applied sciences

MDPI

Article

Release of Antioxidant Compounds of *Zingiber officinale* by Ultrasound-Assisted Aqueous Extraction and Evaluation of Their In Vitro Bioaccessibility

Elizabeth Contreras-López [1], Araceli Castañeda-Ovando [1], Judith Jaimez-Ordaz [1], Nelly del Socorro Cruz-Cansino [2], Luis Guillermo González-Olivares [1], José Sergio Rodríguez-Martínez [3] and Juan Ramírez-Godínez [3,*]

1 Área Académica de Química, Instituto de Ciencias Básicas e Ingeniería, Universidad Autónoma del Estado de Hidalgo, Mineral de la Reforma, Hidalgo 42184, Mexico; elizac@uaeh.edu.mx (E.C.-L.); ovandoa@uaeh.edu.mx (A.C.-O.); jjaimez@uaeh.edu.mx (J.J.-O.); lgonzales@uaeh.edu.mx (L.G.G.-O.)

2 Área Académica de Nutrición, Instituto de Ciencias de la Salud, Universidad Autónoma del Estado de Hidalgo, Circuito Ex Hacienda, La Concepción S/N, Carretera Pachuca Actopan, San Agustín Tlaxiaca, Hidalgo 42060, Mexico; ncruz@uaeh.edu.mx

3 Área Académica de Gastronomía, Instituto de Ciencias Económico Administrativas, Universidad Autónoma del Estado de Hidalgo, Circuito La Concepción Km 2.5 Col. San Juan Tilcuautla, San Agustín Tlaxiaca, Hidalgo 42160, Mexico; sergior@uaeh.edu.mx

* Correspondence: juan_ramirez@uaeh.edu.mx; Tel.: +52-771-7172000 (ext. 2512)

Received: 20 June 2020; Accepted: 15 July 2020; Published: 20 July 2020

Featured Application: Ultrasound has been recognized as a useful technique in the extraction of bioactive compounds from plants. In this case, ultrasound-assisted extraction has been employed for the liberation of antioxidants present in *Zingiber officinale*. The physical conditions to obtain aqueous extract with antioxidant activity were established in this study. These extracts can be used in the food industry due to the different beneficial properties of this rhizome to human health.

Abstract: Ginger rhizome is widely used in culinary preparations and in traditional medicine. Its benefits are associated with its antioxidant properties related to phenolics and terpenoids compounds, which use to be thermolabile. Ultrasound-assisted extraction has been useful for enhancing the release of thermosensitive compounds present in vegetable tissues. Therefore, the aim of this study was to evaluate the influence of ultrasound-assisted extraction on the release of antioxidants from ginger in aqueous media as well as their in vitro bioaccessibility. Central composite rotatable design was applied to obtain the optimal conditions for the extraction; the variables studied were amplitude (80–90%) and temperature (30–50 °C). Total phenolic content, antioxidant capacity (DPPH•, ABTS•+ and FRAP), and in vitro bioaccessibility were determined. Amplitude was the main parameter influencing the extraction of antioxidants. The ginger aqueous extracts showed a bioaccessibility of around 30%. The release of antioxidant compounds from ginger by ultrasound-assisted extraction avoids the use of high temperatures and solvents commonly used in conventional extraction methods.

Keywords: ultrasound-assisted extraction; ginger; antioxidant capacity; aqueous extracts

1. Introduction

Plants and plant-based foods are rich in antioxidants, such as polyphenolic compounds, well-known for their beneficial effects on human health, due to their antioxidant, cardioprotective,

anticancer, anti-inflammatory, and antimicrobial properties [1–7]. In such a context, ginger represents a really challenging bioresource.

Zingiber officinale (ginger) is a rhizome that has been used in traditional medicine to treat different diseases like colds, bronchitis, cough, vomiting, and nausea [8–10]. Its composition includes components of phenolic (6-gingerol and its related compounds like shogaol) and terpene types (α-zingiberene, β-bisabolene), which have antioxidant properties [11–13]. Such properties are of interest from a technological and nutritional point of view. For this reason, the extraction of this kind of compounds from natural sources has encouraged researchers to look for more innovative strategies to maximize the extraction rates from different plant materials due to their promising impact in improving health. To evaluate the use of ginger as a natural source of antioxidants, several studies have reported the use of methods involving different solvents for extraction such as ethanol, methanol, methanol-water, and water, as well as wide range of temperatures and extraction times [14,15]. Among the analyzed variables, temperature is the physical factor that influenced the most in the release of antioxidant compounds, regardless the time of extraction and the quantity of the sample [16]. Recently, emerging technologies such as ultrasound have been successfully used as an alternative method for the extraction of thermosensitive bioactive compounds from plant and fruits [17–19]; nevertheless, there are few studies related to the application of this method for the extraction of antioxidant compounds from ginger in aqueous media.

Ultrasound-assisted extraction (UAE), compared with other techniques, offers some advantages such as significant reduction of extraction time and the use of solvents. This is because the ultrasound has a mechanical effect that allows a better penetration of the solvent into the matrix, expanding the surface of contact between the sample and the liquid phase [20–22]. In recent years, ultrasound has also been used in the extraction of phenolic compounds aiming to preserve its antioxidant activity, avoiding the effect that high temperatures may have when applying conventional solid–liquid extraction methods (infusion, maceration, Soxhlet, etc.) [23–27]. Therefore, the aim of this work was to evaluate the influence of ultrasound-assisted extraction technique on the release of antioxidant compounds from ginger in aqueous media as well as their in vitro bioaccessibility. For that purpose, a central composite rotatable design (CCRD) was used to obtain the optimal physical conditions for antioxidant compounds extraction from ginger in aqueous media.

