified and quantified in the control (untreated blackberry juice), pasteurized and thermoultrasonicated juice were: saturated fatty acids [myristic and stearic (C14:0 and C18:0)], mono-unsaturated fatty acid [oleic (C18:1)] and poly-unsaturated fatty acids [linoleic and linolenic (C18:2 and C18:3)], similar concentration of compounds found in fruits of *Rubus spp* [22]. The results in Table 3 shows that the thermoultrasonication not affected the concentration of fatty acids, except for stearic (C18:0) and oleic (C18:1) acids, where it a decrease is observed in the concentration of these fatty acids in a 42 and 23% respectively, similar case observed in the pasteurization process, in compared to the fatty acid profile obtained for the control juice. Cavitation is likely the main cause of oxidation with thermoultrasound as micro-bubbles form and collapse, resulting in areas of high temperature and pressure. In addition to thermal and shear force-induced oxidation, free radicals may also be generated by sonolysis thus producing an increased release of metabolites [23, 24, 25], this can be seen in Figure 1c where the appearance of new signals are shown, probably the release of phytosterols that may be responsible for the highest antioxidant activity shown by hexane extraction of juice thermoultrasonicated juice (see Table 2) as has already been reported in previous studies [26]. However, in the blackberry juice there is not off-flavors due to this reaction.

Table 3. Fatty acid composition of the oil extracted with hexane from blackberry juice subjected to treatment of pasteurization and thermoultrasonic.

Fatty acid (% w/w)	Blackberry juice oil		
	Control	Pasteurization	Thermoultrasonic
C 14:0	21.78 ± 0.01 [b]	23.75 ± 0.06 [c]	18.01 ± 0.07 [a]
C 18:0	40.45 ± 0.03 [c]	21.12 ± 0.04 [a]	23.22 ± 0.04 [b]
C 18:1	19.09 ± 0.02 [c]	14.56 ± 0.04 [a]	14.69 ± 0.03 [b]
C 18:2 n-6	7.68 ± 0.04 [b]	7.28 ± 0.01 [a]	7.82 ± 0.07 [c]
C 18:3 n-3	10.99 ± 0.05 [b]	14.91 ± 0.06 [c]	7.57 ± 0.06 [a]

[a-b] Different letters in the same line indicate significant difference ($p < 0.05$) between the juices in the same determination. Values are means SD; n=3.

