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Comparison between Ultrasonic Bath and Sonotrode Extraction of Phenolic Compounds from Mango Peel By-Products

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Abstract: Phenolic compounds present in mango peel byproducts have been reported to have several beneficial health properties. In this study, we carried out an optimization of phenolic compounds using ultrasound-assisted extraction via ultrasonic bath and sonotrode. To optimize the variables of extraction, a Box–Behnken design was used to evaluate the best conditions to obtain high total phenolic compound extraction and high antioxidant activity evaluated by different methods (DPPH, ABTS, and FRAP). The optimal ultrasonic bath conditions were 45% ethanol, 60 min, and 1/450 ratio sample/solvent (*w/v*) whereas optimal sonotrode conditions were 55% ethanol, 18 min, and 65% amplitude. The extracts obtained at the optimal conditions were characterized by HPLC–ESI-TOF-MS. A total of 35 phenolic compounds were determined and, to our knowledge, several of them were tentatively identified for the first time in mango peel. The samples were composed mainly by phenolic acids derivatives, specifically of galloylglucose and methylgallate, which represented more than 50% of phenolic compounds of mango peel byproducts. In conclusion, sonotrode is a valuable green technology able to produce enriched phenolic compound extracts from mango peel byproducts that could be used for food, nutraceutical, and cosmeceutical applications.

Keywords: mango waste; polyphenols; Box–Behnken; HPLC-MS; antioxidant activity; ultrasound-assisted extraction

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1. Introduction

Mango fruit belongs to the family *Anacardiaceae* and is one of the four most demanded tropical fruits in the world, whose commercialization continues growing year after year. Mexico, Thailand, Brazil, Peru, and India are the major producers of mangoes, whose importation has increased by 2.9% since 2019 despite COVID-19 [1]. Moreover, mango production worldwide has grown about 14% since 2015. Concretely, in 2020, 54.83 million metric tons of mangoes were produced in the world (www.statista.com (accessed on 17 August 2022)). Nevertheless, there are different byproducts generated from mango processing. Mango peel is the most representative byproduct due to his high weight in the total fruit, between 15% and 20% [2]. It represented a waste of about 11 million metric tons in 2020; thus, the economic losses were high (www.statista.com). Mango peel has several bioactive compounds such as ascorbic acid, carotenoids, and phenolic compounds (phenolic acids, flavonoids, and anthocyanins) [3–5]. In several studies, these compounds were demonstrated to have beneficial health properties such as antioxidative, anticarcinogenic, antiatherosclerosis, antimutagenic, and angiogenesis-inhibitory properties; hence, it can be considered a great source to use to promote health [2,6–9]. Among others, the antioxidant activity reported from mango peel has been highlighted. In this way, phenolic compounds are very important due to their high antioxidant activity which may allow defensive activity

against oxidative stress generated by some degenerative diseases [10]. In recent years, the interest in phenolic compounds has increased. Indeed, mango phenolic compounds could increase the shelf-life of food and improve food packaging when added due to their capacity to reduce free radicals and microbials [11].

There are different techniques to extract bioactive compounds. However, conventional methods of extraction such Soxhlet and maceration present several disadvantages such as the use of high temperature (>60 °C), high solvent consumption, and longer extraction times (several hours) [12,13]. Currently, these problems are being solved with green techniques which are more sustainable and careful with the environment such as ultrasound-assisted extraction (UAE) [12], microwave-assisted extraction (MAE) [14], supercritical fluid extraction (SFE) [15], and pressurized-assisted extraction (PLE) [16]. MAE allows reducing the time needed to carry out various extractions simultaneously and it is cheap; however, this method can overheat the sample [17]. SFE needs less time and temperature than traditional extraction methods; however, it is complex and expensive [17]. PLE reduces the time needed but it needs high temperatures, and the extractions are not simple [18]. Ultrasound-assisted extraction is based on the cavitation of the cell carried out by sound waves which are responsible for sample cell-wall rupture, yielding the compounds of interest in the solvent. The advantages of ultrasonic-assisted extraction are that it is a simple method, is easy to use, and reduces the extraction time, temperature, and solvent required [2,5,19]. Therefore, UAE is a great alternative to conventional methods to obtain polyphenols [12]. Ultrasound power can be applied using two types of devices: an ultrasonic bath or probe ultrasound equipment (sonotrode). The ultrasonic bath is more frequently used than the sonotrode because it is cheaper and more available, and it allows the extraction of different samples at the same time. However, the intensity is attenuated due to the water bath [20]. The sonotrode system is more powerful than the ultrasonic bath because the ultrasound delivery is direct and there is minor energy loss [20]. In a study of olive pomace, both technologies were compared, observing a major recovering of phenolic compounds and antioxidant activity in less time when using sonotrode technology [21].

Therefore, two ultrasound technologies are available at a laboratory scale (ultrasonic bath and sonotrode); accordingly, it is important in terms of quality control to establish the best method of extraction for the determination of these target compounds. There are few articles about the extraction of phenolic compound in mango peel byproducts using ultrasonic-assisted extraction (with ultrasound bath [22] or sonotrode [2]) and about the optimization of different parameters (time, ratio of sample to solvent, percentage ethanol/water, and amplitude) with the aim of achieving better conditions to extract them. Ethanol is used because it is classified as GRAS (generally recognized as safe). Therefore, the goal of this article was to optimize the conditions of extraction via ultrasonic bath and sonotrode in order to obtain the highest phenolic recovery with the highest antioxidant activity in mango peel byproducts. In this way, comparing both ultrasound technologies, using the same mango peel sample, can allow determining if there are advantages in the phenolic compounds extracted and their extraction parameters. The antioxidant activity is determined by three different methods due to their different sensibility to determine phenolic compounds that use the hydrogen atom transfer (HAT) and single-electron transfer (SET) mechanisms. Additionally, in the present article, the compounds of the samples obtained under optimum conditions were characterized by HPLC–ESI-TOF-MS.

2. Materials and Methods

2.1. Chemical and Samples

Mango peels of the *cv.* Palmer proceeding from Brazil were provided by a local industry of juices in February 2022. A total of 10 kg was collected for three consecutive days. The peels were submitted to freeze-drying process using a Zirbus lyophilizer (Bad Grund, Germany) for 120 h at −50 °C with a pressure of 0.8 mbar. To avoid the oxidation of the sensible compounds, the freeze-dryer was covered to create a dark environment. The peels were ground with a knife mill provided by IKA Werke GmbH & Co. KG (Staufen,

Germany) and sieved to 0.2 mm. The sample was stored in a freezer at −32 °C before the analysis.

Gallic acid, Trolox, DPPH, ABTS, and FRAP reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). Na_2CO_3 was purchased from BDH AnalaR (Poole, England). Water was obtained using a Milli-Q system (Millipore, Bedford, MA, USA). 1-O-Galloyl-β-D-glucose (purity > 90%), vanillic acid (purity > 97%), chlorogenic acid (purity > 95%), ferulic acid (purity > 99%), catechin (purity > 99%), quercetin (purity > 95%), and rutin (purity > 95%) were also acquired from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade water, Folin–Ciocâlteu reagent, and other reagents were acquired from Merck KGaA (Darmstadt, Germany).

