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Effects of Ultrasound versus Pasteurization on Whey–Oat Beverage Processing: Quality and Antioxidative Properties

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Abstract: The consumption of functional beverages is rapidly increasing. The improvement in the functional properties of whey after the application of ultrasound is due to the release of bioactive peptides that have antioxidant properties, among others. Bioactive peptides with antioxidant activity have also been found in oats, stimulating the study of whey beverages formulated with oats to obtain functional products. The aim of this study was to determine the influence of ultrasound (24 kHz) at 20 °C for 15 min at 23 W and 154 W on the quality and functional properties of whey–oat (50:50 *v*/*v*) beverages and compare it with pasteurization at 65 °C for 30 min (LTLT). Non-significant effect ($p > 0.05$) of ultrasound intensity (23 W and 154 W) was observed on the physicochemical characteristics and the proximal composition of the whey–oat beverages. The sonicated beverages showed a greater tendency to green and yellow color ($p < 0.05$), higher fat content ($p < 0.05$), and less ash and carbohydrates ($p < 0.05$) than the pasteurized beverage. The antioxidant activity of the mM Trolox equivalent/mL of the sonicated beverages was higher ($p < 0.05$) (4.24 and 4.27 for 23 W and 54 W, respectively) compared to that of the pasteurized beverage (4.12). It is concluded that ultrasound is superior to pasteurization in improving the antioxidant activity of whey–oat beverages without having a detrimental impact on the proximal composition and physicochemical quality. Future studies should evaluate more functional parameters and determine the shelf life of sonicated whey–oat beverages.

Keywords: antioxidant activity; dairy beverages; whey; oats; ultrasound

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1. Introduction

The demand for less-processed foods has boosted the search for and evaluation of conservation technologies that keep or improve the nutritional, functional, and sensory characteristics of food. Among the new technologies that have been developed in the food industry is ultrasound. Ultrasound technology is economical and environmentally friendly [1] and has been applied to a variety of foods such as fruit juices, meat, cereals, and dairy products [2–5]. It has been reported that ultrasound can equal the innocuousness of dairy products that have been pasteurized without a loss or deterioration of physicochemical properties and nutritional quality. The use of ultrasound in whey has been extensively studied to improve its safety and functional and nutritional properties [6,7]. In milk, ultrasound reduces the size of fat globules, which improves the homogenization and stability of the milk during storage [8]. This size reduction is totally dependent on ultrasound power and temperature [9]. In addition, it has been shown that ultrasound pre-treatment has a greater effect on structural proteins than the effect of temperature or exposure time [10]. This effect was due to induced positive structural changes in whey proteins that could make them more susceptible to enzymes. Therefore, ultrasound can

be used as a tool that promotes bioactive peptide production [10]. The improvement in functional properties of whey after ultrasound application is due to the release of bioactive peptides or biopeptides [11].

Whey proteins have certain physiological benefits, which are attributed to special peptide sequences encrypted throughout the parent proteins. These peptide sequences are commonly named biopeptides and are whey protein hydrolysates or partially hydrolyzed α-lactalbumin [12]. Whey biopeptides have antihypertensive, hypocholesterolemic, and antioxidant characteristics [10]. Furthermore, compounds with antioxidant and antihypertensive activity have also been found in oats [13,14], inspiring the study of whey drinks formulated with oats to obtain functional products [15]. Based on the above, the objective of this study was to evaluate the effect of ultrasound on whey–oat beverage processing with a special focus on antioxidant and physicochemical properties of the formulated beverages.

2. Materials and Methods

2.1. Whey Beverage

The whey beverage was made, as reported by Herrera-Ponce et al. [16]. Briefly, the whey was obtained from Holstein cows' milk. The milk was pasteurized at 65 °C for 30 min, left to cool at 42 °C, and $CaCl_2$ (20 g/100 L) was incorporated, stirred, and cooled to 34 °C for renin (Cuamix® CHR Hansen, Mexico City, Mexico) addition (15 mL/100 L). Then, it was left to stand for 90 min at 32 °C. After the curd was set up and cut into 1 cm^2 pieces, it was left to rest for 15 min, and the size of the milk clots was shortened. Then, the temperature was gradually increased to 39 °C and allowed to stand again for 15 min. The curds were drained, and the obtained whey was placed in glass jars and cooled at room temperature.