2. Materials and Methods

2.1. Reagents and Materials

Folin–Ciocalteu reagent (2N), 6-dichloroindophenol sodium salt hydrate (DCPI), 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS•+, ≥98%), 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH•), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox, 97%), sodium bicarbonate, hydrochloric acid, pepsin, pancreatin, sodium cholate hydrate, and sodium deoxycholate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Anhydrous sodium carbonate, gallic acid, potassium persulfate, ethanol, anhydrous sodium acetate, and glacial acetic acid were acquired from Meyer (Mexico City, Mexico). Ferric chloride hexahydrate, 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), hydrochloric acid, and ferrous chloride tetrahydrate were from JT Baker (Center Valley, PA, USA). Dialysis process was carried out into sacks (21 mm, pre-cut, open ended, dry unwashed, length = 30 cm, pore size 12,000 Da), which were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Instruments

All extractions were carried out by using an ultrasonic processor at 1500 W and 20 kHz (VCX-1500, Sonics & Materials, Inc. Newtown, CT, USA). The absorbance measurements were made in a spectrophotometer (PowerWave XS UV-Biotek, Software KC Junior, Winooski, VT, USA).

2.3. Ginger Sample (Zingiber Officinale)

The sample (approximately 30 kg) of ginger (whole rhizomes, previously washed, ground and frozen) was given by the association of organic ginger producers "Productos Orgánicos de Blackberry de la Sierra Norte de Puebla S.C. de R.L." (Puebla, Mexico). The sample was stored in polyethylene bags and kept at −20 °C until use.

2.4. Experimental Design

A central composite rotatable design (CCRD) was used to determine the effect of ultrasound technique in the release of antioxidant components from ginger in aqueous media. The values (lower, central and upper) of independent variables amplitude (80–90%) [17] and temperature (30–50 °C) [18] are shown in Table 1. The design consisted of 13 experiments.

Table 1. Selected experimental factors for release antioxidant compounds from *Zingiber officinale* by ultrasound-assisted extraction at the desired levels.

Experimental Factor	Level				
	$-\alpha$	−1	0	1	$+\alpha$
x_1	78	80	85	90	92
x_2	26	30	40	50	54

Experimental data from the central composite rotatable design were analyzed using a response surface regression (JMP 7.0.2, SAS Institute Inc., Cary, NC, USA, 2007) fitted to a second-order polynomial model (Equation (1)).

$$Y = \beta_0 + \sum_{i=1}^{2} \beta_i x_i + \sum_{i=1}^{2} \beta_{ii} x_i^2 + \sum_i * \sum_{j=i+1} \beta_{ij} x_i x_j \quad (1)$$

where Y was the predicted response, β_0 was the constant coefficient, β_1, β_2 were the linear coefficients, β_{11} and β_{22} were the quadratic coefficients, β_{12} was the cross-product coefficient, x_1 (amplitude) and x_2 (temperature) were the independent variables. Response surface plots were drawn out to show the simultaneous effect of amplitude (x_1) and temperature (x_2) on the experimental dependent parameters (antioxidant capacity and total phenolic content).

2.5. Ultrasound-Assisted Extraction (UAE)

The aqueous extraction of ginger was performed by ultrasound-assisted method (probe system) according to the experimental design described above. The ultrasonic system was immersed in a water bath coupled to a temperature controller to maintain the extraction temperature. Ginger (4 g approximately) was weighed and then placed in an extraction tube. Then, 400 mL of distilled water were added to the extraction tube and each extraction was performed under controlled conditions. To close the system, a probe of 25 mm (amplitude transformer was connected between the converter and the probe) was introduced. Finally, an air flow system was connected to the converter to prevent its overheating. All extractions were carried out for 15 min [17,18] at a pulse mode of 2 s on/4 s off [17]. Later, extracts were centrifuged at 10,000 rpm for 30 min. The supernatants were separated by decantation and kept at −4 °C until the analysis. Antioxidant capacity (DPPH•, ABTS•+ and FRAP assays) and total phenolic content of the extracts were determined. All measurements were carried out in triplicate.

2.6. Measurements of Response Variables

2.6.1. ABTS Assay

This method was carried out according to Thaipong et al. [28]. The radical cation ABTS•+ was produced by the reaction of ABTS•+ (7 mmol L^{-1}) with potassium persulphate (2.45 mmol L^{-1}), which was left to react for 16 h in darkness at room temperature. The ABTS•+ solution was diluted with deionized water until an absorbance measure of 0.70 ± 0.10 at 754 nm. Trolox was used as reference antioxidant and it was used to prepare a calibration curve (10–300 µmol L^{-1}). Then, 60 µL of each standard solution were taken and mixed with 980 µL of a diluted solution of ABTS•+. Solutions were incubated for 7 min at room temperature. Afterward, the absorbance of the mixture was measured at 754 nm. The same procedure was done for each aqueous extract of ginger. All determinations were made by triplicate. The results were expressed as mg Trolox per 100 g of ginger sample (mg Trolox/100 g).

2.6.2. DPPH Assay

Antiradical activity was measured using DPPH method [28]. An ethanolic solution of DPPH• (74 mg L^{-1}) was prepared. A calibration curve was obtained by treatment of a set of Trolox standards (0–300 µmol L^{-1}), which was used as antioxidant reference. Each Trolox standard solution (100 µL) was mixed with DPPH• solution (500 µL). At the same time, a control solution containing ethanol instead of Trolox was prepared. All solutions (standards and control) were incubated at room temperature and darkness for 60 min; the absorbance was measured at 515 nm. The same procedure was performed for aqueous extracts of ginger using Trolox standard solutions to replace the samples. All determinations were made by triplicate, and the results were expressed as mg Trolox per 100 g of ginger (mg Trolox/100 g).