2.2. Experimental Design

A Box–Behnken design and response surface methodology (RSM) were used to optimize the conditions of different variants using bath ultrasonic and sonotrode from mango peel byproducts. The objective of this experimental design was to obtain the major recovery of phenolic compounds and antioxidant activity. The designs were composed by 15 experiments with three different levels (−1, 0, and +1). These experiments were performed in duplicate. The independent variables for ultrasonic bath were X_1: ratio ethanol/water (20:80, 60:40, and 100:0 *v/v*), X_2: time (10, 50, and 90 min), and X_3: ratio sample/solvent (1:70, 1:285, and 1:500 *w/v*). In the case of experiments using a sonotrode, ratio sample/solvent was previously established by our research group; accordingly, the independent variables were X_1: ethanol/water (20:80, 60:40, and 100:0 *v/v*), X_2: time (5, 25, and 45 min), and X_3: amplitude (20%, 60%, and 100%), and a fixed ratio of 1:400 (*w/v*) was established. The dependent variables evaluated were the total phenolic compounds measured using Folin–Ciocâlteu method, and the antioxidant activity measured using DPPH, ABTS, and FRAP. A second-order polynomial model equation was used to adjust the dependent variables (Equation (1)).

$$\Upsilon = \beta_0 + \sum_{i=0}^{4} \beta_i X_i + \sum_{i=0}^{4} \beta_{ii} X_{ii}^2 + \sum_{i=0}^{4} \sum_{j=0}^{4} \beta_{ii} X_i X_j, \quad (1)$$

where Υ represents the response variable: the total phenolic compounds (TPC) or the antioxidant assays ABTS, FRAP, or DPPH. X_i and X_j are the independent factors influencing the response. β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients of the model (interception, linear, quadratic, and interaction terms).

Statistica 7.0 package (StatSoft, Tulsa, OK, USA) was used for analyzing statistical data. Both models were evaluated by ANOVA taking into account its regression coefficients, *p*-values of the regressions, and lack of fit. Additionally, the optimum conditions were established using response surface methodology.

2.3. Extraction of Phenolic Compounds from Mango Peel Byproducts Using Ultrasonic Bath

Mango peel byproducts were extracted using an ultrasonic bath (Bandelin, Sonorex, RK52, Berlin, Germany) which worked at a frequency of 35 kHz. The volume used for extraction was 10 mL of ethanol/water solution following the experimental conditions of the model (Table 1). Then, the samples were centrifuged at 8603× *g* for 10 min and evaporated using a Buchi R-205 rotavapor. To finish, the samples were reconstituted in 2 mL of methanol/water (1:1) and filtered with a 0.2 µm nylon syringe filter.

Table 1. Box–Behnken design with natural and coded values (parenthesis) of the conditions of sonotrode and ultrasonic bath extraction and the experimental results obtained for total phenolic compounds (TPC), and antioxidant assays (DPPH, ABTS, and FRAP) expressed with the average and the standard deviation.

	Sonotrode							Ultrasonic Bath						
	Independent Factors			Dependent Factors				Independent Factors			Dependent Factors			
Run	**Ethanol X_1**	**Time X_2**	**Amplitude X_3**	**TPC**	**DPPH**	**ABTS**	**FRAP**	**Ethanol X_1**	**Time X_2**	**Ratio *w/v* X_3**	**TPC**	**DPPH**	**ABTS**	**FRAP**
1	20 (−1)	5 (−1)	60 (0) (94 W)	5.4 ± 0.4	12.0 ± 0.3	18.5 ± 1.4	15.0± 1.1	20 (−1)	10 (−1)	285 (0)	6.5 ± 0.1	12.8 ± 1.4	20.1 ± 0.9	10.7 ± 0.5
2	100 (1)	5 (−1)	60 (0) (72 W)	4.1 ± 0.4	12.5 ± 0.7	16.4 ± 0.3	15.0 ± 0.3	100 (1)	10 (−1)	285 (0)	3.0 ± 0.3	5.3 ± 0.2	13.2 ± 0.7	6.0 ± 0.1
3	20 (−1)	45 (1)	60 (0) (95 W)	4.6 ± 0.5	15.7 ± 0.8	22.1 ± 0.1	15.9 ± 0.4	20 (−1)	90 (1)	285 (0)	7.0 ± 0.2	14.4 ± 1.1	16.6 ± 0.4	11.5 ± 0.3
4	100 (1)	45 (1)	60 (0) (74 W)	5.9 ± 0.2	16.3 ± 0.9	20.8 ± 1.0	21.1 ± 0.4	100 (1)	90 (1)	285(0)	3.00 ± 0.2	6.4 ± 0.3	11.8 ± 2.5	6.8 ± 0.2
5	20 (−1)	25 (0)	20 (−1) (39 W)	5.6 ± 0.8	14.4 ± 0.1	21.0 ± 0.3	16.6 ± 0.1	20 (−1)	50 (0)	70 (−1)	4.9 ± 0.1	10.8 ± 0.0	10.8 ± 1.7	6.6 ± 0.0
6	100 (1)	25 (0)	20 (−1) (29 W)	3.9 ± 0.1	10.1 ± 0.3	13.6 ± 0.4	13.7 ± 0.3	100 (1)	50 (0)	70 (−1)	1.6 ± 0.0	3.4 ± 0.1	3.9 ± 0.5	4.0 ± 0.3
7	20 (−1)	25 (0)	100 (1) (157 W)	6.0 ± 0.3	18.7 ± 0.6	26.6 ± 1.1	20.3 ± 0.7	20 (−1)	50 (0)	500 (1)	7.4 ± 0.0	17.0 ± 0.5	22.5 ± 1.0	14.1 ± 0.4
8	100 (1)	25 (0)	100 (1) (130 W)	5.5 ± 0.2	17.7 ± 0.4	20.7 ± 0.5	20.6 ± 0.3	100 (1)	50 (0)	500 (1)	4.8 ± 0.2	7.0 ± 0.3	14.0 ± 1.1	7.0 ± 0.4
9	60 (0)	5 (−1)	20 (−1) (34 W)	9.2 ± 0.9	23.2 ± 0.1	32.2 ± 0.5	29.6 ± 0.1	60 (0)	10 (−1)	70 (−1)	4.7 ± 0.2	11.2 ± 0.2	12.0 ± 1.5	6.7 ± 0.0
10	60 (0)	45 (1)	20 (−1) (33 W)	8.3 ± 0.3	22.2 ± 0.4	31.3 ± 1.0	28.5 ± 0.9	60 (0)	90 (1)	70 (−1)	6.0 ± 0.1	11.3 ± 0.1	12.6 ± 1.0	6.8 ± 0.0
11	60 (0)	5 (−1)	100 (1) (151 W)	9.2 ± 0.4	23.8 ± 0.8	34.1 ± 1.2	30.85 ± 0.1	60 (0)	10 (−1)	500 (1)	8.6 ± 0.1	17.8 ± 2.3	24.3 ± 1.5	14.0 ± 0.8
12	60 (0)	45 (1)	100 (1) (142 W)	6.7 ± 0.8	20.8 ± 0.1	30.9 ± 0.1	24.1 ± 0.5	60 (0)	90 (1)	500 (1)	8.7 ± 0.2	17.8 ± 1.2	24.8 ± 1.6	16.5 ± 0.6
13	60 (0)	25 (0)	60 (0) (90 W)	9.2 ± 0.1	28.0 ± 0.7	44.0 ± 0.8	32.9 ± 1.2	60 (0)	50 (0)	285 (0)	7.3 ± 0.3	16.8 ± 1.1	20.7 ± 1.9	13.8 ± 0.0
14	60 (0)	25 (0)	60 (0) (89 W)	9.4 ± 0.4	28.0 ± 0.8	43.9 ± 0.8	33.5 ± 1.0	60 (0)	50 (0)	285 (0)	7.4 ± 0.5	17.1 ± 1.0	20.2 ± 1.1	14.7 ± 0.5
15	60 (0)	25 (0)	60 (0) (90 W)	9.3 ± 0.3	28.7 ± 0.8	44.3 ± 0.5	33.8 ± 1.3	60 (0)	50 (0)	285 (0)	7.4 ± 0.1	16.8 ± 0.8	20.4 ± 1.7	14.4 ± 0.2

Ethanol expressed as *v/v* %, time as min, amplitude as %, ratio sample/solvent as *w/v*, TPC as mg GAE/g dw, and DPPH, ABTS, and FRAP as mg TE/g dw. TPC: total phenolic compounds; GAE: gallic acid equivalent; TE: Trolox equivalent; dw: dry weight.

2.4. Extraction of Phenolic Compounds from Mango Peel Byproducts Using Sonotrode

A fixed ratio of 1:400 (*w/v*) was established to extract mango peel byproducts using a sonotrode (UP400St ultrasonic processor, Hielscher, Germany) according to previous studies. The experiments were carried out following the experimental conditions of the model (Table 1). Then, the samples were centrifuged at 8603× *g* for 10 min and evaporated using a Buchi R-205 rotavapor. Lastly, the samples were reconstituted in 2 mL of methanol/water (1:1) and filtered using a 0.2 µm nylon syringe filter.