2.2. Oats Beverage

The oat beverage was made, as reported by Herrera Ponce et al. [17]. The oat flours were prepared with oat (*Avena sativa*) var. Karma cultivar was purchased from a local market. Oat grains were subjected to soaking and swelling (12 h at 28 ± 2 °C), then the germination was carried out for 72 h at 25 ± 2 °C under artificial lighting. After germination, the oats were dried in an oven (SLGS/12SC Southbend, IN, USA) (3 h at 95 ± 2 °C). The malted oat was ground in a hammer mill (Retsch. Model LC-170. Haan, Germany) and mixed at a 5% w/v in water, and then heated (at 90 °C for 60 min) and cooled (at 50 °C for 30 min) in order to increase the oat starch viscosity [18]. The beverage was stored for 12 h (at 4 °C), decanted, and sterilized (15 min at 120 °C). The oat beverage was allowed to cool at room temperature (28 ± 2 °C) and then refrigerated (4 °C) overnight or until used for the whey–oat beverage preparation.

2.3. Beverages Formulation

All of the whey–oat beverages were prepared with 50% (v/v) of whey and 50% (v/v) oat beverage. During formulation, 1.5% (w/v) of cane sugar (Zulka®, Culiacan, Mexico) and 1.5% (v/v) vanilla (Molina®, Zapopan, Mexico) were added to all of the beverages. Three treatments were designed: 23 W = whey–oat beverage treated with 23 W; 154 W = whey–oat beverage treated with 154 W; LTLT, whey–oat beverage treated with pasteurization at 65 °C for 30 min. The study trial was replicated three times; therefore, the whole experiment was carried out in triplicate.

2.4. Beverages Sonication

The beverage sonication was carried out following the method described by Herrera-Ponce [19]. The whey–oat beverages (500 mL) were placed in glass beakers (1000 mL) and sonicated using an ultrasonic processor (Ultrasonic Processor Hielscher UP400s, Germany) fitted with a titanium sonotrode (Hielscher Sonotrode S24d22D, Teltow, Germany) placed at a 3 cm distance from the medium surface. Power ultrasound was applied at 23 W and 154 W using 24 kHz of frequency [20] for 15 min. The glass beaker containing the beverage

was kept inside an ice-water bath to maintain a constant temperature of 25 °C during HIU application.

2.5. Physicochemical Properties

The pH was recorded randomly at three locations using a pH meter (99163, Hanna Instruments, Woonsocket, RI, USA). The measurement depth was 5 cm. Three random readings were taken, and the averages were recorded. The titratable acidity was determined following the procedure of the A0AC [21]. Briefly, 40 mL of boiled and cooled distilled water and 2 mL of phenolphthalein (prepared at 1% in 95% ethanol) were added to 20 mL of the beverage sample. The mixture was titrated with 0.1 M NaOH until the first color change (to pink) persisted for 30 s.

The surface color was measured with a Minolta Chromameter (CR-400 Konica Minolta Camera, Minolta Sensing, Inc., Tokyo, Japan). Using an 8-mm aperture, the following standards (Illuminant C. Standard observer) C: Y = 94.00, x = 0.3155 and y = 0.3318, were used following the CIE Lab methodology [22] considering L*, a*, b*, and C* coordinates. The color values were expressed as L* (brightness/darkness), a* (redness/greenness), b* (yellowness/blueness), and C* (chroma) $(a^{*2} + b^{*2})^{1/2}$.

The proximal analysis of the whey–oat beverages was performed following the AOAC procedures [21] for the determination of moisture (method 926.08), ash (method 945.46), protein (method 991.20), fat (method 989.05), and carbohydrates (%carbohydrates = 100 − %moisture − %protein − %fat − %ash).

