Effect of solvent composition and its interaction with ultrasonic intensity on the ultrasound-assisted extraction of phenolic compounds from Mango peels (*Mangifera indica* L.).

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Abstract

Ultrasound has been used to intensify the extraction of phenolic compounds from many agro-food products. However, there is still a lack of understanding on how the ultrasonic energy is influenced by blends of different solvents and how this impacts the extraction process. This work studied the effect of ethanol, acetone and hexane blends on the ultrasonic energy generated during the extraction of phenolic compounds from Mango peel, using an ultrasonic-assisted extraction (UAE) and a conventional solvent extraction (CSE). A simplex centroid mixture design and a special cubic regression model were used to evaluate the total phenolic compounds (TPC), antioxidant activity (AA) and ultrasonic intensity (UI) as a function of the solvents proportions. The greatest TPC was obtained with the ethanol-acetone blend (60-40%) for CSE (205.08 mg GAE/100 g DM) and UAE (1493.01 mg GAE/100 g DM). Likewise, an increase (avg. 630%) was observed in TPC when the ultrasound was applied for all solvents and their blends. The TPC showed a good correlation (R^2=0.81) with the UD, with higher UD resulting in larger amounts of TPC extracted. Nevertheless, for the ethanol-acetone blend there was a decrease of 14.2% of the AA for the UAE, which could be due to the sonochemical reactions taking place at the high UD achieved for that blend. The results of this work indicate that the solvent composition and use of ultrasound should be carefully selected to achieve the desired extraction objectives.

Key words: Cavitation, Bioactive Compounds, Physical Properties-Solvents, Mass Transfer.

1. Introduction

Great attention has been paid to the extraction of bioactive compounds from plant materials, since these compounds have the ability to promote benefits to human health. This is due to their potential antioxidant activities that contribute to the prevention of oxidative stress related diseases (Ajila et al., 2007; Guandalini et al., 2019; Lobo et al., 2017). The most common bioactive compounds are secondary metabolites, such as phenolic compounds, which are often present in byproducts obtained from the processing of several fruit products. For example, from Mango (*Mangifera indica* L.) processing, the peels and seeds are the major byproducts with a potential source of phenolic compounds (Gómez-Caravaca et al., 2016; Jahurul et al., 2015; Lobo et al., 2017). Particularly, mango peels contain phenolic compounds such as, flavonol O-glycoside, xanthone C-glycoside, gallotannins, ethyl gallate, mangiferin and benzophenone derivatives (Burton-Freeman et al., 2017; Jahurul et al., 2015; Meneses et al., 2015). The recovery of these compounds from mango peel would generate a sustainable source for the materials and reduce the amount of bio-waste produced during mango production. However, obtaining phenolic compounds from bio-waste depends on the extraction technique utilized and other factors, such as the variables involved in the extraction process (temperature, time of extraction, liquid-solid ratio, particle size, pH, type of solvent). Solvent extraction is the most common method used for isolating phenolic compounds and the yield of the extraction of this compounds have been found to be affected by the nature of solvent (polarity). Therefore, the type of solvent plays a key role in the extraction of phenolic compounds (Rezaie et al., 2015), presenting challenges when attempting to develop a unified standard method for the extraction of phenolic compounds.

Advances have been made in extraction processes with the application of novel technologies. For example, microwave-assisted extraction (Cassol et al., 2019; Rodsamran and Sothornvit, 2019), supercritical fluid extraction (Gallego et al., 2019; Pimentel-Moral et al., 2019), pressurized fluid extraction (Santana et al., 2019) and ultrasonic-assisted extraction (Deng et al., 2017; Wen et al., 2018) have been shown to reduce extraction time

and solvent consumption, in addition to lowering the temperature and energy requirement. These advances have resulted in more efficient and sustainable extraction processes.

Ultrasonic-Assisted Extraction (UAE) is a technique which propagates low frequency ultrasonic waves (i.e. 20 kHz) with a high sound power or sound intensity (generally higher than 1 Wcm^{-2}) into the liquid solvent used for solid-liquid extraction. Ultrasonic assisted extraction is primarily driven by acoustic cavitation although other effects such as acoustic streaming are also present. Acoustic cavitation is the formation, growth, oscillation and powerful collapse of gas bubbles into the solvent. The bubble collapse results in small-scale intense agitation, and facilitates the penetration of the solvent in the natural matrix, affecting its integrity through the cell walls. This enhances the release of the intracellular content to the extraction solvent and improves mass transfer processes (Tiwari, 2015; Wen et al., 2018).