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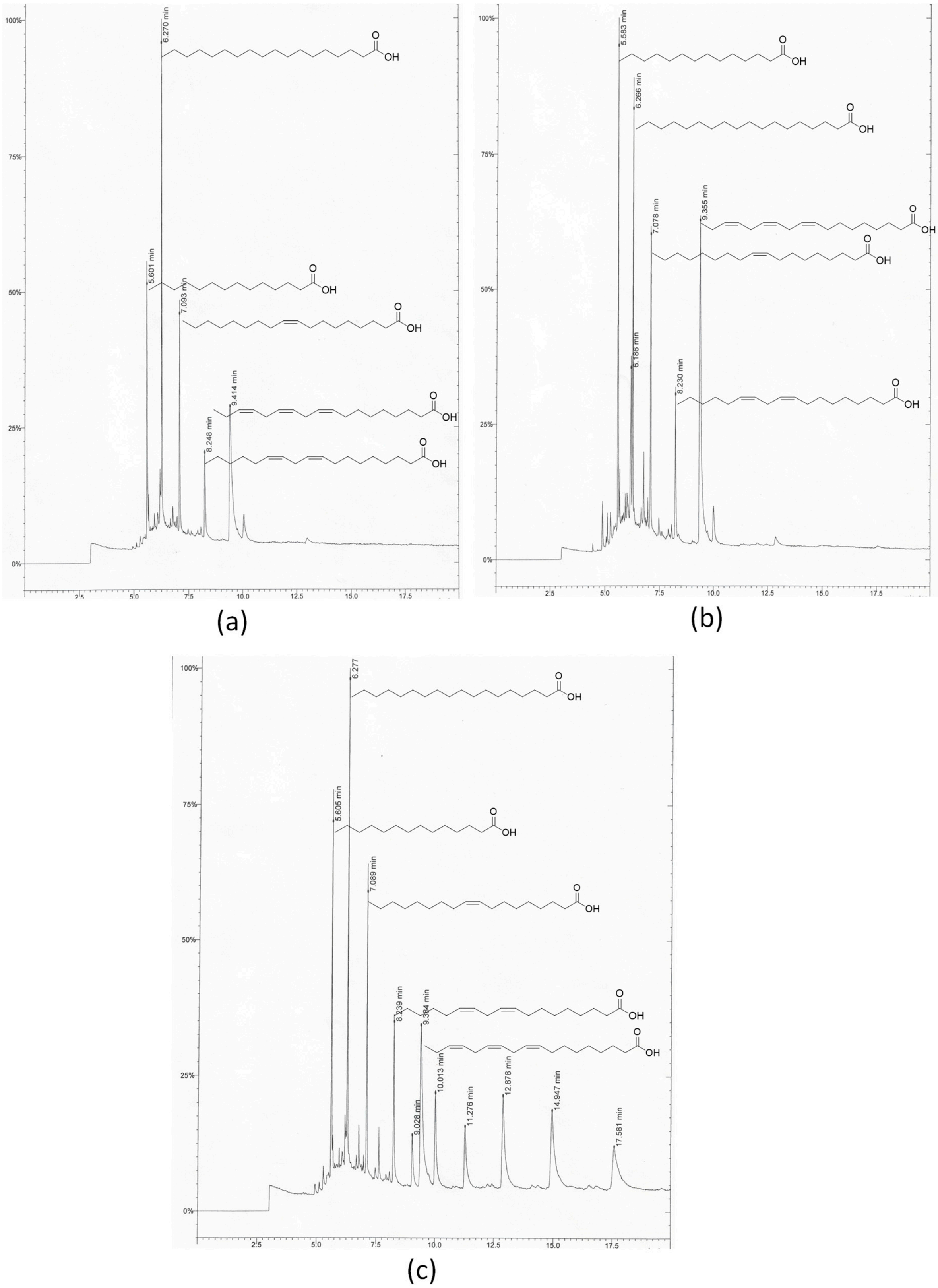

Figure 1. Chromatograms of the lipid profile of the hexane extractions performed in juices: (**a**) Control juice; (**b**) Pasteurized juice; (**c**) Thermoultrasonicated juice.

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3. Materials and Methods

3.1 Materials

Blackberries (*Rubus fruticosus spp*) were supplied from a local market in Atotonilco, Hidalgo, México, during the winter of 2014. Folin–Ciocalteu 2N reagent (Sigma-Aldrich, St. Louis, MO, USA), anhydrous sodium carbonate (Meyer, Tláhuac, DF, Mexico), gallic acid (Meyer), potassium chloride (Sigma-Aldrich), anhydrous sodium acetate (Meyer), hydrochloric acid (Reasol, Iztapalapa, DF, Mexico), 2,21azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt (ABTS) ≥ 98% (Sigma-Aldrich), potassium persulfate crystals (Meyer), Trolox 97% (Sigma-Aldrich), absolute ethanol (Meyer), 1,1-di-phenyl-2-picrylhydrazyl (DPPH) (Sigma-Aldrich). All chemicals and reagents used in the study were of analytical grade (AG).

3.2. Instruments

Ultrasound (VCX-1500, Sonics & Materials, Inc., Newtown, CT, USA), industrial blender (38BL52 LBC10, Waring Commercial, Torrington, CT, USA), refrigerated centrifuge (Allegra 25™, Beckman Coulter, Palo Alto, CA, USA), lyophilizer (7753020, LABCONCO, Kansas City, MO, USA), mill analytical (IKA® A11 basic,Wilmington, NC, USA), water bath (1210610, Cole-Parmer, Vernon Hills, IL, USA), overhead stirrer (HS-50A, Wisestir® Wisd, laboratory instruments, Seongbuk-gu, Seoul, Korea), spectrophotometric microplate reader (Power Wave XS UV-Biotek, software Gen5 2.09, Winooski, VT, USA).