2.6. Soluble Extract

The soluble extract for the antioxidant activity determination was prepared following the method used by Herrera-Ponce et al. [19]. Then, 40 mL of whey samples were collected, stirred, and centrifuged twice (Beckman Coulter. Model Avanti J-26 XP1. Series JXT12H22. Indianapolis, IN, USA) at 4000× *g* for 30 min at 4 °C. The supernatant was passed through 2.5-µm Whatman filter paper and then filtered through membrane filters with a pore size of 2 µm. The supernatant was collected in conical tubes and stored at −20 °C until further analysis.

2.7. Antioxidant Activity

The antioxidant activity was carried out by the 1,1-diphenyl-2-picrilhydrazil (DPPH) radical method [23] used by Herrera-Ponce [19]. Briefly, a 0.6 mM methanolic stock solution of DPPH (J.T. Baker®, Aguascalientes, Mexico) was prepared, and stored in an amber bottle at −20 °C until use. Ten milliliters of the stock solution were taken and mixed with 45 mL of methanol to reach an absorbance of 1.1 ± 0.02. For the determination, 150 µL of the sample (or standard: Trolox) and 2850 µL of the DPPH working solution were placed in amber flasks and left to stand for 24 h in the dark at 25 °C. Subsequently, the absorbance at 515 nm was read on a UV spectrophotometer (UV-1800. Shimadzu, Japan). The standard curve was linear between 25 and 800 mM Trolox. The antioxidant activity was expressed as the equivalent of Trolox concentration by the regression analysis of the plotted chemiluminescence peak time versus sample dry matter (mM Trolox/g).

2.8. Statistical Analysis

All of the analyses were carried out in triplicate. The data on pH, titratable acidity, color (L*, a* and b*), proximal analysis, and the antioxidant activity in the whey–oat beverages were analyzed by one-way analysis of variance (ANOVA) using the GLM procedure of the Statistical Analysis Software (v9.0, SAS Institute Inc, Cary, NC, USA) [24], with a confidence interval of 95%. The difference between the samples was determined using Tukey tests, and the significance was declared to be $p < 0.05$.

3. Results and Discussion

3.1. Physicochemical Properties

The effects of ultrasound power and pasteurization on the physicochemical characteristics of whey–oat beverages are shown in Table 1. Except for luminosity (L*), titratable acidity, and pH, there was a difference ($p < 0.05$) between the sonicated and the pasteurized beverages in most of the properties analyzed. In the current study, the ultrasonic beverages had a higher greenness and yellowness than the control beverage. This effect could be due to the carotenoids released during the breakdown of fat globules, as these pigments are responsible for the yellowish color in milk [25]. Guimarães et al. [26] applied ultrasound (19 kHz for 3 min at 200, 400, or 600 W) or pasteurization (75 °C for 15 s) to whey and inulin beverages and found that the sonicated samples had lower L*, a*, and C*, and higher b* than the untreated or pasteurized samples. The same was reported by Komes et al. [8] when applying ultrasound (30 kHz and 60% amplitude) for 5 or 10 min to goat's milk. The application of ultrasound for 10 min decreased the milk color parameters (L*, a*, and b*) compared to 5 min of ultrasound. Therefore, high-power ultrasound could negatively impact the milk color, and this could make the final product undesirable to consumers. The sensory analysis could help to explain such an effect. Additionally, some additives could counter the negative effect of HIU on beverage color. Recently, Krasulya et al. [27] reported that food systems containing milk whey subjected to HIU treatment for 15 min after adding a fruit filler (in particular, raspberry puree) have a more intense bright color compared to a sample containing untreated milk whey and fruit filler.

Table 1. Effect of ultrasound power and pasteurization on physicochemical characteristics of whey–oat beverages.

Characteristic [1]	Treatment [1]		
	23 W	154 W	LTLT
L*	24.80 ± 0.19 [a]	24.66 ± 0.16 [a]	25.00 ± 0.34 [a]
a*	−0.34 ± 0.05 [a]	−0.39 ± 0.04 [a]	−0.42 ± 0.07 [b]
b*	4.62 ± 0.03 [a]	4.59 ± 0.03 [a]	4.42 ± 0.07 [b]
C*	4.64 ± 0.03 [a]	4.61 ± 0.03 [ab]	4.44 ± 0.07 [b]
Titratable acidity (%)	0.01 ± 0.00 [a]	0.01 ± 0.00 [a]	0.01 ± 0.00 [a]
pH	6.37 ± 0.02 [a]	6.40 ± 0.01 [a]	6.39 ± 0.01 [a]