2.6.3. FRAP Assay

The FRAP reagent was prepared by mixing 50 mL of acetate buffer (300 mmol L^{-1} at pH 3.6), 5 mL of ferric chloride hexahydrate (20 mmol L^{-1}), and 5 mL of TPTZ (10 mmol L^{-1} in HCl 40 mmol L^{-1}). A calibration curve was prepared (0 to 100 mmol L^{-1}) by dilution of 0.1 mmol L^{-1} Fe^{2+} solution (from $FeCl_2 \bullet 4H_2O$ in HCl 40.0 mmol L^{-1}). To each standard solution of the curve, 1 mL of FRAP reagent was added, and the resulting mixture was taken to a final volume of 10 mL with distilled water. All the solutions were incubated at 37 °C for 4 min and absorbance was read at 593 nm [28]. The antioxidant capacity of the sample was measured using the same procedure for the standards, replacing the Fe^{2+} solution with 250 µL of each aqueous extract of ginger. All determinations were done by triplicate and the results were expressed as mg of Fe^{2+} per 100 g of ginger (mg Fe^{2+}/100 g).

2.6.4. Determination of Total Phenolic Content

The total phenolic content in aqueous extracts of ginger using UAE was determined in accordance with the method proposed by Stintzing et al. [23]. Gallic acid (GA) was used as reference phenolic compound. First, standard solutions of GA (3–15 mg L^{-1}) were prepared. A mixture of 100 µL of standard solution and 500 µL of Folin-Ciocalteu's reagent was prepared (1:10). Afterward, 400 µL of Na_2CO_3 (75 g L^{-1}) were added. The mixtures were incubated at room temperature for 30 min, then, the absorbance readings were made at 765 nm. Later, total phenolic content in the aqueous extracts of ginger was determined following the same procedure described above but replacing GA with the sample. All determinations were made by triplicate and the results were expressed as mg gallic acid per 100 g of ginger (mg GA/100 g).

2.7. Optimization and Validation

The model was analyzed using the Minitab V. 17 software (State College, PA, USA). A polynomial quadratic regression (Equation (1)) was used to determine the effects of the selected factors (amplitude and temperature). Linear, squared, and interaction coefficients were calculated.

Confirmatory Experiments

The optimum extraction point was determined through the statistical procedure applied. The desired goals for each variable (amplitude and temperature) and response (DPPH•, ABTS•+, FRAP and total phenolic content) were chosen. To validate the polynomial model, three replicates of aqueous extract of ginger (confirmatory experiments) were prepared under the optimized levels of factors. The experimental values for each response were compared to the predicted data from the mathematical model.

2.8. In Vitro Bioaccessibility Test

This assay was done only for the confirmatory experiments. The in vitro digestion model was followed by dialysis, according to the methodology proposed by Moreda-Piñeiro et al. [29] with some modifications. An aliquot of 10 mL of ginger extract was homogenized, and pH was adjusted at 2 with HCl (6 mol L^{-1}); later, 120 µL of pepsin solution (40 mg mL^{-1} of pepsin in 0.1 mol L^{-1} HCl) were added. The mixture was incubated at 37 °C for 2 h with constant stirring (100 rpm). Afterward, 1.5 mL of pancreatin-cholate-deoxycholate solution (5 mg of pancreatin, 12.5 mg of sodium cholate hydrate, and 12.5 mg of sodium deoxycholate in 0.1 mol L^{-1} $NaHCO_3$) were added.

Digestion product was transferred into a dialysis sack, which was placed in 200 mL of $NaHCO_3$ solution (pH 7.5, 0.1 mol L^{-1}) for 16 h. Bioaccessibility was calculated from DPPH•, FRAP and total phenolic content values, which were measured before and after the digestion process. The bioaccessibility was expressed as bioaccessibility index (BI), which was calculated as the percentage of the tested compound remaining in the bioaccessible fraction related to the original non-digested sample (Equation (2)) [30].

$$BI = \frac{C_{DS}}{C_{FS}} \times 100 \quad (2)$$

where C_{DS} is concentration of antioxidant capacity from aqueous extract of ginger at the end of the digestion process and C_{FS} is the initial concentration.

3. Results and Discussion

3.1. Experimental Design

To optimize the UAE of the antioxidant capacity and the total phenolic content from aqueous ginger's extracts, a CCRD was used. Table 2 shows the CCRD matrix and the total phenolic content corresponding responses. Data show the effect of amplitude and temperature (X_1 and X_2) on the antioxidant capacity and total phenolic content extracted from ginger. Responses for Y_1 (DPPH•) and Y_2 (ABTS•+) ranged from 13 to 157 mg Trolox/100 g. Lower values corresponded to ABTS method. For Y_3 (FRAP) and Y_4 (total phenolic content), values were from 93 to 168 mg Fe^{2+}/100 g and from 8 to 17 mg GA/100 g, respectively.

Table 2. Experimental results of the central composite rotatable design (CCRD) for the optimization of the variables involved (x_1 and x_2) in the ultrasound-assisted extraction of antioxidants from ginger for the four responses, Y_1, Y_2, Y_3, and Y_4.

Variable Coded Values		Responses			
x_1	x_2	Y_1	Y_2	Y_3	Y_4
−1	−1	154.62	20.63	149.30	14.33
+1	−1	128.21	13.28	109.77	11.74
−1	+1	141.15	18.88	140.70	13.62
+1	+1	125.91	13.10	108.20	10.38
−α	0	138.48	17.95	136.23	12.94
+α	0	109.65	12.23	93.18	8.18
0	−α	157.15	22.53	168.80	17.11
0	+α	139.80	16.59	130.92	13.54
0	0	133.03	13.58	138.92	12.40
0	0	133.14	14.24	139.65	12.80
0	0	134.06	14.40	136.77	12.07
0	0	132.05	13.64	137.35	11.99
0	0	134.61	14.07	136.70	12.62

x_1, amplitude; x_2, temperature; Y_1, DPPH in mg Trolox/100 g; Y_2, ABTS in mg Trolox/100 g; Y_3, FRAP in mg Fe^{2+}/100 g; and Y_4, total phenolic content in mg GA/100 g. Variables, natural values and ranges were given in Table 1.