3.3. Sample and treatments

Only fruits without external injuries were selected and washed. Blackberries juice was obtained by stirring the fruit using industrial blender (38BL52 LBC10, Waring Comercial, USA) and passed through a strainer to remove seeds and peel. Juice was then clarified by centrifugation at 10 000 rpm (Allegra 25™, Beckman Coulter, California, USA) for 30 min at 4 °C. A sample of 400 mL was introduced and heated in a jacket vessel at of 45 ± 1°C, overheating of the sample was prevented by running cold wáter (Cole Parmer, USA) through the treatment chamber at 4 °C and was treated by ultrasound (VCX-1500, Sonics&Materials, Inc., Newtown, USA) at 1500 W, with constant frequency of 20 kHz at 80% amplitude for 25 min with pulse duration 4 seconds on and 2 seconds off using a probe of 13 mm. This process condition of amplitude and time were obtained from a study of response surface, in which the optimal treatment conditions for juice was established where thermoultrasound process completely inactivated microorganisms and had high content of antioxidant capacity, phenolic and anthocyanins content (1076.2 and 17126.3 umol TE/L, 3318.4 mg GAE/L and 949.2 mg Cy-3-Gl/L, respetively) [27]. Untreated juice was selected as control and other juice was pasteurized (70 °C, 30 min) and included to compare results. A solid phase extraction using a 3 cc-Oasis-HLBTM cartridge (Waters Corp., MA, USA) was carried out on the juices to eliminate interferences. The filtrate was used to determinate ascorbic acid, total phenol, anthocyanins and antioxidant activity by ABTS and DPPH. On the other hand, the juices were frozen (-32 °C) and lyophilized (Labconco VWR26671-581 139 154, USA), the extractions were performed subsequently with n-hexane in the control, pasteurized and thermoultrasonicated juices. This extraction was performed with 25 g of lyophilized juices and 250 mL of n-hexane, then filtered and the supernatant concentrated yields extraction compounds were of 0.031 g, 0.042g and 0.024g to control, pasteurized and thermoultrasonicated juice, respectively, then fractions with n-hexane were obtained to analyze antioxidant activity by ABTS and DPPH, as well as, fatty acids profile.

3.4. Determination of ascorbic acid content

Acid ascorbic content of the juice was determined with 2,6-Dichloroindophenol sodium salt hydrate (DCPI).Thesamplewasdiluted in 0.4% oxalicacid, briefly, 100 µL of the juice was mixed with 100 µL of acetate buffer and 800 µL of DCPI. The absorbance of the mixture was measured at 520 nm in a microplate reader (Power Wave XS UV-Biotek, software KC Junior, USA). Ascorbic acid was

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used as a reference standard and the results were expressed as mg ascorbic acid per liter (mg AA/L) [28].

3.5. Determination of total phenolic content

Total phenolic content of the juice was determined with Folin-Ciocalteu reagent. 100 µl sample was mixed with 500 µL of 1:10 diluted Folin-Ciocalteu. After 400 µL (7.5%) of sodium carbonate was added and the mixture was incubated for 30 min at room temperature. The absorbance of the mixture was measured at 765 nm using a microplate reader (Power Wave XS UV-Biotek, software KC Junior, USA). Gallic acid was used as a reference standard and the results were expressed as mg gallic acid equivalents per liter (mg GAE/L) [29].

