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Research Article

Optimization of Ultrasound Extraction of Cactus Pear *(Opuntia ficus indica)* Seed Oil Based on Antioxidant Activity and Evaluation of Its Antimicrobial Activity

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The purpose of the present study was to determine the optimal ultrasound conditions (amplitude level and time) for the extraction of cactus pear seed oil with the highest antioxidant activity using a closed system. Seed oil was analyzed for yield, antioxidant activity by ABTS and DPPH, and antimicrobial activity. Conventional extraction methods were assessed for comparison. Amplitude level significantly affected antioxidant activity in linear terms ($p < 0.0001$ DPPH and $p < 0.001$ ABTS, resp.) so, at lower amplitudes, the higher antioxidant activity was achieved. The optimum ultrasound extraction conditions were of 78% amplitude for 10 min and yielded antioxidant activity values of 66.25 mg AAE/100 g and 289 μmol TE/100 g for ABTS and DPPH, respectively. Compared with conventional extraction methods, ultrasound exhibited lower oil yield and antioxidant activity but had the potential to achieve comparable results if multiple ultrasound extractions are performed in the time needed by conventional methods. Seed oils showed similar antimicrobial activity despite the extraction method and were more effective against *Escherichia coli*. The results demonstrated that ultrasound can be an alternative extraction method of seed oils from fruits such as cactus pear.

1. Introduction

Cactus pear fruit *(Opuntia ficus indica)* is common in arid and semiarid regions around the world [1]. This fruit that is mainly consumed fresh in Mexico [2] is composed by pulp, peel, and seeds [3]. According to several studies cactus pear fruit has bioactive compounds [4, 5] with high antioxidant and antimicrobial activity [6, 7]. Some of these compounds are found in the seeds [8], which comprise 3 to 15% of the cactus pear pulp [9] and are usually considered waste after pulp processing [7]. Seeds also have a high content of oil (98.8 g/kg) [10] characterized by high levels of linoleic and oleic acids [7] and other components as phenols [11], all which may contribute to human health [12]. Currently, seed oils have been used as natural agents for food preservation [13], and many have exhibited antimicrobial and antioxidant activity; some of these oils are from pumpkin [14], apple [15], black cumin [16], and basil [17] among other seeds. Cactus pear seeds from *Opuntia dillenii* also have a high antioxidant activity derived from bioactive compounds such as polyphenols and polyunsaturated fatty acids [18]. Some polyunsaturated fatty acids have also been identified in seeds from *Opuntia ficus indica* [19, 20] implying that these seeds may also have high antioxidant activity.

Seed oil is usually extracted by means of conventional methods such as Soxhlet and maceration, using heat, agitation, or long extraction times [21]. Microwave, supercritical fluids, and ultrasonic assisted extraction are unconventional methods that exert a physical effect on the sample [22]. Ultrasound has been used to extract antioxidants from many food materials including seeds. Ultrasound and ultrasound-assisted extractions use sound waves to produce cavitation

microbubbles that collapse violently in the sample and facilitate the release and extraction of several compounds [23–25]. Some researchers had evaluated ultrasound-assisted extraction, in an open system, using a sonicator probe directly on the liquid sample to obtain seed oil from flaxseed [26], Korean pine [27], and pomegranate [28]. The purpose of the present study was to optimize the extraction conditions of cactus pear seed oil using ultrasound in a closed system based on antioxidant activity and using response surface methodology. Yield extraction and antioxidant and antimicrobial activity were compared with conventional methods.

2. Materials and Methods

2.1. Sample. Green cactus pear *(Opuntia ficus indica)*, Reyna variety, was provided by the Mexican Association CoMeN-Tuna (Consejo Mexicano del Nopal y la Tuna A.C. of Actopan, Hidalgo, México) in spring of 2012. The green cactus pear seeds were obtained after several washes with water that removed the pulp and residues. The seeds were leaved to dry at ambient temperature until they reached a moisture of 6.43%. After the seeds were crushed using an industrial mill (Cyclotec 1093, Tecator, Höganäs, AB, Sweden), the powder was passed through a mesh sieve to obtain a particle size of approximate 0.5 mm and then stored in sealed plastic bags at room temperature and dark conditions.