[1] 23 W = whey–oat beverage treated with 23 W; 154 W = whey–oat beverage treated with 154 W; LTLT, whey–oat beverage treated with pasteurization at 65 °C for 30 min. [a,b] Mean with different literal within the same row denote significant difference ($p < 0.05$). L* is a measure of darkness to lightness (greater L* values indicate a lighter color); a* is a measure of redness or greenness (positive a* values indicate a redder color, negative a* values indicate a greener color); and b* is a measure of yellowness or blueness (positive b* values indicate a more yellow color and negative b* values indicate a bluer color). Chroma (C*) is a measure of the total or vividness of color (greater C* values indicate greater total color and/or a more vivid color). Expressed as the percentage of lactic acid concentration. Data = mean ± standard error of three repetitions.

The ultrasound power had no effect on titratable acidity (0.1%) or pH (6.37–6.40) (Table 1). Similar results were reported by Komes et al. [8], who did not find a significant impact ($p > 0.05$) of ultrasound (30 kHz and 60% amplitude for 5 min) on goat's milk pH, with pH values around 6.6. Furthermore, Jambrak et al. [28] did not observe differences in the pH of the samples with an ultrasound application (20 kHz and 43–48 W/cm^2 for 15 or 30 min). Barukčić et al. [29] reported similar acidity values of 0.074% for whey fermented with a pasteurization pre-treatment (65 °C for 30 min) or ultrasound (20 kHz) at 45 °C for 8 min and 480 or 600 W. From this, it can be concluded that whey–oat beverages do not change in pH and acidity by ultrasound application, and this enhances its shelf life.

3.2. Proximal Composition

The proximal composition of the whey–oat beverages is shown in Table 2. There was no effect ($p > 0.05$) of ultrasound power on the water content of the beverages, with values of 93.92 ± 0.018% and 93.94 ± 0.024%, for 23 and 154 W, respectively. However, these

values were higher ($p < 0.05$) than the water content of the pasteurized beverage, with a content of 93.58 ± 0.173%. It has been reported that high-intensity ultrasound changes the structure of proteins in such a way that the internal hydrophilic parts are exposed [30–32]. In addition to this, Jambrak et al. [33] used ultrasound (20 kHz, 43–48 W/cm^2) for 15 or 30 min in whey protein and found that the molecular weight of the proteins decreased. They observed changes in the three-dimensional structure of proteins, which caused an increase in the charged groups (NH_4^+, COO^-), showing that the water–protein interactions increased. This means that ultrasound helped to increase water retention from beverages.

Table 2. Effect of ultrasound power and pasteurization on proximal composition of whey–oat beverages.

Component (%)	Treatment [1]		
	23 W	**154 W**	**LTLT**
Water	93.92 ± 0.02 [a]	93.94 ± 0.02 [a]	93.58 ± 0.17 [b]
Fat	0.99 ± 0.04 [a]	0.92 ± 0.04 [a]	0.76 ± 0.07 [b]
Ash	0.29 ± 0.01 [b]	0.29 ± 0.01 [b]	0.33 ± 0.01 [a]
Protein	3.32 ± 0.07 [a]	3.33 ± 0.07 [a]	3.21 ± 0.06 [a]
Carbohydrates	1.36 ± 0.11 [b]	1.55 ± 0.09 [b]	2.36 ± 0.04 [a]

[1] 23 W = whey–oat beverage treated with 23 W; 154 W = whey–oat beverage treated with 154 W; LTLT, whey–oat beverage treated with pasteurization at 65 °C for 30 min. [a,b] Mean with different literal within the same row denote significant difference ($p < 0.05$). Data = mean ± standard error of three repetitions.