The CCRD allowed us to estimate the effects of the factors (amplitude and temperature) and their second order interactions (quadratic and cross-product effects) which are presented in Table 3. In general, amplitude (x_1) presented a positive effect in all responses, except for Y_2 (ABTS•+), permitting a higher extraction of bioactive compounds. It is well known that amplitude plays an important role in the intensification of the extraction due to its impact in cavitation [31]. On the other hand, temperature had a negative effect in all responses since it can damage the structure and properties of thermosensitive antioxidant and phenolic compounds promoting higher degradation rates [31,32].

Table 3. Parametric results of the second-order polynomial equation of Equation (2) for ultrasound-assisted extraction (UAE) of antioxidants from ginger for the four responses, Y_1, Y_2, Y_3, and Y_4 and fitting coefficients obtained after applying the central composite rotatable design.

Parameter	Response			
	Y_1	Y_2	Y_3	Y_4
Intercept				
β_0	−615	230	−3084	−239.6
Linear effect				
β_1	25.33	−3.25	83	6.71
β_2	−11.53	−2.86	−7.52	−0.91
Quadratic effect				
β_{11}	−0.1743	0.0136	−0.5160	−0.0406
β_{22}	0.0785	0.0257	0.0467	0.0137
Cross-product effect				
β_{12}	0.0558	0.0079	0.0351	−0.0032
R^2	0.9905	0.9178	0.9300	0.9601
R^2-adj	0.9837	0.8591	0.8800	0.9317

Y_1, DPPH in mg Trolox/100 g; Y_2, ABTS in mg Trolox/100 g; Y_3, FRAP in mg Fe^{2+}/100 g; and Y_4, total phenolic content in mg GA/100 g. Variables, natural values and ranges were given in Table 1.

Regarding to the correlation coefficient values (Table 3), these were superior to 0.93, and Y_2 (ABTS•+) showed the lowest R^2 (0.9178). Therefore, CCRD turns out to be useful to optimize the ultrasound-assisted aqueous extraction conditions of antioxidant compounds from ginger.

Data obtained from CCDR were used to plot the response surfaces showed in Figure 1A–D. In this research, amplitude (X_1) played a relevant role in the release of antioxidant compounds from ginger. Figure 1A shows DPPH• antioxidant activity responses; it is observed that ginger extracts under amplitudes of 80 to 82.5% presented the highest values (>160 mg Trolox/100g). In the case of extracts measured through ABTS•+ (Figure 1B), using an amplitude of 80%, the highest quantity of antioxidant components (>24 mg Trolox/100g) was obtained. Regarding the liberation of antioxidant compounds determined by FRAP method, levels higher than 165 mg Fe^{2+}/100g were obtained in the aqueous extracts applying an amplitude of 85% and a temperature of 26 °C (Figure 1C). The same trend as DPPH• method was observed. Figure 1D indicates that by exposing the extracts of ginger at amplitudes of 80 and 85% and a temperature of 25 °C, the higher liberation of compounds of phenolic type was obtained (17 mgAG/100g sample).

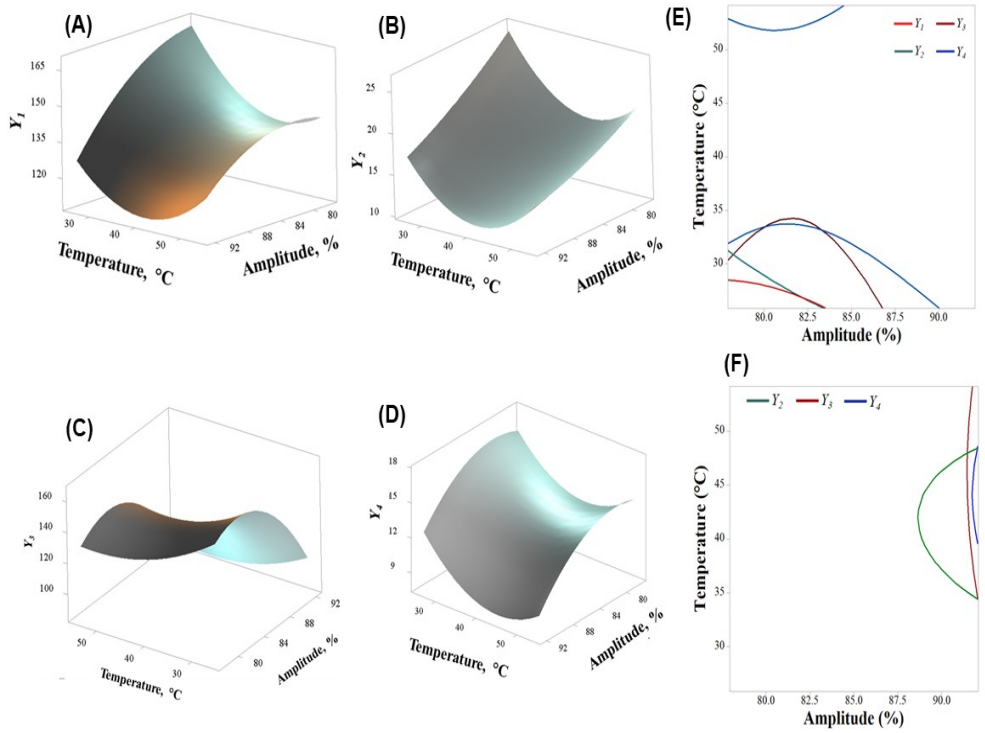

Figure 1. Representation of the response surfaces for surface models for Y_1, DPPH in mg Trolox/100 g (**A**); Y_2, ABTS in mg Trolox/100 g (**B**); Y_3, FRAP in mg Fe^{2+}/100 g (**C**); Y_4, total phenolic content in mg GA/100 g (**D**) and of overlaid contours at upper (**E**), and lowest values of responses (**F**).