2.2. Ultrasound Extraction. Ultrasound (VCX-1500, Sonic & Materials, Inc. Newtown, CT, USA) at 1500 W, with a constant frequency of 20 kHz and a probe of 25 mm, was used for the extraction of green cactus pear seeds oil. Extraction from milled and sieved seeds (20 g) was carried out at an amplitude and time ranges of 80 to 90% and 5 to 15 min, respectively, and a fixed outlet temperature of 25°C. A sample of 400 mL was introduced in a jacketed vessel with water at 4°C circulating through the secondary layer [29]. After extraction, the aqueous and solid phases were separated by filtration using a vacuum pump (DOA-P704-AA, GAST Manufacturing, Inc., Benton Harbor, MI, USA). Both phases, aqueous and solid (this last was dried) (Weston 74-1001-w, Weston Products, L.L.C. Strongsville, OH, USA), were mixed with hexane for 30 min and then separated from the solvent by filtration. The aqueous phase was centrifuged (Allegra 25R, Beckman Couler, CA, USA) at 10,000 rpm for 30 min at 4°C and was stored in plastic containers and kept frozen until analysis. The solid phase was stored in hermetically sealed bags in the dark. The solvent obtained from the two phases was evaporated (BÜCHI Labortechnik AG, Flawil, SG, Switzerland) at 40°C to obtain the oil.

2.3. Soxhlet Extraction. Soxhlet extraction was performed according to the AOAC [30]. Milled and sieved seeds (5 g), hexane (120 mL), and a universal fat extraction system (Büchi Labortechnik AG, Flawil, SG, Switzerland) were used.

2.4. Maceration Extraction. Milled and sieved seeds (10 g) were introduced in a previously defatted cotton bag and then immersed in 200 mL of hexane in a closed glass at a temperature of ≈25°C. After the sample was stored in a dark place for 24 hrs, the oil was obtained after solvent evaporation using a rotary evaporator (BÜCHI Labortechnik AG, Flawil, SG, Switzerland) at 40°C.

2.5. Yield. Oil yield was determined according to Chougui et al. [31], using the following equation:

$$\text{Oil}\,(\%) = \left(\frac{M_1 - M_0}{M_2}\right) \times 100, \quad (1)$$

where M_0 is the weight of the empty Eppendorf tube (g), M_1 is the weight of the Eppendorf tube after evaporation (g), and M_2 is the weight of the milled seeds (g).

The oil was stored in 2 mL amber Eppendorf tubes at −32°C until analysis.

2.6. Determination of Antioxidant Activity

2.6.1. ABTS Assay. Antiradical capacity by ABTS was measured according to Kuskoski et al. [32]. The radical cation 2,2′azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$) was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate in the dark at room temperature for 16 hrs before being used. The $ABTS^{\bullet+}$ solution was diluted with deionized water to an absorbance of 0.70 ± 0.10 at 754 nm. An aliquot of 20 μL of sample was added to 980 μL of the diluted $ABTS^{\bullet+}$ solution, and absorbance readings were taken after 7 min incubation at room temperature. The absorbance of the mixture was measured at 754 nm in the microplate reader (Power Wave XS UV-Biotek, software KC Junior, VT, USA), and antioxidant capacity was expressed as mg ascorbic acid equivalent per 100 g of oil (mg AAE/100 g).

2.6.2. DPPH Assay. Antiradical activity was measured using 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) radical as described by Morales and Jiménez-Pérez [33]. A methanol-acetate solution (7.4 mg/100 mL) of the stable $DPPH^{\bullet}$ radical was prepared. A sample aliquot of 100 μL was placed into vials and 500 μL of $DPPH^{\bullet}$ solution was added before the mixture was left to sit at room temperature for 1 hr. Finally, absorbance was measured at 520 nm in the microplate reader (Power Wave XS UV-Biotek, software KC Junior, Winooski, VT, USA), and antioxidant activity was expressed as μmol of Trolox equivalents per 100 g of oil (μmol TE/100 g).