2.5. Antioxidant Activity

The antioxidant capacity of mango peel byproducts was determined through three different methods (DPPH, FRAP, and ABTS). The DPPH assay was performed following the protocol of Brand-Williams et al. (1995) [23]. In this way, 0.1 mL of the extract was added to 2.9 mL of 100 µM DPPH solution in MeOH/H_2O 1/1 (*v/v*), and the absorbance was measured after 30 min at 517 nm. The protocol of Re et al. (1999) [24] was carried out for the ABTS assay. ABTS radical cation (ABTS+) was added to ethanol to reach an absorbance of 0.7 ± 0.02 at 734 nm. Thus, 10 µL of extract was added to 1 mL of ABTS reagent, and its absorbance was determined after 10 min. The FRAP assay was undertaken according to the process described by Pulido et al. (2000) [25]. For this, 30 µL of the extracts was added to 0.9 mL of water and 0.9 mL of FRAP reagent. The absorbance was observed after 30 min at 595 nm. For the three assays, the results were expressed as mg Trolox equivalent/g dry weight (dw). Each sample was processed in duplicate.

2.6. Determination of Total Phenolic Compound Using Folin–Ciocâlteu Assay

Total phenolic compounds were performed following the Folin–Ciocâlteu spectrophotometric method [26]. In this way, 100 µL of the extract was added to 500 µL of Folin–Ciocâlteu reagent and 6 mL of MilliQ water. The flask was agitated, and then 2 mL of 15% (*w/v*) Na_2CO_3 was added before flushing the flask to 10 mL with MilliQ water. Lastly, it was stored in dark conditions, and, after 2 h, absorbance was measured at 750 nm using a UV–visible spectrophotometer (Spectrophotometer 300 Array, UV–Vis, single beam, Shimadzu, Duisburg, Germany). To calculate the total phenolic compounds, the results were compared to a standard curve of gallic acid. For this reason, results were expressed as mg gallic acid equivalents (GAE)/g dry weight (dw).

2.7. Determination of Phenolic Compounds by HPLC–ESI-TOF-MS

The extracts of mango peel byproducts obtained using the optimized conditions from the Box–Behnken design were analyzed in duplicate on an ACQUITY Ultra Performance LC system (Waters Corporation, Milford, MA, USA) coupled to an electrospray ionization (ESI) source operating in the negative mode and a time-of-flight (TOF) mass detector (Waters Corporation, Milford, MA, USA). The phenolic compounds were separated using a BEH Shield RP18 column (1.7 µm, 2.1 mm × 100 mm; Waters Corporation, Milford, MA, USA) at 40 °C using a gradient previously stated by Verni et al. (2020) [27] (Supplementary Figure S1). The data were processed using MassLynx 4.1 software (Waters Corporation, Milford, MA, USA).

For the quantification of the phenolic compounds in mango peel samples, six different calibration curves of 1-O-Galloyl-β-D-glucose, chlorogenic acid, ferulic acid, quercetin, catechin, and rutin were used. They were elaborated by using the peak areas of each standard measured by HPLC at different concentrations. The identified compounds were classified in function of their structure and molecular weight. Appendix A Table A1 contains the standards used, with their calibration ranges and curves, the regression coefficients, and the limits of detection (LOD) and quantification (LOQ). These curves were good according to their good linearity ($R^2 > 0.99$). Calibration ranges were determined previously according to the LOQ values. LOD ranged between 0.16 and 2.24 µg/mL, and LOQ ranged between 0.54 and 7.48 µg/mL.

3. Results and Discussion

3.1. Fitting the Models

The total phenolic compounds and the antioxidant capacity assays were utilized to optimize the different ultrasonic models (ultrasonic bath and sonotrode) using a Box–Behnken design and response surface methodology (RSM). Table 1 shows all the experiments carried out for both models with the results obtained for all the responses.

The values obtained for TPC ranged between 1.6 and 8.7 mg GAE/g dw and between 3.9 and 9.4 mg GAE/g dw in the ultrasonic bath and sonotrode, respectively. The lowest ultrasonic bath and sonotrode values were obtained when 100% ethanol was used; this confirmed that the lower polarity of the organic solvent compared with water was not enough to recover the targeted compounds. On the other hand, the highest content of phenolic compounds was observed in the ultrasonic bath model with 60% ethanol/water (*v/v*), 90 min, and a 1:500 ratio of sample to solvent (*w/v*), whereas the conditions used to obtain the highest value of TPC in the sonotrode model were 60% ethanol/water (*v/v*), 25 min, and 60% amplitude. These data confirmed that the mixture with water (with higher polarity compared with 100% ethanol) was able to extract high amounts of phenolic compounds.

For antioxidant assays, ultrasonic bath values ranged from 3.4 to 17.8 mg TE/g dw, from 3.9 to 24.8 mg TE/g dw, and from 4.0 to 16.5 mg TE/g dw according to the DPPH, ABTS, and FRAP methods, respectively, whereas sonotrode values ranged from 10.1 to 28.7 mg TE/g dw, from 13.6 to 44.3 mg TE/g dw, and from 13.7 to 33.8 mg TE/g dw, respectively. The trend of antioxidant assay results was very similar to the TPC results; moreover, the lowest and highest values were obtained with the same TPC assay conditions in both models, confirming that the phenolic compounds with high antioxidant activity were better recovered using an ethanol/water mixture.

According to the Box–Behnken design, the experimental data were fitted to second-order polynomial equations, and the regression coefficients are shown in Table 2 for each variable response of the model designs of ultrasonic bath and sonotrode.

The models were tested using ANOVA, setting a statistical significance of confidence model at 95%. At $p < 0.05$, we rejected the null hypothesis that there was no difference between the means and concluded that there was a significant difference. For this reason, the terms with $p > 0.05$ were classified as nonsignificant, and the models were recalculated only with significant terms. The tendency and magnitude of influence on the response variables depended on the sign and value of effect. Positive values favored TPC, DPPH, ABTS, and FRAP, whereas negative values indicated an inverse relationship. Furthermore, the value of effect had a direct relationship with the magnitude on the response variables [22]. In the ultrasonic bath model (Table 2), all the linear terms were significant for all the response variables studied except for the linear effect of time (β_2) in the FRAP assay. Considering the crossed terms, all showed a significant influence except for the crossed effect β_{13} in TPC and ABTS responses. Additionally, the quadratic term β_{11} for TPC, DPPH, and FRAP, β_{22} for ABTS, and β_{33} for DPPH and FRAP showed no significant effect. In Table 2, the different effects of the variants and their correspondent *p*-values can be observed in the sonotrode model. Accordingly, the linear effects were all significant except for ethanol/water (X_1) in DPPH and FRAP methods and time (X_2) in TPC and FRAP assays. In the case of quadratic effects, all of them were significant for all the response variables studied. Lastly, considering the crossed effects, all showed a significant influence except for the crossed effect β_{12} in DPPH and ABTS responses, the crossed effect β_{13} in DPPH, ABTS and FRAP assays, and the crossed effect β_{23} in the DPPH response. In both models (ultrasonic bath and sonotrode), a high correlation between dependent and independent factors was obtained whose quadratic correlation coefficients (R^2) were higher than 0.96; therefore, the model had substantial response variability. Furthermore, the lacks of fit were not significant ($p > 0.05$); hence, the models fitted well.

Table 2. Estimated regression coefficients of the adjusted second-order polynomial equation and analysis of variance (ANOVA) of the sonotrode and ultrasonic bath model.