Several works in the literature have used different solvents and the application of ultrasound for the extraction of phenolic compounds from different matrices including dry date pits (Liu et al., 2018), bene fruit (Rezaie et al., 2015) and rice grains (Setyaningsih et al., 2019). The results have shown that solvent composition and ultrasound both have effects on the extraction processes which are related to the chemical affinity between the solid matrix and the solvent, and by the increased mass transfer caused by the application of ultrasound. The previous research indicated that the polarity, selectivity, viscosity, vapor pressure and surface tension are important physicochemical properties that should be considered when selecting a suitable solvent for the ultrasound assisted extraction.

Nevertheless, only pure solvents at varying concentration were studied and therefore the interaction between solvents with different physicochemical properties, which might affect ultrasonic cavitation, and the effect of solvent mixtures on extraction has not been investigated.

Therefore, it is important to understand the relationship between the solvent type and their properties and how they influence cavitation within the solvent. In this sense, the impact of cavitation on extraction processes is a function of the ultrasonic power or intensity or density conveyed into the medium, usually expressed in Wcm^{-2} or Wcm^{-3}, respectively. However, cavitation in solvents is affected by absorption phenomena such as viscous or frictional interactions between molecules of the medium in which cavitation occurs and therefore, the ultrasonic intensity highly depends on the physical properties of the solvent being irradiated (Da Porto et al., 2013; Tiwari, 2015). In spite that different solvents have been used for the ultrasonic-assisted extraction of phenolic compounds, no research has evaluated the interactions of the blend of solvents and the generated ultrasonic intensity, on the extraction efficacy. Therefore, the aim of this study was to determine the effect of different solvent blends on the ultrasonic intensity achieved in the ultrasonic-assisted solid-liquid extraction process of Mango (*Mangifera indica* L.) peels and to assess its influence on extraction of phenolic compounds and their antioxidant activity.

2. Materials and methods

2.1. Raw materials for extractions and reagents

Mangoes (*Mangifera indica* L.) were purchased in a local market (Puebla, México) and the fruits were chosen randomly with a uniform yellow peel color, without bumps or marks on the peels. Then, the fruits were washed and the peel removed. The peels were dehydrated (35±1 °C) to constant weight in a convective flow oven (RF 53-UL. Redline by Binder. Tuttlingen, Germany) and then ground and sieved to a particle size below 500 µm. This powder was kept in hermetic plastic bags and stored in the dark at 25±1 °C, to avoid possible oxidation.

Ethanol (99%), acetone (99%) and hexane (99%) were used as the extraction solvents. The reagents used in this study were Folin–Ciocalteu reagent (2N), 2,2-Diphenyl-

1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), gallic acid, potassium persulphate and sodium carbonate. All chemicals used in the experiments were of analytical grade (Reyma-Merck. Puebla, México).

2.2. Extraction methods.

2.2.1. Conventional Solvent Extraction (CSE).

The extracts were obtained by adding 5 g of mango peel powder to 100 mL of solvent. The solvent was prepared according to a simplex-centroid design (section 2.6), which was composed of 10 different experimental assays, where the solvent types (ethanol, acetone and hexane) were the varying factors. The extraction was performed in a glass vessel covered with aluminum foil to avoid loss of solvent. The extraction was performed for 15 min at a temperature of 20±1 °C, with constant stirring at 1000 rpm, in a ceramic stirring plate (SP131325. Cimarec Thermo Scientific Digital. New Jersey, United States). Following extraction the samples were centrifuged (UNIVERSAL 320 R. Hettich Lab. Tuttlingen, Germany) for 10 minutes (1350×g) at 4±1 ºC and filtered through Whatman No.1 filter paper. The extracts were stored at 4 °C until analysis. Experiments were run in triplicate.

2.2.2. Ultrasound-assisted extraction (UAE)

For the ultrasound experiments an ultrasonic probe system (UP400S. Hielscher. Teltow, Germany) was used. The mango peel powder (5 g) was mixed with 100 mL of solvent, using the compositions specified in the experimental simplex-centroid design (section 2.6) in a jacketed reactor (volume 250 ml; diameter 5.6 cm) (Flow cell-GD22K. Hielscher. Teltow, Germany). The reactor worked under controlled temperature conditions (25±1 °C), recirculating ethylene glycol (20%) with the aid of a recirculating bath (AD07R-20-AA1B. PolySciencie. Illinois, United States). The probe (2 cm diameter, 3.8 cm^2), was submerged

1.5 cm under the surface of the solvent. The experiments were performed at the maximum power settings of the transducer (100%, 400 W), at 24 kHz, for 15 minutes. After each extraction, the solvent/mango peel powder mixture was centrifuged for 10 minutes (1350×g) at 4 ºC, filtered through Whatman No.1 filter paper and stored in opaque vials at 4 °C until analysis. Experiments were carried out in triplicate.