2.7. Experimental Design

2.7.1. Optimization. The optimization of the ultrasound extraction conditions was performed using the response surface methodology (RSM) with a central composite rotatable design for two independent extraction variables at five levels. The independent extraction variables (amplitude level: 80–90%; time: 5–15 min) were determined based on preliminary experiments where higher antioxidant activity by ABTS and DPPH was observed. Design consisted in thirteen combinations with five central points replicates (Table 1). Experimental data were subjected to multiple nonlinear regression

Table 1: Experimental design matrix.

Number[a]	Pattern	Amplitude level (%) X_i	Time (min) X_j
(1)	00	85	10
(2)	+−	90	5
(3)	++	90	15
(4)	00	85	10
(5)	A0	92	10
(6)	− −	80	5
(7)	00	85	10
(8)	a0	78	10
(9)	0A	85	17
(10)	−+	80	15
(11)	00	85	10
(12)	00	85	10
(13)	0a	85	3

[a]Nonrandomized.

analysis (JMP 7.0.2, SAS Institute Inc., Cary, NC, USA) fitted to a second-order polynomial model:

$$Y = \beta_0 + \sum_{i=1}^{2} \beta_i X_i + \sum_{i=1}^{2} \beta_{ii} X_i^2 + \sum_{i} \sum_{j=i+1} \beta_{ij} X_i X_j, \quad (2)$$

where Y is the predicted response, β_0 is the constant coefficient, β_i is the lineal coefficient, β_{ii} is the quadratic coefficient, and β_{ij} is the interaction coefficients. In this model, X_i and X_j are the independent extraction variables, amplitude level (%), and time (min), respectively.

The adequacy of the mathematical model was determined using the coefficient R^2. The significance of the model regression coefficients was evaluated using an analysis of variance. Three-dimensional curves from the response surface plots were obtained to interpret the effects of the interaction between independent variables on the response variables. Contour plots were generated to represent the extrapolation and interpret the optimization of the extraction variables, using the Sigma Plot 12.3 graphing software (SYTAT software Inc., Richmond, CA, USA).

2.7.2. Treatment Comparison. Comparison between extraction methods (ultrasound-optimized Soxhlet and maceration) was carried out by a one-way analysis of variance (ANOVA). All determinations were performed in triplicate and significant differences between means were determined by Duncan test ($p \leq 0.05$) using the SPSS program (15.0, SPSS Inc., Chicago, IL, USA).

2.8. Scanning Electron Microscopy. Scanning electron microscopy (SEM) was used to examine the morphological alterations caused to the cactus pear seeds before and after the ultrasound extraction. Samples deposited on the silicon wafer were coated with a thin layer of gold (Denton Vacuum Desk V, Moorestown, NJ, USA) applying 20 millitorr and 20 mA during 4 min. Samples were observed in a scanning electronic microcopy (JEOL JSM-6300, Peabody, MA, USA) at 1,000 and 500 amplifications and micrographs were taken to establish the structural comparison between both samples.

2.9. Antimicrobial Activity. The green cactus pear seed oil was tested against one Gram-positive bacteria, *Staphylococcus aureus* (ATCC 1654), and two Gram-negative bacteria, *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). All microorganisms were obtained from the Mexican Microbial Culture Collection of CINVESTAV of the National Polytechnic Institute (Mexico). For each microorganism, bacterial suspensions were made in a soybean-casein digest medium to a concentration of approximately 10^8 CFU/mL.