Finally, Figure 1E,F shows the upper and lowest values of the overlaid contour plot of the responses measured. Figure 1E indicates that using an amplitude <85% and a temperature <40 °C allows a higher antioxidant capacity of aqueous ginger extract measured by DPPH• (Y_1), ABTS•+ (Y_2) and FRAP (Y_3). On the contrary, total phenolic content (Y_4) can be increased using 85% of amplitude and temperarure >50 °C (Figure 1F). Some authors have found that total phenolic content increases at high temperature, due to complex polyphenolics degrade and produce simple phenolic compounds [33]. In addition, high percentage of ultrasound amplitude can produce break of bonds in the polyphenolic bonds [34].

3.2. Antioxidant Capacity

Regarding the antioxidant activity of ginger's extracts determined through the ABTS•+ technique, the results were from 12.23 mg to 22.53 mg Trolox/100 g. The highest value corresponded to the extracts of ginger obtained at 80% of amplitude and 30 °C; these conditions coincide with the ones observed in the DPPH assay. The antioxidant capacity of the extracts analyzed by DPPH assay (109.65–157.15 mg Trolox/100 g) were similar to the results found by Kaur and Kapoor (147.6 mg Trolox/100 g) [14], Chohan

et al. (115.1 mg Trolox/100g) [35], and Chan et al. (119.3 mg Trolox/100 g) [36] using ethanol-water, water, and methanol as extractants agents, respectively. In the cited researches, the release of antioxidant compounds was mainly dependent of temperature and time of extraction but in our case, amplitude was the most important factor.

Despite UAE being reported as an effective extraction method for the release of thermosensitive and antioxidant compounds [31–33], in our work, the antioxidant activity values measured by DPPH were lower than those reported by Shan et al. [37]. This difference is attributed to the conditions used for the extraction. In that research, authors used methanol-water mixture as extractant under agitation for 24 h. It has been demonstrated than organic solvents as methanol, can allow a higher extraction of compounds with antioxidant properties [38]. In the present research, we avoided the use of organic solvents since the purpose was to evaluate the antioxidant capacity of aqueous ginger extracts, which can be used directly in the food industry.

With regard to the antioxidant capacity determined by FRAP assay, the values observed were lower (8.18–17.11 gallic acid/100 g) than those reported previously (147–1237 mg Fe^{2+}/100 g) [16,39]. In FRAP assay, the capacity to reduce Fe(III) can be attributed to two factors: (1) the donation of hydrogen from phenolic compounds, or (2) the number and position of the hydroxyl group of phenolic compounds [3] so, a low content of phenolic compounds will leads to a low antioxidant capacity measure. Additionally, several factors involving extraction step such as media, method (conventional, and assisted by ultrasound or microwave), time and ginger variety affect the yield of antioxidant compounds released [14,16,31,32,39].

3.3. Total Phenolic Content

All values of total phenolic content from aqueous extracts of ginger obtained by ultrasound were lower than others reported previously using conventional extraction methods (from 102 to 2350 mg GA/100 g) [36,39]. It should be highlighted that those studies involved the use of organic solvents as extractants. As far as we know, there are no reports of the total phenolic content of ginger extracts using UAE and water as extractant, and it has been explained the influence of the solvent in the antioxidant compounds yield extraction. In spite of that, our results are in agreement with data reported by Anese et al. [40], who observed a decrease in the total phenolic content after an ultrasound-assisted process in tomato pulp. It could be explained for the formation of new hydrogen chains promoted by ultrasound, which cause the bonding of released phenols, and therefore, their aqueous extraction is inhibited.

3.4. Model Validation

The optimization plot is showed in Figure 2, Y_2 (ABTS•+) was not considered in this step since this response presented the lowest correlation coefficient (0.9178), which can affect the accuracy of the experimental values. According to the optimization plot, the optimal conditions were: 82% of amplitude and 26 °C (temperature). Three confirmatory extractions were carried out under the optimal conditions and the responses (Y_1, Y_3, and Y_4) were measured. The experimental values were: 157.15 ± 13.29 mg Trolox/100 g (Y_1), 168.80 ± 2.45 mg Fe^{2+}/100 g (Y_3), and 17.11 ± 0.53 mg GA/ 100 g (Y_4). These results are similar to predicted values (Figure 2); therefore, the extraction model is accurate and can be useful for the extraction of antioxidant compounds from ginger using water as solvent.

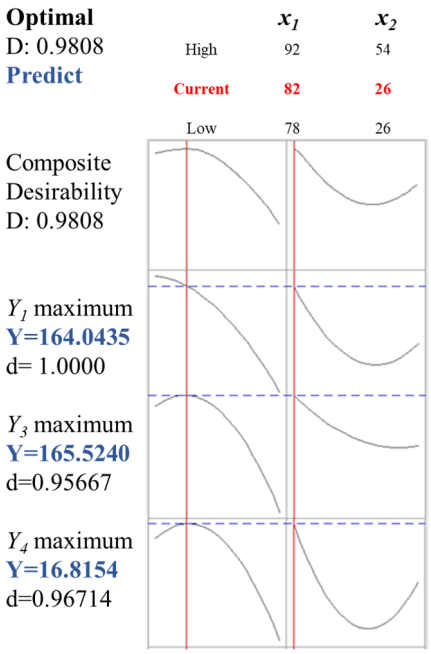

Figure 2. Optimization plot for the responses: Y_1, DPPH in mg Trolox/100 g; Y_3, FRAP in mg Fe^{2+}/100 g, and Y_4, total phenolic content in mg GA/100 g.

3.5. In-Vitro Bioaccessibility Evaluation

This assay was carried out using the aqueous extract of ginger obtained under optimal conditions from the experimental design applied. Bioaccessibility indices were calculated from each response evaluated (Y_1, Y_3, and Y_4), and the values found were 31.54% from DPPH assay (Y_1), 35.10% from FRAP test (Y_3), and 35.77% from total phenolic content (Y_4). Considering the DPPH measure (lipophilic character) and the lower total phenolic content, terpenoids can be the main antioxidant compounds present in the aqueous ginger extract.