	Ultrasonic Bath								**Sonotrode**							
	TPC		**DPPH**		**ABTS**		**FRAP**		**TPC**		**DPPH**		**ABTS**		**FRAP**	
	Effect	***p*-Value**	**Effect**	***p*-Value**	**Effect**	***p*-Value**	**Effect**	***p*-Value**	**Effect**	***p*-Value**	**Effect**	***p*-Value**	**Effect**	***p*-Value**	**Effect**	***p*-Value**
β_0	5.5355 *	0.0000	11.2496 *	0.0000	15.4920 *	0.0000	9.2175 *	0.0002	6.2021	0.0000	17.2753 *	0.0000	24.0143 *	0.0000	20.9256 *	0.0000
Lineal																
β_1	−3.4867 *	0.0003	−8.1799 *	0.0002	−6.4299 *	0.0007	−4.7598 *	0.0040	0.3547 *	0.0228	−0.5233	0.2061	−3.3403 *	0.0026	1.2959	0.0594
β_2	0.3973 *	0.0197	0.9010 *	0.0188	−1.4636 *	0.0136	0.9540	0.0961	−0.2332	0.0505	1.8276 *	0.0232	2.0101 *	0.0071	1.0430	0.0876
β_3	3.0925 *	0.0003	5.4419 *	0.0005	11.6027 *	0.0002	6.3139 *	0.0025	0.4140 *	0.0169	3.8119 *	0.0054	4.4745 *	0.0014	3.0140 *	0.0118
Crossed																
β_{12}	2.3981 *	0.0003	6.0902 *	0.0002	5.3633 *	0.0005	4.3031 *	0.0027	1.3334 *	0.0030	0.0030	0.8862	0.4361	0.1958	2.6155 *	0.0276
β_{13}	0.0899	0.1510	1.1203 *	0.0060	−0.3348	0.1086	1.2314 *	0.0312	0.6125 *	0.0140	0.0140	0.0501	0.8154	0.0700	1.5791	0.0707
β_{23}	0.2633 *	0.0217	1.2760 *	0.0047	2.3397 *	0.0026	2.0726 *	0.0114	0.7910 *	0.0084	0.0084	0.1193	−1.1366 *	0.0379	−2.8725 *	0.0230
Quadratic																
β_{11}	−0.2178	0.1033	−0.2790	0.2391	1.0865 *	0.0425	−0.0160	0.9736	3.6936 *	0.0001	10.7067 *	0.0003	18.1533 *	0.0000	13.5639 *	0.0003
β_{22}	0.3454 *	0.0452	−1.2767 *	0.0169	−0.7717	0.0793	−2.2605 *	0.0341	0.5824 *	0.0042	3.4036 *	0.0034	6.4731 *	0.0003	3.0676 *	0.0053
β_{33}	−0.5770 *	0.0169	−0.0248	0.8964	−0.0240	0.9269	1.2136	0.1053	0.3732 *	0.0102	2.3264 *	0.0072	5.4665 *	0.0005	2.0479 *	0.0125
R^2	0.9928		0.9955		0.9903		0.9648		0.9915		0.9786		0.9977		0.9828	
p model	0.0000		0.0000		0.0000		0.0001		0.0031		0.0008		0.0000		0.0012	
p lack of fit	0.1026		0.1504		0.1022		0.2068		0.0505		0.1075		0.1083		0.0707	

* = Significant at $\alpha \leq 0.05$. (1) Ethanol/water ratio (*v*/*v*); (2) time; (3) amplitude in sonotrode model and ratio sample/solvent (*w*/*v*) in ultrasonic bath model.

3.2. Optimization of Extraction Conditions

In order to establish the optimal conditions of phenolic compound extraction via ultrasonic bath and sonotrode, the different response surface graphics (Figures 1 and 2) were observed and studied. In Figure 1, it can be observed that the highest content of phenolic compounds and antioxidant activity could be reached with an intermediate value of the range of ethanol/water ratio (*v/v*). However, the highest ratio of sample/solvent (*w/v*) was needed to get these results. Thus, the optimal bath ultrasonic conditions established were 45% ethanol, 60 min, and a sample/solvent ratio of 1:450 (*w/v*) (Table 3). The results obtained with these optimal conditions were in concordance with predicted conditions obtained by the mathematical model. The coefficients of variation (CV) that existed between the predicted and the experimental data were lower than 10% in all cases. In the Folin–Ciocâlteu assay and all antioxidant assays (DPPH, ABTS, and FRAP), the CV was less than 4. For these reasons, the ultrasonic bath model optimization was established with these conditions. After the analysis of the different graphics performed by response surface methodology (Figure 2), it was observed that the parameters studied to optimize the sonotrode extraction conditions were found in the middle of the plots. Therefore, the ethanol/water (*v/v*), time (min), and amplitude (%) values needed to obtain an optimized model were in the intermediate ranged used to carry out the different assays of the model. In this way, the optimal sonotrode conditions used were 55% ethanol, 18 min, and 65% amplitude (Table 3). This model can be considered optimized for these parameters. The time influence depended on the other variables (ethanol/water percentage, the sample/solvent ratio (*w/v*), and amplitude); however, in Figures 1 and 2, it can be observed that intermediate time values were good to extract phenolic compounds.

Table 3. Optimal conditions selected and models predicted values with the obtained values expressed with the mean and the standard deviation of ultrasonic bath and sonotrode.

	Parameter	**Optimal Conditions Ultrasonic Bath**			**Parameter**	**Optimal Conditions Sonotrode**		
	Ethanol/water (*v/v*)	45			Ethanol/water (*v/v*)	55		
	Time (min)	60			Time (min)	18		
	Ratio sample/solvent (*w/v*)	1/450			Amplitude (%)	65		
	TPC (mg/g)	DPPH (mg/g)	ABTS (mg/g)	FRAP (mg/g)	TPC (mg/g)	DPPH (mg/g)	ABTS (mg/g)	FRAP (mg/g)
Predicted value (mg/g dw)	8.6 ± 0.5	19.5 ± 1.2	24.0 ± 1.6	16.8 ± 1.5	9.5 ± 0.5	27.7 ± 2.6	43.3 ± 1.6	33.7 ± 3.1
Obtained value (mg/g dw)	8.3 ± 0.2	19.0 ± 1.4	24.9 ± 0.9	17.6 ± 1.7	9.7 ± 0.2	27.7 ± 1.3	43.2 ± 1.5	33.5 ± 0.8
	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

N.S. = not significant.

According to the literature, ethanol can be used in the food system due to it is classified as GRAS (generally recognized as safe) [28]. Some authors studied the extraction of phenolic compounds by mango peels using ethanolic maceration with and without stirring but needing large times and with unsatisfactory results [15,29]. García-Mendoza et al. (2015) [15] and Sai-Ut et al. (2015) [29] needed 24 h and 221 min to complete their extractions by maceration, respectively. Safdar et al. (2017) [5] reported a higher content of total phenolic compounds and antioxidant capacity when using ethanol (80%) instead of methanol, acetone, or ethyl acetate by maceration and ultrasound-assisted extraction with sonotrode. However, when they used 50% ethanol, no significant differences were found according to the antioxidant extraction. Comparing both techniques, the sonotrode method for 60 min allowed extracting 13% higher total phenolic content than maceration for 20 h.