To evaluate the antimicrobial activity and the minimum inhibitory concentration (MIC), the disk diffusion method was used. Each bacterial suspension (100 μL from the 10^8 CFU/mL) was spread on prepared agar plates (sterile Standard Methods Agar for bacteria). Filter sterile paper discs (6 mm in diameter) were impregnated with 66.67, 50, 33.33, and 16.67 μL of the undiluted oil and were placed on the inoculated plates. The negative control was hexane, while ampicillin (10 μg) and streptomycin (10 μg) were used as positive controls. The plates where incubated at 37°C (Arsa AR-130, Felisa, Jalisco, Mexico) for 24 hrs. The diameters of the inhibition zones were measured in millimeters and the results of MIC were expressed as μg/μL.

3. Results and Discussion

3.1. Extraction Yield. Table 2 shows the oil yields achieved by the ultrasound treatments. The extraction yield varied from 3.75 to 6% and the maximum yield was obtained at the highest amplitude level of 92%. Oil yield strongly depended on amplitude level probably because at high amplitudes the cavitation effect increases [21] and induces physical changes on the structure of the seed such as disruption of the cell walls, reduction of the particle size, and increase of exposure area. These conditions may facilitate the penetration of the solvent and thus the extraction of oil [26, 28, 34]. The maximum yield was achieved after 10 min of treatment, and longer times (15 min) at high amplitudes (90%) did not increase oil extraction (Table 2). Albeit time is an important variable, after yield reaches a peak, a longer treatment does not maximize extraction; the same was described by Zhang et al. [27] for Korean pine seed, who demonstrated that oil yield increased with time, but when it reached a maximum, yield equilibrated and then decreased gradually. This may be attributed to an initial complete fracture of the cell walls during the first minutes of the cavitation effect [26, 28, 34].

3.2. Antioxidant Activity of Green Cactus Pear Seed Oil Extracted by Ultrasound. Due to the complexity of the oxidation processes, it is advisable to perform more than one method to obtain the antioxidant profile of a sample [35]. In order to determine the antioxidant activity of the green

Table 2: Extraction yield and antioxidant activity of green cactus pear seed oil extracted by ultrasound under different conditions.

Extraction conditions			Antioxidant activity	
Amplitude level (%)	Time (min)	Oil yield (%)	ABTS mg AAE/100 g	DPPH μmol TE/100 g
85	10	5.75 ± 0.03	66.15 ± 4.07	246.10 ± 0.92
90	5	4.15 ± 0.01	55.98 ± 3.13	141.76 ± 3.90
90	15	4.85 ± 0.02	59.93 ± 4.45	167.02 ± 1.14
85	10	5.25 ± 0.01	68.12 ± 6.59	236.01 ± 3.22
92	10	6.00 ± 0.06	55.84 ± 2.36	101.63 ± 4.68
80	5	5.25 ± 0.01	66.07 ± 2.17	267.07 ± 7.37
85	10	3.75 ± 0.00	68.37 ± 3.92	245.05 ± 2.37
78	10	5.40 ± 0.10	65.99 ± 5.01	289.26 ± 0.26
85	17	5.60 ± 0.06	65.78 ± 0.37	260.09 ± 1.33
80	15	5.40 ± 0.00	66.91 ± 3.20	284.96 ± 0.93
85	10	5.75 ± 0.00	67.73 ± 0.40	277.55 ± 1.71
85	10	5.30 ± 0.10	67.44 ± 3.89	245.08 ± 2.92
85	3	5.50 ± 0.10	67.63 ± 0.00	250.05 ± 1.99

±: standard deviation.

Table 3: Antioxidant activity regression coefficients of the ultrasound extraction conditions.

Coefficient	ABTS	DPPH
β_0	67.562964[a]	249.96199[a]
β_i	−3.927762[b]	−63.57459[a]
β_j	0.2724311	7.1702572
β_{ij}	0.77572	1.8421052
β_{ii}	−3.719624[b]	−29.77102[b]
β_{jj}	−0.826106	0.0425804
R^2_{adj}	0.93	0.96

β_i: amplitude level; β_j: time; significance levels: [a] $p < 0.0001$; [b] $p < 0.001$.

cactus pear seed oil, two parameters were evaluated: antioxidant and scavenging capacity by ABTS and DPPH, respectively. The results and experimental design are described in Table 2. Antioxidant activity ranged from 55.84 to 68.37 mg AAE/100 g for ABTS and 101.63 to 289.26 μmol TE/100 g for DPPH. A R^2 value closest to one or at least of 0.80 indicates a good fit of the model [36]. The R^2 values for ABTS and DPPH were 0.93 and 0.96, respectively (Table 3), indicating that the averages obtained adjusted to the mathematical response surface model.