There are no previous studies on the bioaccessibility of aqueous ginger extracts; however, the values obtained in the present study are lower than results found by Ramírez-Moreno et al. [41] from pulp of green and purple cactus pear (69–83%) and Bouayed et al. [42] from four varieties of apple (65%). Some authors have suggested that the low bioaccessibility of phenolic compounds could be because they are found linked to macromolecular compounds, such as proteins, polysaccharides, or soluble fiber or forming mineral complexes with reduced solubility [42–46].

The low bioaccessibility of antioxidant compounds from ginger aqueous extracts (DPPH and FRAP assay) is related to the absorption of phenolic compounds (flavonoids or terpenes); which can suffer structural changes or produce secondary metabolites during the digestion process [42–45]. Additionally, the bioaccessibility of phenolic compounds can be influenced by the change of pH during gastric and intestinal digestion (acid to alkaline media) that produce alterations in the phenolic polarity; the linkage with other substances; and the hindrance of large molecules to cross the cell membrane semipermeable barrier [47,48].

4. Conclusions

The central composite rotatable design was useful to study the release of antioxidant compounds from ginger under ultrasound-assisted extraction in aqueous media. The overall results indicate that UAE favors the release of phenolic compounds from ginger aqueous extracts. Amplitude was the main variable influencing the liberation of antioxidant compounds of *Zingiber officinale*; nevertheless, temperature must not be discarded. Further research is needed to increase the amount of antioxidants and total phenolic content in aqueous ginger extracts.

Author Contributions: Coordination of the study, J.R.-G. Chemical analysis, E.C.-L, A.C.-O., N.d.S.C.-C., J.S.R.-M., and J.R.-G. Revision of results, J.J.-O. and L.G.G.-O. Writing—original draft, E.C.-L., A.C.-O., J.J.-O. and J.R.-G. Review and editing, E.C.-L., A.C.-O., J.J.-O. and J.R.-G. All authors have read and agreed to the published version of the manuscript.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments: For the support to CONACYT and Productos Orgánicos de Blackberry de la Sierra Norte de Puebla S.C. de R.L.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Carocho, M.; Ferreira, I.C. A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food Chem. Toxicol.* **2013**, *51*, 15–25. [CrossRef] [PubMed]
2. Lawson, M.; Jomova, K.; Poprac, P.; Kuca, K.; Musilek, K.; Valko, M. Free Radicals and Antioxidants in Human Disease. In *Nutritional Antioxidant Therapies: Treatments and Perspectives*; Springer Science and Business Media LLC: Berlin/Heidelberg, Germany, 2017; pp. 283–305.
3. Di Mauro, M.D.; Fava, G.; Spampinato, M.; Aleo, D.; Melilli, B.; Saita, M.G.; Centonze, G.; Maggiore, R.; D'Antona, N. Polyphenolic Fraction from Olive Mill Wastewater: Scale-Up and in Vitro Studies for Ophthalmic Nutraceutical Applications. *Antioxidants* **2019**, *8*, 462. [CrossRef] [PubMed]
4. Nile, S.H.; Park, S.W. Chromatographic analysis, antioxidant, anti-inflammatory, and xanthine oxidase inhibitory activities of ginger extracts and its reference compounds. *Ind. Crop. Prod.* **2015**, *70*, 238–244. [CrossRef]
5. Tapia-Hernández, J.A.; Rodríguez-Felix, F.; Juárez-Onofre, J.E.; Ruiz-Cruz, S.; Robles-García, M.A.; Borboa-Flores, J.; Wong-Corral, F.J.; Cinco-Moroyoqui, F.J.; Castro-Enríquez, D.D.; Del-Toro-Sánchez, C.L. Zein-polysaccharide nanoparticles as matrices for antioxidant compounds: A strategy for prevention of chronic degenerative diseases. *Food Res. Int.* **2018**, *111*, 451–471. [CrossRef] [PubMed]
6. Shukla, Y.; Singh, M. Cancer preventive properties of ginger: A brief review. *Food Chem. Toxicol.* **2007**, *45*, 683–690. [CrossRef] [PubMed]
7. Chung, H.Y.; Arulkumar, R.; Bang, E.; Noh, S.-G.; Yokozawa, T. Role of Garlic and Ginger in Anti-oxidative and Anti-inflammatory Effects in Aging. *SDRP J. Food Sci. Technol.* **2019**, *4*, 788–795. [CrossRef]
8. Chen, I.N.; Chang, C.C.; Ng, C.C.; Wang, C.Y.; Shyu, Y.T.; Chang, T.L. Antioxidant and Antimicrobial Activity of Zingiberaceae Plants in Taiwan. *Plant Foods Hum. Nutr.* **2007**, *63*, 15–20. [CrossRef]
9. Srinivasan, K. Ginger rhizomes A spice with multiple health beneficial potentials. *PharmaNutrition* **2017**, *5*, 18–28. [CrossRef]
10. Mahboubi, M. Zingiber officinale Rosc. essential oil, a review on its composition and bioactivity. *Clin. Phytosci.* **2019**, *5*, 6. [CrossRef]
11. Lu, D.L.; Li, X.Z.; Dai, F.; Kang, Y.; Li, Y.; Ma, M.; Ren, X.-R.; Du, G.W.; Jin, X.L.; Zhou, B. Influence of side chain structure changes on antioxidant potency of the [6]-gingerol related compounds. *Food Chem.* **2014**, *165*, 191–197. [CrossRef]
12. Koch, W.; Kukula-Koch, W.; Marzec, Z.; Kasperek, E.; Wyszogrodzka-Koma, L.; Szwerc, W.; Asakawa, Y. Application of Chromatographic and Spectroscopic Methods towards the Quality Assessment of Ginger Rhizomes from Ecological Plantations. *Int. J. Mol. Sci.* **2017**, *18*, 452. [CrossRef] [PubMed]
13. De Oliveira, C.S.; Bisinella, R.Z.B.; Bet, C.D.; Beninca, C.; Demiate, I.M.; Schnitzler, E. Physicochemical, Thermal, Structural and Pasting Properties of Unconventional Starches from Ginger (Zingiber officinale) and White Yam (sp.). *Braz. Arch. Boil. Technol.* **2019**, *62*, 1–12. [CrossRef]
14. Kaur, C.; Kapoor, H.C. Anti-oxidant activity and total phenolic content of some Asian vegetables. *Int. J. Food Sci. Technol.* **2002**, *37*, 153–161. [CrossRef]
15. Huda-Faujan, N.; Noriham, A.; Norrakiah, A.S.; Babji, A.S. Antioxidant activity of plants methanolic extracts containing phenolic compounds. *Afr. J. Biotechnol.* **2009**, *8*, 484–489.