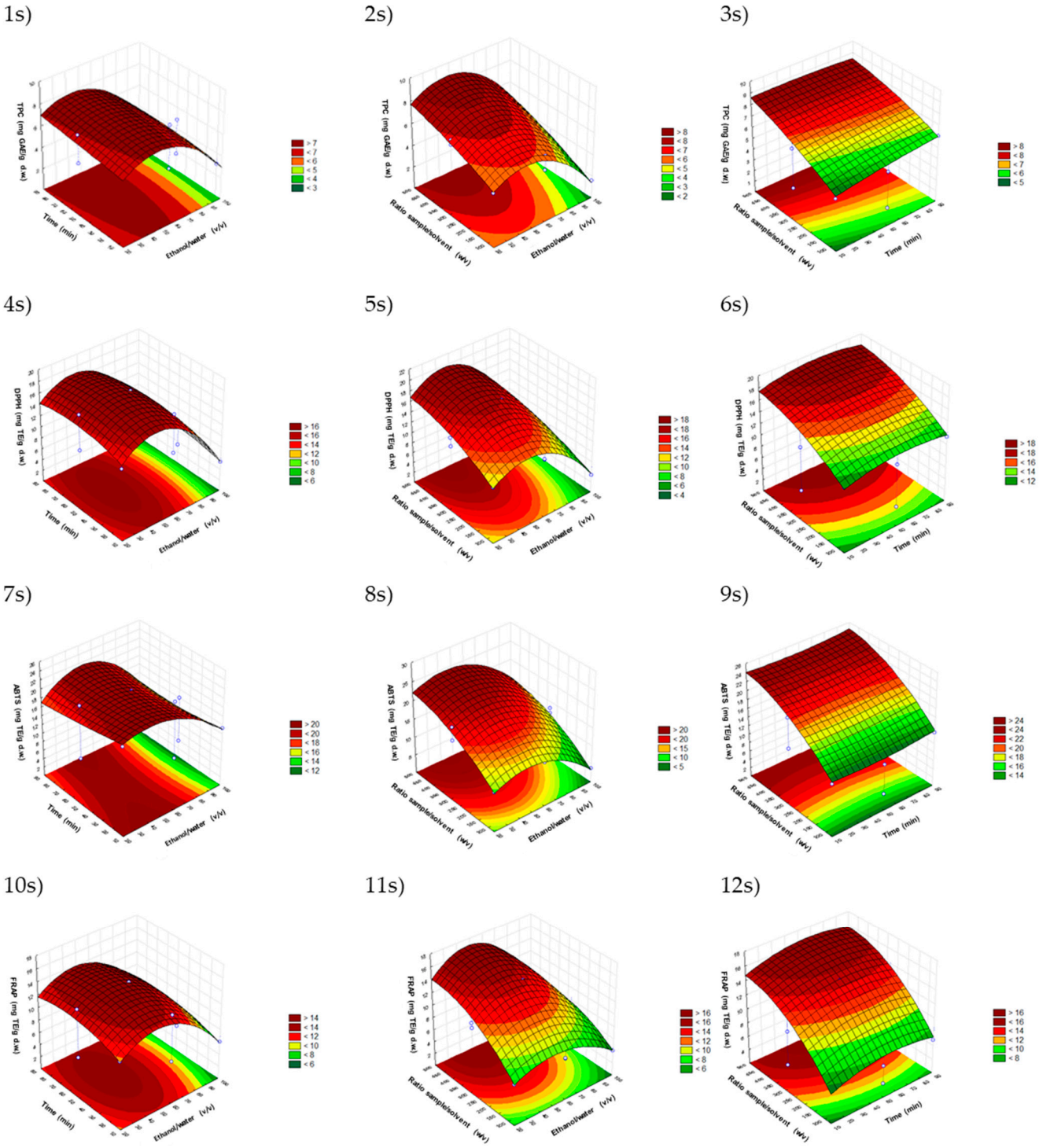

Figure 1. Response surface graphs (1s–12s) of ultrasonic bath model showing the combined effects of the process variables: ethanol/water (*v*/*v*), time (min), and ratio sample/solvent (*w*/*v*) for TPC (mg GAE/g dw), for DPPH antioxidant assay (mg TE/g dw), for ABTS antioxidant assay (mg TE/g dw), and for FRAP antioxidant assay (mg TE/g dw).

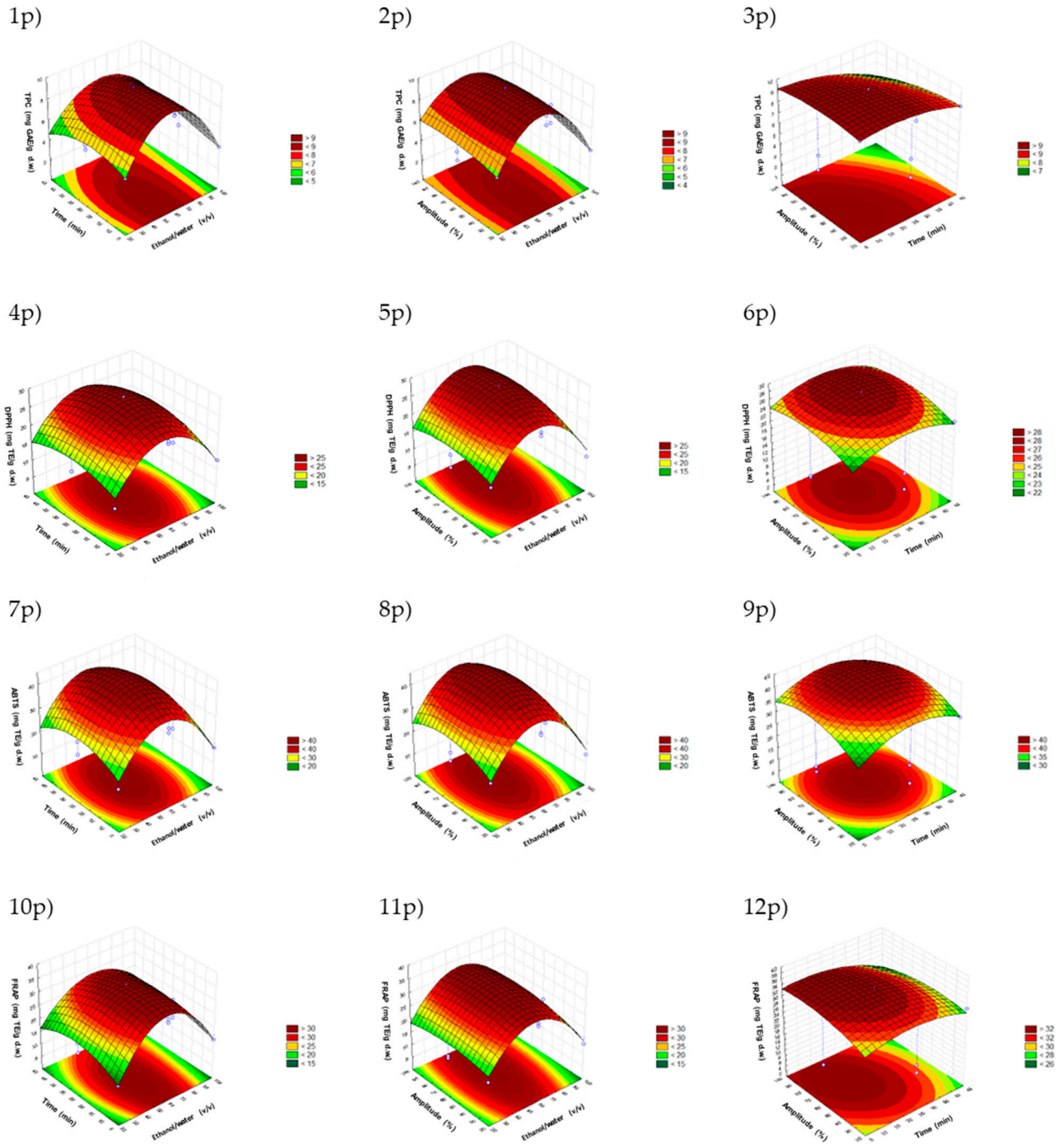

Figure 2. Response surface graphs of sonotrode model (1p–12p) showing the combined effects of the process variables: ethanol/water (*v*/*v*), time (min), and amplitude (%) for TPC (mg GAE/g dw), for DPPH antioxidant assay (mg TE/g dw), for ABTS antioxidant assay (mg TE/g dw), and for FRAP antioxidant assay (mg TE/g dw).