3.3. Effect of Ultrasound Extraction Conditions on the Antioxidant Activity of Cactus Pear Seed Oil. In this study, a second-order polynomial model for predicting the antioxidant activity of green cactus pear seed oil was obtained by multiple linear regression analysis of the experimental data. Table 3 shows the regression coefficients and significant probabilities of the linear, quadratic, and interaction effects of the ultrasound conditions on the seed oil antioxidant activity. Both ABTS and DPPH values were significantly affected by amplitude level in linear term (β_i) at $p < 0.0001$ DPPH and $p < 0.001$ ABTS, respectively, as well as in its quadratic term (β_{ii}) at $p < 0.001$ DPPH and ABTS. The three-dimensional surface plots constructed to observe the effect of ultrasound processing (Figure 1) demonstrated that antioxidant activity was higher when the applied amplitude decreased.

3.4. Optimization of the Ultrasound Extraction Conditions of Cactus Pear Seed Oil. Optimal extraction conditions were selected from the overlapped contour plots in which the effect of amplitude level and time on the antioxidant activity of green cactus pear seed oil was considered.

Figure 2 shows the optimal zone where the highest antioxidant activity by ABTS and DPPH was achieved. The conditions corresponded to amplitude of 78% applied for 10 min. In this zone the values for antioxidant activity were of 66.25 mg AAE/100 g and 289 μmol TE/100 g for ABTS and DPPH, respectively.

3.5. Comparison between Methods

Extraction Yield and Antioxidant Activity. Figure 3 compares the oil yields obtained by the three extraction methods. The results revealed that the yield obtained using the optimized ultrasound extraction was significantly lower than the obtained with the Soxhlet and maceration procedures. The higher oil yield obtained by Soxhlet could be attributed to the constant and extended contact (4 to 6 hrs) of the sample with the solvent at high temperatures, in addition to the repeated washing cycles [37]. The ultrasound yield was closer to that of maceration method but this last one required longer time (24 hrs) as compared to 10 min ultrasound treatment.

The results of antioxidant activity by ABTS and DPPH are shown in Figure 4. Both parameters were significantly higher in the oil extracted by Soxhlet (54.33 ± 0.84 mg AAE/100 g and 266.60 ± 1.97 μmol TE/100 g, resp.) compared to the other extraction methods. The ultrasound and maceration extractions exhibited similar antioxidant activity by ABTS, while DPPH was significantly higher for the ultrasound seed