16. Ramírez-Godínez, J.; Jaimez-Ordaz, J.; Castañeda-Ovando, A.; Añorve-Morga, J.; Salazar-Pereda, V.; González-Olivares, L.G.; Contreras-López, E. Optimization of Physical Conditions for the Aqueous Extraction of Antioxidant Compounds from Ginger) Applying a Box-Behnken Design. *Plant Foods Hum. Nutr.* **2016**, *72*, 34–40. [CrossRef]
17. Zafra-Rojas, Q.Y.; Cruz-Cansino, N.S.; Quintero-Lira, A.; Gómez-Aldapa, C.A.; Alanís-García, E.; Cervantes-Elizarrarás, A.; Güemes-Vera, N.; Ramírez-Moreno, E. Application of Ultrasound in a Closed System: Optimum Condition for Antioxidants Extraction of Blackberry (Rubus fructicosus) Residues. *Molecules* **2016**, *21*, 950. [CrossRef]
18. Rehman, T.; Arshad, M.U.; Ahmad, R.S.; Rasool, B.; Hussain, G.; Saeed, F.; Shahbaz, M.; Ahmed, A.; Imran, M.; Khan, M.A.; et al. Reconnoitring the impact of different extraction techniques on ginger bioactive moieties extraction, antioxidant characterization and physicochemical properties for their therapeutic effect. *Pak. J. Pharm. Sci.* **2019**, *32*, 2223–2236.
19. Chemat, F.; Vian, M.A.; Fabiano-Tixier, A.-S.; Nutrizio, M.; Jambrak, A.R.; Munekata, P.E.S.; Lorenzo, J.M.; Barba, F.J.; Binello, A.; Cravotto, G. A review of sustainable and intensified techniques for extraction of food and natural products. *Green Chem.* **2020**, *22*, 2325–2353. [CrossRef]
20. Teh, S.-S.; Birch, E.J. Effect of ultrasonic treatment on the polyphenol content and antioxidant capacity of extract from defatted hemp, flax and canola seed cakes. *Ultrason. Sonochem* **2014**, *21*, 346–353. [CrossRef]
21. Chemat, F.; Rombaut, N.; Sicaire, A.-G.; Meullemiestre, A.; Fabiano-Tixier, A.-S.; Abert-Vian, M. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrason. Sonochem.* **2017**, *34*, 540–560. [CrossRef]
22. Santos, M.P.; Souza, M.C.; Sumere, B.R.; Da Silva, L.C.; Cunha, D.T.; Bezerra, R.M.N.; Rostagno, M.A. Extraction of bioactive compounds from pomegranate peel (*Punica granatum* L.) with pressurized liquids assisted by ultrasound combined with an expansion gas. *Ultrason. Sonochem.* **2019**, *54*, 11–17. [CrossRef] [PubMed]
23. Stintzing, F.C.; Herbach, K.M.; Mosshammer, M.R.; Carle, R.; Yi, W.; Sellappan, S.; Akoh, C.C.; Bunch, R.; Felker, P. Color, Betalain Pattern, and Antioxidant Properties of Cactus Pear (Opuntia spp.) Clones. *J. Agric. Food Chem.* **2005**, *53*, 442–451. [CrossRef] [PubMed]
24. Altemimi, A.B.; Choudhary, R.; Watson, D.G.; Lightfoot, D.A. Effects of ultrasonic treatments on the polyphenol and antioxidant content of spinach extracts. *Ultrason. Sonochem.* **2015**, *24*, 247–255. [CrossRef] [PubMed]
25. Deng, J.; Xu, Z.; Xiang, C.; Liu, J.; Zhou, L.; Li, T.; Yang, Z.; Ding, C. Comparative evaluation of maceration and ultrasonic-assisted extraction of phenolic compounds from fresh olives. *Ultrason. Sonochem.* **2017**, *37*, 328–334. [CrossRef] [PubMed]
26. Rincón, E.; Balu, A.M.; Luque, R.; Cantador, L.S. Mechanochemical extraction of antioxidant phenolic compounds from Mediterranean and medicinal Laurus nobilis: A comparative study with other traditional and green novel techniques. *Ind. Crop. Prod.* **2019**, *141*, 111805. [CrossRef]
27. Sasikala, P.; Chandralekha, A.; Chaurasiya, R.S.; Chandrasekhar, J.; Raghavarao, K. Ultrasound-assisted extraction and adsorption of polyphenols from Ginger Rhizome (*Zingiber officinale*). *Sep. Sci. Technol.* **2017**, *53*, 439–448. [CrossRef]
28. Thaipong, K.; Boonprakob, U.; Crosby, K.; Cisneros-Zevallos, L.; Byrne, D.H. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J. Food Compos. Anal.* **2006**, *19*, 669–675. [CrossRef]
29. Moreda-Piñeiro, J.; Herbello-Hermelo, P.; Domínguez-González, M.R.; Bermejo-Barrera, P.; Moreda-Piñeiro, J. Bioavailability assessment of essential and toxic metals in edible nuts and seeds. *Food Chem.* **2016**, *205*, 146–154. [CrossRef]
30. Ah-Hen, K.S.; Mathias-Rettig, K.; Gómez-Pérez, L.S.; Riquelme-Asenjo, G.; Lemus-Mondaca, R.; Muñoz-Fariña, O. Bioaccessibility of bioactive compounds and antioxidant activity in murta (Ugni molinae T.) berries juices. *J. Food Meas. Charact.* **2017**, *12*, 602–615. [CrossRef]
31. Medina, N.; Ayora-Talavera, T.; Espinosa-Andrews, H.; Sánchez-Contreras, A.; Pacheco, N. Ultrasound Assisted Extraction for the Recovery of Phenolic Compounds from Vegetable Sources. *Agronomy* **2017**, *7*, 47. [CrossRef]
32. Mokrani, A.; Madani, K. Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (*Prunus persica* L.) fruit. *Sep. Purif. Technol.* **2016**, *162*, 68–76. [CrossRef]