Martínez-Ramos et al. (2020) [30] compared a conventional solvent extraction and an ultrasound-assisted extraction with sonotrode. They also carried out extractions with different solvent mixtures of acetone, ethanol, and n-hexane. They reported the best mixture as ethanol/acetone 60%/40% due to the capacity of ethanol to extract glycosidic and non-glycosidic phenolic compounds, whereas acetone only extracts non-glycosidic phenolic compounds. The results of phenolic compounds obtained with the sonotrode ultrasound-assisted method in this article were in the same range of magnitude as those

found in our study using a similar time. In fact, we only used ethanol as an organic solvent combined with water. Borrás-Enríquez et al. (2021) also optimized the extraction of phenolic compounds from mango peel using an ultrasonic bath. Optimal conditions were obtained with 50% ethanol/water (*v/v*) [22]. The ethanol/water (*v/v*) percentage was very similar to the ultrasonic bath percentages of our study. In our case, we needed a longer time due to their bath frequency being 80 kHz in contrast to our bath with a frequency of 35 kHz [22]. Kaur et al. (2021) [2] used ultrasound-assisted extraction by sonotrode, finding that the highest extraction of phenolic compounds was obtained with an amplitude and time lower than those obtained in our work.

Mango peel can be blended with liquid nitrogen due to it being an excellent freezing agent that may control enzymatic browning. Then, it can be used in other food matrices such as potato puree [31]. Jirasuteeruk and Theerakulkait [31] extracted phenolic compounds after pretreatment using distilled water and a sample/solvent ratio (*w/v*) of 1:6. A better time was obtained at 15 min using an ultrasonic bath at 50 kHz combined with 15 min of stirring. The total phenolic compounds extracted were in the same range as our research (972 mg/100 g dw); however, in our case, this pretreatment was not used.

In addition, in other investigations, new technologies were used such as ultrasound/microwave-assisted extraction, microwave-assisted extraction [14,32], microwave-assisted extraction with deep eutectic solvents [33], supercritical CO_2 extraction [34], and sequential extraction steps [15].

All previous research on other technologies used for extracting phenolic compounds from the mango peel byproduct are summarized in Table 4.

Table 4. Previous research about other technologies used for extracting phenolic compounds from the mango peel byproduct with the conditions used and the total phenolic compounds (TPC) obtained.

Technology Used	Optimum Conditions	TPC (mg GAE/g dw)	Ref.
Maceration	80% ethanol/water (*v/v*), 1:15 sample-to-solvent ratio (*w/v*), 40 °C, 20 h	59.7 mg GAE/ g of powder extract	[5]
Maceration	49% ethanol/water (*v/v*), 1:30 sample-to-solvent ratio, 61 °C, 221 min	1.1 mg GAE/g dry sample	[29]
Conventional solvent extraction	1:20 sample to solvent ratio (*w/v*) ethanol–acetone blend (60–40%), 15 min, 1000 rpm stirring	2.0 mg GAE/g dry sample	[30]
Maceration	3.33 ratio of sample/solvent (*w/v*) with ethanol 100%, magnetic stirring for 24 h, 25 °C	41.6 mg GAE/g of extract	[15]
Ultrasound-assisted extraction (ultrasonic bath)	50% ethanol/water (*v/v*), 1:50 ratio of solvent to solid (*w/v*), 20 min, 60% amplitude, 200 W, 80 kHz	18.1 mg/g d.w. sample	[22]
Ultrasound-assisted extraction (ultrasonic bath) +stirring	Liquid nitrogen + distilled water 1:6 sample/solvent (*m*/*V*), 25 °C, 15 min ultrasound extraction +15 min stirring, 50 kHz, 160 W	9.7 mg/g d.w. sample powder	[31]
Ultrasound-assisted extraction (ultrasonic probe)	50% ethanol/water 1:30 sample/solvent (*v/w*), 45 °C, 10 min, 30% amplitude	35.5 mg GAE/g of raw sample	[2]
Ultrasound-assisted extraction (sonicator)	80% ethanol, sample-to-solvent ratio of 1:20, 45 °C, 60 min, 35 kHz, 100% amplitude	67.6 mg/g of extract	[5]
Ultrasound-assisted extraction (ultrasonic probe)	Ethanol–acetone blend (60–40%), 1:20 sample to solvent ratio (*w/v*), 24 kHz, 15 min	14.9 mg GAE/g dry sample	[30]

There are few studies about the antioxidant activity of mango byproducts; however, those that evaluated the radical-scavenging activity mostly used the DPPH and ABTS techniques. Martínez-Ramos et al. [30] reported values of 26.4 mg TE/100 g dw and 21.1 mg TE/100 g dw for DPPH and ABTS assays, respectively, in mango peels extracts obtained by ultrasound-assisted extraction with ethanol/acetone 60/40 (*v/v*) for 15 min at 24 kHz. Sai-Ut et al. [29] reported values slightly higher but they used maceration as the extraction technique with temperature (60 °C) and needing longer time (220 min), yielding 102.57 mg TE/100 g for the DPPH method, 108.8 mg TE/100 g for the ABTS technique, and 52.72 mg TE/100 g for the FRAP assay. These results were in the same range of magnitude as those reported here. Nevertheless, Castañeda-Valbuena et al. [12], in mango peel from the Haden variety, reported results in the range of 65.9 to 249.3 mg TE/g for the DPPH method, between 239.1 and 1155.8 mg TE/g for the ABTS technique, and between 7.1 and 100.9 mg TE/g for the FRAP assay, representing the same range of magnitude as the results obtained here for mango peels of the Palmer variety.

3.3. Identification of Polar Compounds Using HPLC–MS-ESI-TOF

Phenolic compounds of mango peel byproducts were identified from the samples extracted in the optimal conditions using HPLC-ESI-TOF-MS. It was possible to compare the different mass data and fragment ions with the literature, commercial standards, and various databases. Determination of phenolic compounds from mango peel byproducts took into account the retention time, experimental and calculated *m/z*, the error (ppm), score (%), mainly in source fragment ions, and molecular formula.

Table 5 presents a total of 35 identified polar compounds from the sonotrode and ultrasonic bath optimized samples, including two organic acids, 25 phenolic acid derivatives, 10 flavonoids, one monoterpenoid, and another polar compound. The studies of Gómez-Caravaca et al. [4] and Pierson et al. [35] were used to identify most phenolic compounds.

Firstly, two organic acids were identified at 0.39 min and 0.49 min (peaks 3 and 4) corresponding to quinic and citric acid, respectively [4,35].

Phenolic acids were the main representative phenolic compounds found in mango peel. Peak 5 and peak 9 corresponded with galloylglucose isomers a and b, respectively. The compound at 0.803 min (peak 6) was identified as gallic acid. Peak 7 corresponded to 3-galloylquinic. Peak 8 had a molecular formula of $C_{19}H_{25}O_{15}$ and was proposed to be galloyl diglucoside. Peak 10 was identified as p-hydroxybenzoic acid glucoside. Peak 11 and 27 were methylgallate isomers. Peak 12 corresponded with digalloylglucose. The peak at 4.331 min (peak 15) and with a molecular formula of $C_{16}H_{20}O_9$ was tentatively identified as ferulic acid hexoside. Peak 18 corresponded to syringic acid. Peak 19 was identified as sinapic acid hexoside-pentoside. Peak 20 was assigned to dihydro sinapic acid hexoside-pentoside, and peak 22 corresponded to the hydroxybenzoyl galloyl glucoside. Peak 23 had a molecular formula of $C_{27}H_{24}O_{18}$ and was identified as trigalloyl glucose. Peak 26 with *m/z* 477.1017 corresponded to coumaroyl galloyl glucoside. Peak 28 was identified as methyl-digallate ester, and peak 33 was protocatechuic acid. Peak 35 with $C_{16}H_{14}O_9$ as the molecular formula corresponded to ethyl 2,4-dihydroxy-3-(3,4,5-trihydroxybenzoyl) oxybenzoate. Peak 36 at 9.972 min with a fragment at 124 and $C_9H_{10}O_5$ as the molecular formula was identified as syringic acid [4,35]. Furthermore, some new phenolic acids were tentatively identified in mango peel for the first time. Firstly, peak 1 at 0.32 min, *m/z* 341 and fragment ions 191, 165, and 113 corresponded to hexosyl-hexose, identified previously in mango seed samples [36]. Peak 2 at 0.34 min with *m/z* 377.0817 and fragment ion 341 was tentatively identified as a caffeic acid derivative previously described in *Lysimachia* species [37]. Peaks 16 and 17 were isomers with *m/z* 401.1448 and, according to fragment ions 313, 125, and 161, they were classified as 6-pentyl-O-galloyl-beta-d-glucose according to the PUBCHEM database. Peak 21 at 5.367 min, with *m/z* 533.1869, $C_{23}H_{34}O_{14}$ as the molecular formula, and a fragment ion at 401.1404, was considered to be dicaffeoylhexaric acid, which was previously identified in *Helichrysum italicum* samples [38].