Regarding the amount of fat, there was no difference ($p > 0.05$) between applying ultrasound (40 kHz and 11 W/cm^2 for 15 min) at 23 or 154 W (0.99 and 0.92%, respectively). On the other hand, the amount of fat in the ultrasonicated beverages was significantly higher ($p < 0.05$) than that of the pasteurized beverages (0.76% fat) (Table 2). Previous studies have found that pasteurization has no effect on milk fat [34], while ultrasound reduces the size of fat globules in milk [35] due to the implosion of bubbles generated by acoustic cavitation, which generates high-intensity shock waves. This leads to homogenization, causing a greater number of fat globules per unit of volume but which are smaller in size. So, this was probably the cause of the higher fat content in the sonicated samples. It has been reported that the decrease in the size of fat globules depends mostly on time [35,36] and the amplitude of ultrasound [37].

Gregersen et al. [9] observed that the degree of homogenization depends on temperature and ultrasound power. These authors used a power of 10, 30, or 50 W with temperatures of 27, 50, or 70 °C. In the present study, the temperature of the beverages remained less than 25 °C. In fact, those authors commented that the reduction in fat was minimal at a low temperature (27 °C) and/or power (10 W). Shanmugam et al. [35] found no difference in the size of the fat globules of ultrasonic milk (20 kHz) at 90 or 18 W for 15 min. However, Monteiro et al. [38] found differences in the size of the fat globules when applying 160 W for 937 s or 720 W for 208 s at 19 kHz. This can lead to differences in the percentage of the fat content, which can be confirmed, for example, by comparing the results of this research with a fat range of 0.92 to 0.99% versus 0.43% in the study by Tranjan et al. [39] in whey beverages with strawberry and mango, without ultrasonic treatment.

The ash content was greater ($p < 0.05$) in the pasteurized beverage (0.33%) than the ultrasonic beverages (0.29%) with no difference among the sonicated ones ($p > 0.05$) (Table 2).

The use of high-intensity ultrasound is needed to produce a cavitation effect, which increases the solubility of organic or inorganic salts [40], and this is reflected in the decrease in ash contents observed in ultrasound-treated samples from this study.

Additionally, Monteiro et al. [38] found no difference in the mineral content when applying 160 or 720 W of ultrasound (19 kHz) for 937 or 208 s, respectively, in a milk drink with chocolate. A similar value of 0.30% ash was reported by Baba et al. [41] in a drink with 30% v/v whey and 70% v/v pineapple juice, and no effect on the ash content was reported due to the amount of ultrasound energy applied.

There was no effect of ultrasound or pasteurization ($p > 0.05$) on the protein content of whey–oat beverages, showing values of 3.32% and 3.33% when sonicated at 23 W or at 154 W, respectively. In contrast, the pasteurized beverage showed 3.21% protein (Table 2). Similarly, Gregersen et al. [9] found no difference in the protein content, as well as in α-lactalbumin and β-lactoglobulin, when the milk was sonicated (20 kHz) for 30 min at 10, 30, or 50 W. These authors found that the denaturation of α-lactalbumin and β-lactoglobulin is not directly proportional to the power used. Jambrak et al. [28] reported that the solubility of the whey proteins increased with ultrasound (20 kHz) by 15 and 30 min and was attributed to changes in the tertiary structure of the proteins. On the contrary, Barukčić et al. [29] found different protein contents in the whey after applying pasteurization (65 °C per 30 min) or ultrasound (20 kHz) at 60 W, 45, or 55 °C per 10 min. The protein contents observed were 0.56 ± 0.13% and 0.43 ± 0.09%, respectively. However, on the basis that the protein was quantified by the Kjeldahl method [21], which only determines the amount of nitrogen and not the proportion of denatured proteins, it cannot be concluded whether the treatments applied to the whey–oat beverages influenced the denaturation.