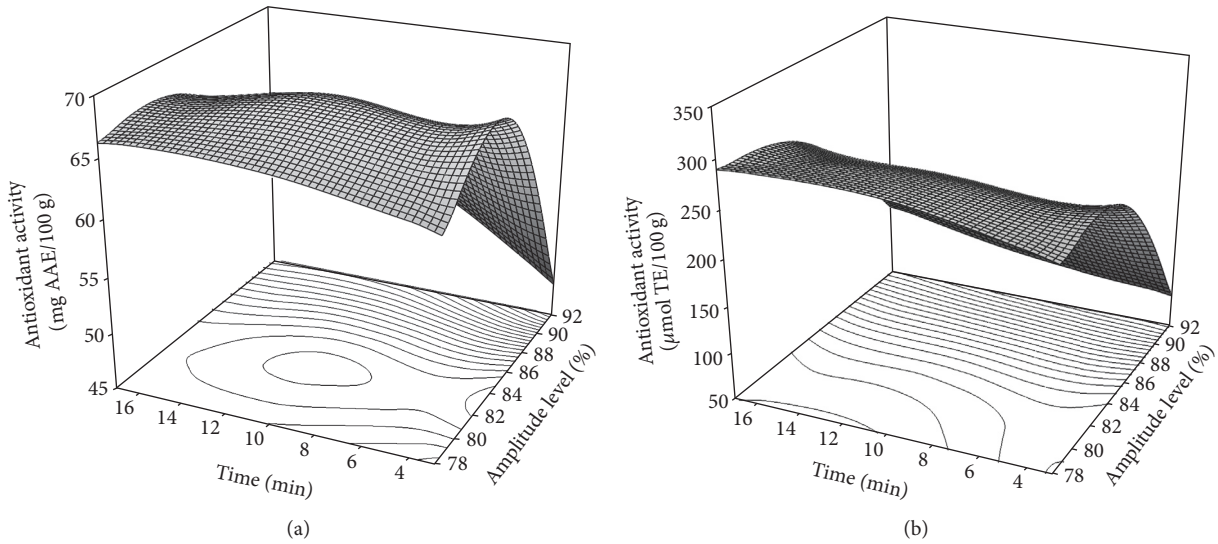

Figure 1: Effect of the ultrasound extraction on the antioxidant activity of green cactus pear seed oil. (a) ABTS; (b) DPPH.

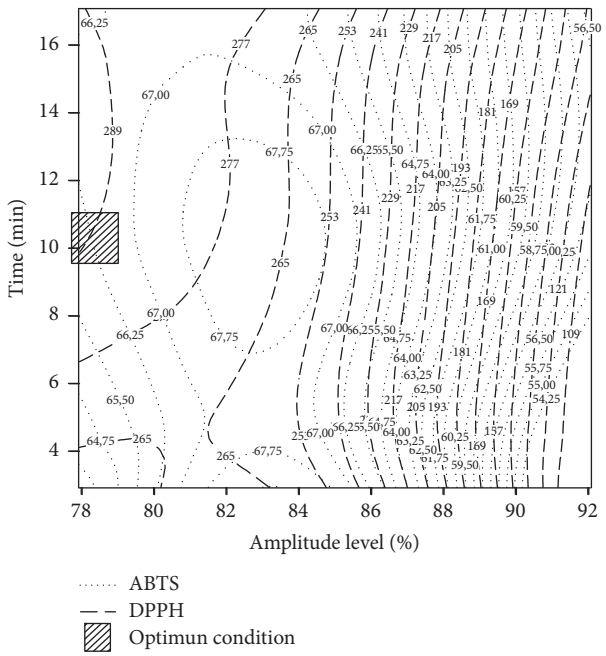

Figure 2: Optimal ultrasound extraction conditions of green cactus pear seed oil based on the highest antioxidant activity.

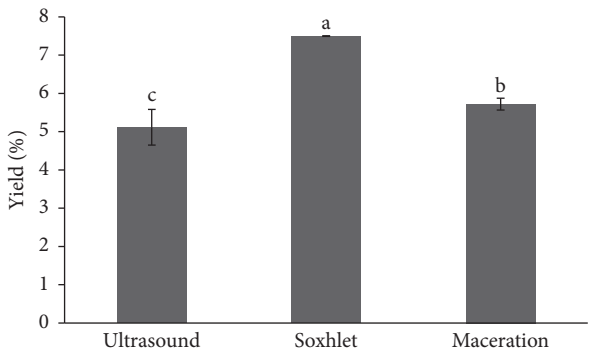

Figure 3: Cactus pear seed oil yield achieved by different extraction methods. [a,b,c]Different letters mean significant differences between methods ($p < 0.05$).