Table 5. Identified compounds from optimum ultrasonic bath and sonotrode mango peel byproduct samples using HPLC–MS.

No.	Retention Time (min)	*m*/*z* Experimental	*m*/*z* Calculated	Error (ppm)	Score (%)	Fragments	Molecular Formula	Compound	Ultrasonic Bath	Sonotrode
1	0.319	341.1073	341.1084	−3.2	98.42	191.0537, 165.0367, 113.0212	$C_{12}H_{22}O_{11}$	Hexosyl-hexose	X	X
2	0.34	377.0877	377.0873	1.1	94.83	341.1050, 191.0179	$C_{18}H_{18}O_9$	Caffeic acid derivative	X	X
3	0.39	191.0549	191.0556	−3.7	100	111.0016	$C_7H_{12}O_6$	Quinic acid	X	X
4	0.4895	191.0186	191.0192	−3.1	100	111.0063	$C_6H_8O_7$	Citric acid	X	X
5	0.659	331.0656	331.0665	−2.7	90.28	169.0085, 125.0135	$C_{13}H_{16}O_{10}$	Galloylglucose isomer I	X	X
6	0.803	169.0129	169.0137	−4.7	100	125.0271	$C_7H_6O_5$	Gallic acid	X	X
7	1.006	343.0663	343.0665	−0.6	90.12	169.013, 191.0536	$C_{14}H_{16}O_{10}$	3-Galloylquinic acid	X	X
8	1.097	493.1197	493.1193	0.8	91.64	169.0131, 125.0214	$C_{19}H_{26}O_{15}$	Galloyl diglucoside	X	X
9	1.217	331.0652	331.0665	−3.9	99.8	169.0112, 125.0219	$C_{13}H_{16}O_{10}$	Galloylglucose isomer II	X	X
10	1.498	299.0769	299.0767	0.7	100	137.0212	$C_{13}H_{16}O_8$	*p*-Hydroxybenzoic acid glucoside		X
11	2.359	183.029	183.0293	−1.6	99.7	124.0117	$C_8H_8O_5$	Methylgallate	X	X
12	3.278	483.076	483.0775	−3.1	92.58	169.0083, 125.0222	$C_{20}H_{20}O_{14}$	Digalloylglucose	X	X
13	3.865	289.0698	289.0712	−4.8	94.78	123.0458	$C_{15}H_{14}O_6$	Catechin	X	X
14	3.878	443.191	443.1917	−1.6	99.25	137.0241, 101, 151,213,303	$C_{21}H_{21}O_{10}$	Unknown	X	X
15	4.331	355.1043	355.1029	3.9	91.12	193.0481, 134.0353	$C_{16}H_{20}O_9$	Ferulic acid hexoside	X	X
16	4.465	401.1445	401.1448	−0.7	96.24	313.0528, 125.0237	$C_{18}H_{26}O_{10}$	6-pentyl-O-galloyl-beta-d-glucose isomer I	X	X
17	4.718	401.1435	401.1448	−3.2	90.2	161.0387	$C_{18}H_{26}O_{10}$	6-pentyl-O-galloyl-beta-d-glucose isomer II		X
18	4.854	197.0445	197.045	−2.5	99.91	124.0143, 125.0232, 169.0094	$C_9H_{10}O_5$	Syringic acid	X	X
19	5.164	517.2298	517.2285	2.5	96.76	153.0894, 205.1173, 385.1864	$C_{24}H_{38}O_{12}$	Sinapic acid hexoside-pentoside	X	X

Table 5. *Cont.*

No.	Retention Time (min)	*m/z* Experimental	*m/z* Calculated	Error (ppm)	Score (%)	Fragments	Molecular Formula	Compound	Ultrasonic Bath	Sonotrode
20	5.305	519.2421	519.2442	−4	97.11	387.2, 225.1477	$C_{24}H_{40}O_{12}$	Dihydro sinapic acid hexoside-pentoside	X	X
21	5.367	533.1869	533.187	−0.2	90.47	401.1404	$C_{23}H_{34}O_{14}$	Dicaffeoylhexaric acid	X	X
22	5.404	451.0872	451.0877	−1.1	90.42	169.0043	$C_{20}H_{20}O_{12}$	Hydroxybenzoyl galloyl glucoside	X	X
23	5.768	635.0894	635.0884	1.6	92.79	169.0086	$C_{27}H_{24}O_{18}$	Trigalloyl glucose		X
24	5.859	443.1904	443.1917	−2.9	99	314.043	$C_{21}H_{32}O_{10}$	Cynaroside A		X
25	7.27	553.1551	553.1557	−1.1	90.82	391.621	$C_{25}H_{30}O_{14}$	Ligustrosidic acid	X	X
26	7.063	477.1017	477.1033	−3.4	91.41	313.0555, 163.0370, 119.0459, 169.0117	$C_{22}H_{22}O_{12}$	Coumaroyl galloyl glucoside	X	X
27	7.99	183.0291	183.0293	−1.1	99.4	124.0122	$C_8H_8O_5$	Methylgallate isomer I	X	X
28	8.057	335.0391	335.0403	−3.6	96.35	183.0244, 124.0123	$C_{15}H_{12}O_9$	Methyl-digallate ester		X
29	8.995	463.0875	463.0877	−0.4	99.81	300.0253	$C_{21}H_{20}O_{12}$	Quercetin glucoside	X	X
30	9.17	463.0868	463.0877	−1.9	96.82	300.0253	$C_{21}H_{20}O_{12}$	Quercetin galactoside	X	X
31	9.707	433.075	433.0771	−4.8	99.95	271.0219, 241.0106, 300.0254	$C_{20}H_{18}O_{11}$	Quercetin xyloside	X	X
32	9.757	447.0913	447.0927	−3.1	90.17	300.0252, 271.0215	$C_{21}H_{20}O_{11}$	Quercetin 3-rhamnoside isomer I	X	X
33	9.79	153.091	153.0916	−3.9	n/a	149.6901	$C_9H_{14}O_2$	Protocatechuic acid	X	X
34	9.844	433.0754	433.0771	−3.9	93.23	300.0242	$C_{20}H_{18}O_{11}$	Quercetin arabinopyranoside	X	X
35	9.906	349.0544	349.056	−4.6	95.21	124.014, 197.04	$C_{16}H_{14}O_9$	Ethyl 2,4-dihydroxy-3-(3,4,5-trihydroxybenzoyl)oxybenzoate		X
36	9.972	197.0443	197.045	−3.6	93.87	124.0131	$C_9H_{10}O_5$	Syringic acid	X	X
37	10.092	447.092	447.0927	−1.6	94.18	284.025, 255.0225, 227.0386	$C_{21}H_{20}O_{11}$	Quercetin 3-rhamnoside isomer II	X	X
38	10.293	447.0925	447.0927	−0.4	n/a	255.0284	$C_{21}H_{20}O_{11}$	Quercetin 3-rhamnoside isomer III	X	X
39	11.156	477.1035	477.1033	0.4	90.36	299.0186	$C_{22}H_{22}O_{12}$	Rhamnetin hexoside	X	X

Moreover, some flavonoids were found in the mango peel samples according to Gómez-Caravaca et al. (2016) [4] and Pierson et al. (2014) [35]. Peak 13 was identified as catechin, and peaks 29 and 30 corresponded to quercetin glucoside and quercetin galactoside, respectively. Peak 31 with a molecular formula of $C_{20}H_{18}O_{11}$ was identified as quercetin xyloside. Peaks 32, 37, and 38 were isomers with fragment ions at 300, 271, 284, 255, and 227, which were identified as quercetin 3-rhamnoside. Peak 34 at 9.844 min, with *m/z* 433.0754, corresponded to the compound quercetin arabinopyranoside. Peak 39 with a molecular formula of $C_{22}H_{22}O_{12}$ corresponded to rhamnetin hexoside. In addition to these flavonoids, special attention was paid to those identified in mango peel for the first time. Peak 24 at 5.859 min, with *m/z* 443.1904 and the fragment ion 314, was tentatively named cynaroside A in concordance with the PUBCHEM database.