3.6. Determination of anthocyanins

The total monomeric anthocyanins content was measured according to the pH differential method [14]. Anthocyanin concentration was calculated based on the molecular weight (449.2) and extinction coefficient (26,900) for cyanidin-3-glucoside (Cy-3-Gl). The absorbance was measured at 510 and 700 nm in a microplate reader and the results were expressed as mg of Cy-3-Gl equivalent per liter (mg Cy-3-GlE/L).

3.7. Antiradical capacity by ABTS$^{\bullet+}$

Antiradical capacity by ABTS was measured with the radical cation 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS$^{\bullet+}$), which was produced by reacting 7 mmol/l of ABTS$^{\bullet+}$ stock solution with 2.45 mmol/L potassium persulfate under dark conditions at room temperature for 16 h before use. The ABTS$^{\bullet+}$ solution was diluted with deionized water to an absorbance of 0.70 ± 0.10 read at 754 nm measured in the microplate reader (Power Wave XS UV-Biotek, soft-ware KC Junior, USA). The results were expressed as mg Vitamin C equivalent antioxidant capacity per liter (mg VCEAC/L) of juice [30].

3.8. Antiradical capacity by DPPH•

Antiradical activity was measured using 1,1-diphenyl-2-picrylhydrazyl (DPPH•) radical as described by Morales and Jiménez-Pérez (2001) [31]. The sample was diluted in deionized water (1:50). An ethanolic solution (7.4 mg/100 mL) of the stable DPPH• radical was prepared. Then 100 µl of the sample was taken into vials and 500 µL of DPPH• solution was added, and the mixture was left to stand at room temperature for 1 h. The solution was stirred and centrifuged at 3000 rpm during 10 min. Finally, absorbance was measured at 520 nm using a microplate reader (Power Wave XS UV-Biotek, soft-ware KC Junior, USA) and the result was expressed as µmol trolox equivalent per liter (µmol TE/L).

3.9. Fatty acids profile by Gases coupled to mass spectroscopy

Fatty acids contained in n-hexane extractions of the three samples (control, pasteurized and thermoultrasonicated) of blackberry juice were identified by gas chromatograph detector temperature. MS data were acquired in EI mode, and 70 eVon a Hewlett Packard 5890 Series II spectrometer with a scan range of 20 – 601m/z. Identification was confirmed by the retention time lock (RTL) of the fatty acids samples compared with a standard of 37-components (Food Industry FAMEs Mix, Restek).The standard product used was a mix of methyl esters with chains C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C14:1, C15:0, C15:1, C16:0, C16:1, C17:0, C17:1, C18:0, C18:1n-9c, C18:1n-9t, C18:2n-6c, C18:2n-6t, C18:3n-6, C18:3n-3, C20:0, C20:1n-9, C20:2, C20:3n-6, C20:3n-3, C20:4n-6, C20:5n-3, C21:0, C22:0, C22:1n-9, C22:2, C22:6n-3, C23:0, C24:0 and C24:1n-9) to comparison of fragmentation patterns. All determinations were carried out in triplicates.

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3.10. Statistical analysis

All values were obtained by triplicate and expressed as mean ± standard deviation (SD). Data were analyzed by one-way analysis of variance (ANOVA), and differences among means were determined using Tukey test with a level of significance of $p < 0.05$. The statistical package SPSS® System for WINTM version 15.0 was used.

4. Conclusions

Blackberry juice is a valuable raw material that can be utilized for oil recovery. The thermoultrasound process could be recommended as a method of conservation of the blackberries juice, however more research is required about the impact on the quality of the oils, plus if this technology retains all its nutritional properties.

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Author Contributions: J. Jesús Manríquez-Torres and Esther Ramírez-Moreno conceived, designed the experiments and analyzed the data, in addition to serving as directors of thesis J. Antonio Sánchez-Franco; J. Antonio Sánchez-Franco performed the experiments; J. Alberto Ariza-Ortega and J. Martín Torres-Valencia contributed reagents/materials/analysis tools; N. S. Cruz-Cansino contributed to valuable discussion.

Conflicts of Interest: The authors have declared no conflict of interest.

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