33. Xu, G.; Ye, X.; Chen, J.; Liu, D. Effect of Heat Treatment on the Phenolic Compounds and Antioxidant Capacity of Citrus Peel Extract. *J. Agric. Food Chem.* **2007**, *55*, 330–335. [CrossRef] [PubMed]
34. Sharayei, P.; Azarpazhooh, E.; Zomorodi, S.; Ramaswamy, H.S. Ultrasound assisted extraction of bioactive compounds from pomegranate (*Punica granatum* L.) peel. *LWT* **2019**, *101*, 342–350. [CrossRef]
35. Chohan, M.; Forster-Wilkins, G.; Opara, E. Determination of the Antioxidant Capacity of Culinary Herbs Subjected to Various Cooking and Storage Processes Using the ABTS*+ Radical Cation Assay. *Plant Foods Hum. Nutr.* **2008**, *63*, 47–52. [CrossRef] [PubMed]
36. Chan, E.W.C.; Lim, Y.; Wong, L.; Lianto, F.; Wong, S.; Lim, K.; Joe, C.; Lim, T. Antioxidant and tyrosinase inhibition properties of leaves and rhizomes of ginger species. *Food Chem.* **2008**, *109*, 477–483. [CrossRef]
37. Shan, B.; Cai, Y.Z.; Sun, M.; Corke, H. Antioxidant Capacity of 26 Spice Extracts and Characterization of Their Phenolic Constituents. *J. Agric. Food Chem.* **2005**, *53*, 7749–7759. [CrossRef]
38. Chen, S.; Zeng, Z.; Hu, N.; Bai, B.; Wang, H.; Suo, Y. Simultaneous optimization of the ultrasound-assisted extraction for phenolic compounds content and antioxidant activity of *Lycium ruthenicum* Murr. fruit using response surface methodology. *Food Chem.* **2018**, *242*, 1–8. [CrossRef]
39. Ghasemzadeh, A.; Jaafar, H.Z.E.; Rahmat, A. Antioxidant Activities, Total Phenolics and Flavonoids Content in Two Varieties of Malaysia Young Ginger (*Zingiber officinale* Roscoe). *Molecules* **2010**, *15*, 4324–4333. [CrossRef]
40. Anese, M.; Mirolo, G.; Beraldo, P.; Lippe, G. Effect of ultrasound treatments of tomato pulp on microstructure and lycopene in vitro bioaccessibility. *Food Chem.* **2013**, *136*, 458–463. [CrossRef]
41. Ramírez-Moreno, E.; Hervert-Hernández, D.; Sánchez-Mata, M.D.C.; Díez-Marqués, C.; Goñi, I. Intestinal bioaccessibility of polyphenols and antioxidant capacity of pulp and seeds of cactus pear. *Int. J. Food Sci. Nutr.* **2011**, *62*, 839–843. [CrossRef]
42. Bouayed, J.; Hoffmann, L.; Bohn, T. Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastro-intestinal digestion and dialysis of apple varieties: Bioaccessibility and potential uptake. *Food Chem.* **2011**, *128*, 14–21. [CrossRef] [PubMed]
43. Tagliazucchi, D.; Verzelloni, E.; Bertolini, D.; Conte, A. In vitro bio-accessibility and antioxidant activity of grape polyphenols. *Food Chem.* **2010**, *120*, 599–606. [CrossRef]
44. Cilla, A.; Perales, S.; Lagarda, M.J.; Barberá, R.; Clemente, G.; Farré, R. Influence of storage and in vitro gastrointestinal digestion on total antioxidant capacity of fruit beverages. *J. Food Compos. Anal.* **2011**, *24*, 87–94. [CrossRef]
45. Kopustinskiene, D.M.; Jakstas, V.; Savickas, A.; Bernatoniene, J. Flavonoids as Anticancer Agents. *Nutrients* **2020**, *12*, 457. [CrossRef]
46. Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jiménez, L. Polyphenols: Food sources and bioavailability. *Am. J. Clin. Nutr.* **2004**, *79*, 727–747. [CrossRef]
47. Liang, L.; Wu, X.; Zhao, T.; Zhao, J.; Li, F.; Zou, Y.; Mao, G.; Yang, L.-Q. In vitro bioaccessibility and antioxidant activity of anthocyanins from mulberry (*Morus atropurpurea* Roxb.) following simulated gastro-intestinal digestion. *Food Res. Int.* **2012**, *46*, 76–82. [CrossRef]
48. Hilary, S.; Tomás-Barberán, F.A.; Martinez-Blazquez, J.A.; Kizhakkayil, J.; Souka, U.; Al-Hammadi, S.; Habib, H.; Ibrahim, W.; Platat, C. Polyphenol characterisation of *Phoenix dactylifera* L. (date) seeds using HPLC-mass spectrometry and its bioaccessibility using simulated in-vitro digestion/Caco-2 culture model. *Food Chem.* **2020**, *311*, 125969. [CrossRef]