Additionally a secoiridoid monoterpenoid was found at 7.27 min (peak 25), with *m/z* 553.1551, tentatively named as ligustrosidic acid according to a previous study of the herbal medicine *Ligustri Lucidi Fructus* [39]. Furthermore, another polar compound was detected corresponding to peak 35 with a molecular formula of $C_{16}H_{14}O_9$ and fragment ions 124 and 197, tentatively named ethyl 2,4-dihydroxy-3-(3,4,5-trihydroxybenzoyl)oxybenzoate [40].

3.4. Quantification of Phenolic Compounds in Mango Peel Byproducts by HPLC–MS-ESI-TOF

A total of 22 and 15 compounds could be quantified in sonotrode and ultrasonic bath samples, respectively (Table 6).

Table 6. Quantification of phenolic compounds in sonotrode and ultrasonic bath of mango peel byproducts by HPLC–MS expressed as μg/g dw.

Compound	Ultrasonic Bath (μg/g dw)	Sonotrode (μg/g dw)
Caffeic acid derivate	995.3 ± 0.6	843.4 ± 0.2
Galloylglucose isomer I	2736.6 ± 0.2	2795.4 ± 0.3
Galloylglucose isomer II	2291.9 ± 0.7	2246.9 ± 0.6
Gallic acid	141.3 ± 0.1	270.0 ± 0.02
p-Hydroxybenzoic acid glucoside	<LOQ	39.7 ± 0.01
Methylgallate	237.7 ± 0.05	2037.0 ± 0.3
Digalloylglucose	<LOQ	536.8 ± 0.2
Catechin	<LOQ	37.5 ± 0.01
6-pentyl-O-galloyl-beta-d-glucose isomer I	<LOQ	68.4 ± 0.05
6-pentyl-O-galloyl-beta-d-glucose isomer II	n.d.	32.3 ± 0.03
Syringic acid	37.3 ± 0.01	1014.7 ± 0.05
Sinapic acid hexoside-pentoside	651.9 ± 0.2	482.5 ± 0.03
Dicaffeoylhexaric acid	96.7 ± 0.002	40.8 ± 0.002
Hydroxybenzoyl galloyl glucoside	1268.8 ± 0.3	1043.8 ± 0.1
Cynaroside A isomer I	247.1 ± 0.2	316.2 ± 0.01
Ligustrosidic acid	128.7 ± 0.003	70.8 ± 0.002
Coumaroyl galloyl glucoside	112.3 ± 0.002	119.2 ± 0.03
Quercetin glucoside	225.7 ± 0.09	232.0 ± 0.03
Quercetin galactoside	68.6 ± 0.05	54.2 ± 0.009
Quercetin xyloside	15.2 ± 0.02	45.6 ± 0.005
Protocatechuic acid	<LOQ	29.1 ± 0.003
Quercetin arabinopyranoside	<LOQ	12.5 ± 0.002
Rhamnetin hexoside	<LOQ	<LOQ
Sum of phenolic compounds	9225.1 ± 0.8	12368.8 ± 0.9
Sum of flavonoids	556.6 ± 0.3	968.0 ± 0.1
Sum of phenolic acids	8698.5 ± 0.5	11670.8 ± 0.3

n.d., not detected; <LOQ, lower than limit of quantitation.

As expected, the value obtained via HPLC–TOF-MS, which is a specific method for the determination of phenolic compounds, and that obtained via the Folin–Ciocâlteu assay, which is a spectrophotometric method, were a little bit different but in the same order of magnitude.

According to another study [4], the main compounds on sonotrode and ultrasonic bath samples were galloylglucose and methylgallate, a phenolic acid derivative of gallic acid. The amount of galloylglucose extracted by the two ultrasound techniques were quite similar. In sonotrode extracts, a higher content in methylgallate was detected, which was

more than eight times higher than in the bath ultrasound extract. The sum of galloylglucose isomers and methylgallate was higher in sonotrode samples (7.1 vs. 5.3 mg/g dw). In both cases, the sum of galloylglucose isomers and methygallate represented more than 50% of the total phenolic compounds, specifically, 57.2% in sonotrode and 56.9% in ultrasonic bath. The study of López-Cobo et al. (2017) [41] found 11–15% galloylglucose isomer I, 8–10% methyldigallate ester isomer, and 5–7% methylgallate in three different varieties of mango peel, being the main representative compounds of the samples; however, it should be taken into account that, in this work, we analyzed a different variety from different origin. According to Hu et al. (2018) [42], gallic acid content was higher in mango peel in comparison with mango pulp. Syringic acid was the third highest compound in the sonotrode sample, representing about, 8% whereas the values obtained in ultrasonic bath were very low, around 0.4%. Syringic acid was one of main compounds in mango peel according to Ajila et al. [43] who extracted with 80% acetone. In the article of Peng and coworkers [44], the main phenolic compounds were quantified, observing that syringic acid was the highest compound present in the peel of Kensington Pride variety (17.78 mg/g dw) and the third highest in Keitt variety (9.3 mg/g dw). Hydroxybenzoyl galloyl glucoside was found in higher amounts in ultrasonic bath samples (1.27 mg/g dw). On the other hand, the flavonoids were found in the same order of magnitude; however, the sonotrode extract reported the highest amounts. Quercetin-glucoside was the main flavonoid, representing 40.5% and 33.2% of the total flavonoids in the bath and sonotrode extracts, respectively.

4. Conclusions

A comparison of the extraction of phenolic compounds and determination of antioxidant activity of mango peel byproducts obtained via ultrasonic bath and sonotrode was carried out for the first time to our knowledge.

The optimal ultrasonic bath conditions were 45/55 ethanol/water (*v/v*), 60 min, and 1/450 ratio of sample/solvent (*w/v*), whereas the optimal sonotrode conditions were 55/45 ethanol/water (*v/v*), 18 min, and 65% amplitude. A total of 35 polar compounds were identified by HPLC–ESI-TOF-MS, from which six were tentatively described here for the first time. In both extractions, phenolic acid derivatives from gallic acid were the majority, representing more than 50% of phenolic compounds from mango peel byproducts. Galloylglucose was the main phenolic compound in both extracts, and the amounts obtained with both techniques were in the same order of magnitude. The total amount of phenolic compounds was higher in the sonotrode sample (+33%) needing a lower time to reach this value. In conclusion, the optimized method via sonotrode extraction is useful to analyze mango peel byproducts for food and nutraceutical applications. In addition, sonotrode ultrasound technology can be scaled up at a pilot and industrial level.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae8111014/s1: Figure S1. HPLC-MS chromatograms of ultrasonic and sonotrode extracts extract.

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Appendix A

Table A1. Standard analytes used for elaborating the calibration curves with the range used, equations, R^2, LOD, and LOQ of each compound for the phenolic compound analysis.

Standard	LOD (μg/mL)	LOQ (μg/mL)	Calibration Ranges (μg/mL)	Calibration Curves (μg/mL)	R^2
1-O-Galloyl-β-D-glucose	2.2443	7.4812	LOQ-237	y = 6.1459x + 122.9	0.9976
Chlorogenic acid	0.2160	0.7201	LOQ-247	y = 63.853x + 135.09	0.9978
Ferulic acid	1.1142	3.7139	LOQ-227	y = 12.38x + 92.068	0.9980
Catechin	0.2184	0.7281	LOQ-230	y = 63.149x + 124.93	0.9921
Rutin	0.7026	2.3420	LOQ-220	y = 19.632x + 403.42	0.9924
Quercetin	0.1631	0.5436	LOQ-227	y = 84.589x + 287.32	0.9957

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