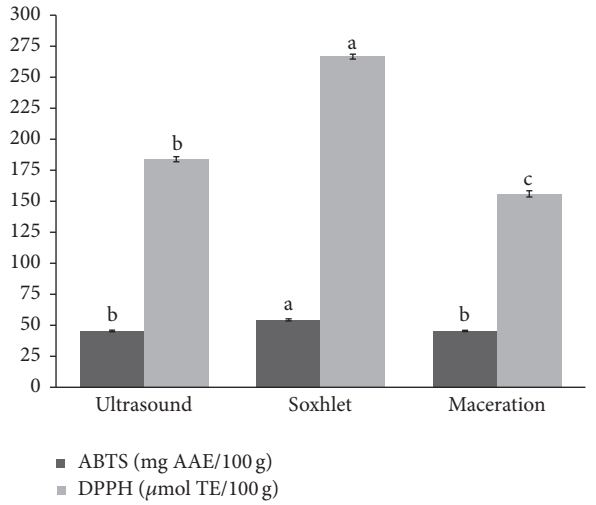

Figure 4: Antioxidant activity by ABTS and DPPH of cactus pear seed oil extracted by different methods. [a,b,c]Different letters mean significant differences between methods ($p < 0.05$).

oil. Albeit the oil extracted by the Soxhlet method presented higher yield and antioxidant activity, the ultrasound-assisted extraction may be enhanced if multiple extractions are carried out in a time comparable to the required by the Soxhlet procedure (4–6 hrs).

3.6. Antimicrobial Activity. *Escherichia coli* and *Staphylococcus aureus* are distributed in nature (water, soil, and vegetation) and are also part of the human intestinal

Table 4: Antimicrobial activity of cactus pear seed oil extracted by different methods.

	Diameter of inhibition zone (mm)		
	Oil*	(+) control	(−) control
Staphylococcus aureus (Gram-positive)			
Ultrasound	9.17 ± 0.29[a]	18.89 ± 1.54	ND
Soxhlet	9.50 ± 0.87[a]	18.78 ± 1.64	ND
Maceration	9.78 ± 0.69[a]	18.11 ± 1.17	ND
Escherichia coli (Gram-negative)			
Ultrasound	7.78 ± 0.19[a]	15.56 ± 2.12	ND
Soxhlet	7.56 ± 0.19[a]	15.33 ± 2.65	ND
Maceration	7.56 ± 0.38[a]	14.00 ± 2.33	ND
Pseudomonas aeruginosa (Gram-negative)			
Ultrasound	ND	12.56 ± 0.38	ND
Soxhlet	ND	12.22 ± 0.38	ND
Maceration	ND	12.78 ± 0.38	ND

*Seed oil: 66.67 μL; (+) control: ampicillin (disc 10 μg; *Staphylococcus aureus* and *Escherichia coli*) and streptomycin (disc 10 μg; *Pseudomonas aeruginosa*); (−) control: hexane; ND: not detected; ±: standard deviation; [a] Same superscripts indicates that there is no significant difference ($p > 0.05$).

microbiota [38]. *Pseudomonas aeruginosa*, besides being present in the intestinal microbiota [39], is a bacteria found in the soil, fertilizers, and water used for food production [40] and thus it can contaminate fresh or processed food, which is an indicator of inadequate sanitation or improper handling during food production [41]. Table 4 summarizes the antimicrobial activity of green cactus pear seed oil. Extraction method did not have a significant effect on the antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, but the effect was lower than the positive controls. These results may be explained by the similar seed oil concentration and combined action of compounds on the structure of microbial cells [42, 43], despite the extraction method. Seed oil did not exhibit antimicrobial activity against *Pseudomonas aeruginosa*, probably due to the oil chemical composition, the type of microorganism, and the own characteristics of the bacteria [42–44]. The antimicrobial activity of oils is generally more effective against Gram-positive bacteria in comparison to Gram-negative bacteria, which are more resistant mainly because their outer membrane is less permeable [42, 43, 45, 46]. The results suggest that *Pseudomonas aeruginosa* was more resistant than *Escherichia coli*, probably due to the lipopolysaccharides present in the outer membrane that restrict the diffusion of compounds making it less permeable [45]. The resistance can also be caused by systems of exclusion pumps that eject antimicrobial compounds from the inside of the bacteria before they can cause damage [39, 44].