3.3. Antioxidant Activity

The antioxidant activity (mM Trolox equivalent/mL) of the whey–oat beverages is shown in Table 3. The antioxidant activity of the sonicated whey–oat beverages was higher ($p < 0.05$) than that of the pasteurized beverage (4.12) without the effect ($p > 0.05$) of the ultrasound power (4.24 and 4.27 for 23 W or at 154 W). Several studies have reported that ultrasound generates a higher antioxidant capacity than pasteurization. Guimarães et al. [42] observed that the antioxidant activity of a prebiotic drink of soursop and whey increases with ultrasound power. In their study, the best antioxidant activities were observed at 600 and 400 W (79.5% and 78%, respectively) compared to that at 200 W (63%). This activity was significantly lower ($p < 0.05$) when applying pasteurization at 75 °C for 15 s with 46 ± 2.0% of antioxidant activity. Abadía-García et al. [10] reported that ultrasound (20 kHz and 750 W) could be used as a pre-treatment for the enzymatic hydrolysis of whey protein with papain. These authors found that the conditions that generated the greatest antioxidant capacity were 52.5 °C at 0.092 W/mL for 15 min (22.31%) and 12 °C at 220 W/mL for 15 min (22.10%), while the control, without pre-treatment, showed less antioxidant activity (19.62%). Liu et al. [43] reported a lower ($p < 0.05$) antioxidant activity when a whey protein isolate was heated for 180 min than when thermosonication (20 kHz, 110 W/cm^2) was applied at 90 °C for 60 min with values of 38.97% and 44.85%, respectively. Interestingly, the antioxidant activity values observed in the present study were higher than those reported in the whey protein hydrolysates in the milk beverage system [44].

Table 3. Effect of ultrasound power and pasteurization on the antioxidant activity of whey–oat beverages (mean ± SE).

Treatment [1]	23 W	154 W	LTLT
Antioxidant activity [2] (mM Trolox equivalent/mL)	4.24 ± 0.03 [a]	4.27 ± 0.01 [a]	4.12 ± 0.03 [b]
Antioxidant activity[2] (%)	77.05 ± 0.01 [a]	76.51 ± 0.01 [a]	74.25 ± 0.01 [b]

[1] 23 W = Ultrasound application at 23 W (24 kHz for 15 min). 154 W = Ultrasound application at 154 W (24 kHz for 15 min); LTLT = Pasteurization at 65 °C for 30 min. [2] Measured by the ABTS radical method. [a,b] Means with different literal denote significant difference ($p < 0.05$). Data = mean ± standard error of three repetitions.

HIU induces the degradation and structural destruction of whey proteins, enhancing the generation of peptides with antioxidant activity [45]. Therefore, it could be expected that an increase in bioactive peptides due to an ultrasound treatment to whey, and this is a great advantage compared to pasteurized beverages.

Two techniques based on free radicals that are commonly used to assess in vitro antioxidant activity are 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) [46]. In the present study, we reported the antioxidant

activity using the ABTS methodology; however, we also used DPPH and observed that the antioxidant activity of whey–oat drinks obtained with the ABTS was greater than DPPH (data not shown in Table). The antioxidant activity values obtained with DPPH were 0.361 ± 0.023, 0.321 ± 0.034 and 0.141 ± 0.012 mM Trolox equivalent/mL in ultrasound-treated drinks at 154 W and 23 W and pasteurized drink, respectively. However, it is relevant to mention that the same statistical tendency was observed with both techniques, where the pasteurized samples show lower values than the sonicated samples. Floegel et al. [47] demonstrated that ABTS was better than that of DPPH in products containing hydrophilic compounds and lipophilic and antioxidant pigments. In addition, most of the studies that use the ABTS technique report the results as a percentage of the inhibition instead of reporting data based on a compound with high antioxidant activity such as Trolox or gallic acid.

4. Conclusions

Today there is a growing interest in assisting food processing with ultrasound, which leads to positive or minimal effects on the nutritional and functional properties of foods. The aim of the present study was to determine the effect of ultrasound on whey–oat beverage processing with a special focus on the antioxidant and physicochemical properties.

Our study demonstrated that HIU was superior to pasteurization for improving the antioxidant activity of whey–oat beverages without detrimental effects on the physicochemical and proximal quality. Ultrasound application either at 23 W or 154 W significantly increases the antioxidant activity of whey–oat beverages promoting, in this way, the functionality of the beverages. The novelty of this work relies firstly on the combination of whey and oat to formulate a new beverage product and secondly on the application of ultrasound as a non-thermal processing method for enhancing beverage quality.

Finally, it is recommended to evaluate more of the functional parameters, compare the sensory characteristics, and determine the shelf-life of the sonicated whey–oat beverages.

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