3.7. Effect of Ultrasound on the Physical Structure. Scanning electron micrographs of the green cactus pear seeds powder before and after the ultrasound treatment at magnifications factors of 1000x and 500x are shown in Figure 5. Before the ultrasound treatment and despite the previous milling process, in the control sample it was possible to identify intact structures of the seeds cell as well as some starch granules (Figure 5(a)). After the ultrasound treatment, the cell structural damage and the variations in the shape and size of the particles were observed (Figure 5(b)). For instance, starch granules were not observed because sonication fragmented these particles while cavitation phenomenon disrupted the cell structures of the seeds [47, 48].

4. Conclusions

This study demonstrated that response surface methodology and a second-order polynomial model were effective tools to determine the optimum processing conditions of ultrasound-assisted extraction based on the maximum values of antioxidant activity. The results demonstrated that cactus pear seed oil has good antioxidant and antimicrobial properties. Ultrasound-assisted extraction was comparable to maceration but a single ultrasound process yielded less oil and lower antioxidant activity than solvent extraction (Soxhlet). Ultrasound can be considered an alternative technology for the extraction of seed oil but further research is required to determine the uses of the seed oil and the technology within the food industry and the potential of several ultrasound cycles at the optimized conditions.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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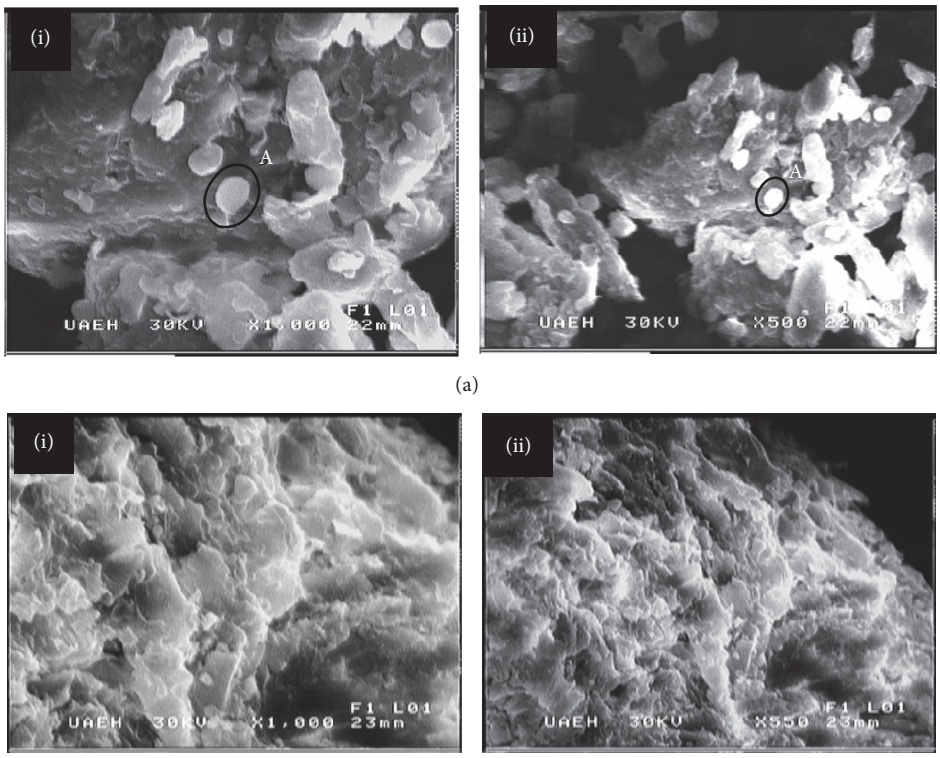

Figure 5: Scanning electron micrographs of green cactus pear seed powder before (a) and after (b) ultrasound treatment (i) 1,000x, (ii) 500x. A. The black circle shows a starch granule.

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