A calorimetric procedure was used to determine the ultrasonic power *P* (W) transferred by the probe into the medium (González-Centeno et al., 2014) (Eq. 1).

$$P = mC_p \left[\frac{dT}{dt}\right]_{t=0} \quad (1)$$

Where Cp (Jg^{-1} $°C^{-1}$) is the heat capacity of the solvent, m (g) is the mass of solvent and dT/dt is the temperature rise per second ($°Cs^{-1}$). The heat capacity of mixed solvents was calculated according to the equation (Eq. 2) reported by Teja (1983):

$$Cp_{mixture} = \sum_i Cp_i x_i T_R \quad (2)$$

where x_i is the mole fraction of each pure component and T_{Ri} is the temperature of the mixture.

Subsequently, the applied ultrasonic density (UD) was determined from the calculated power (Eq. 3).

$$UD = \frac{P}{V} \quad (3)$$

Where UD is the ultrasonic density (Wcm^{-3}), *P* is the ultrasonic power (W) and *V* is the sample volume (cm^{-3}) (Chemat et al., 2017; Cheng et al., 2014., Tiwari, 2015).

2.3. Determination of total phenolic compounds (TPC)

Total phenolic content was measured using the Folin–Ciocalteu method (Khemakhem et al., 2017; Singleton et al., 1999). A gallic acid standard was utilized. The total content of phenolic compounds within the extracts was expressed as mg gallic acid equivalents (GAE)/100 g of dry matter of mango peel powder. All analyses were carried out in triplicate.

2.4. $ABTS^{\bullet+}$ scavenging ability

The $ABTS^{\bullet+}$ scavenging ability was determined according to the method described by (Butkhup et al., 2013) and (Fu et al., 2011). The free radical scavenging activity of extracts was expressed as mg of Trolox equivalents (TROLOX)/100 g of dry matter.

2.5. $DPPH^{\bullet}$ radical scavenging activity.

The antioxidant activity was measured via the ability to donate hydrogen to the stable free radical $DPPH^{\bullet}$ of the phenolic components (Dubie et al., 2013). The free radical scavenging activity of the extracts was expressed as mg of Trolox equivalents (TROLOX)/100 g of dry matter.

2.6. Simplex-Centroid Mixture Design (SCMD).

The simplex-centroid mixture design method, provided by Statistica® 13.0 software (Statsoft Inc. Tulsa, Oklahoma, USA) was employed to determine the effect of the solvent composition (mixtures of ethanol (x_1), acetone (x_2) and hexane (x_3)) on the extraction of phenolic compounds from Mango peel, and their antioxidant activity as affected by the ultrasonic intensity. This method establishes a surface model which evaluated the interactions between the variables to determine the optimal combination to maximize the desired result. In the design of the present work, the factors considered were the solvents (x_1, x_2, x_3), their levels was restricted as their sum must equate to 1. Thus, a 3-component simplex-centroid design was established with three added points. This consists of $2^3 - 1$ distinct design points, which are the three permutations of (1, 0, 0) or single-component blends, the C_3^2 permutations of (1/2, 1/2, 0) or all binary mixtures, and the C_3^3 permutation of (1/4, 1/4, 1/2), (1/4, 1/2, 1/4), (1/2, 1/4, 1/4) and the (1/3, 1/3, 1/3) or ternary mixtures. A ~~S~~special cubic regression model was fitted for variations of each of the three responses variables ($\hat{y}_{TPC}$: total phenolic content, $\hat{y}_{AA}$: antioxidant activity, $\hat{y}_{UD}$: ultrasonic density) as a

function of significant ($p<0.05$) interaction effects between the solvents proportions. The special cubic regression model for each response variable is represented by the Eq. 4.

$$\hat{y}_n = \sum_{1\leq i\leq n} \beta_i x_i + \sum_{1\leq i\leq j\leq n} \beta_i \beta_j x_i x_j + \sum_{1\leq i\leq j\leq k\leq n} \beta_i \beta_j \beta_k x_i x_j x_k \quad (4)$$

where $\hat{y}$ is the predicted response, $x_i x_j$ are the independent variables; β_i is the regression coefficient for each linear effect term; $\beta_i \beta_j$ and $\beta_i \beta_j \beta_k$ are the binary and ternary interaction effect terms, respectively (Montgomery, 2017; Dias et al 2015). Analysis of variance (ANOVA) was performed to determine the individual linear, quadratic and interaction regression coefficients (β) using Statistica® 13.0 software. The contour plots were carried out using the regression coefficients to determine the optimum region for each response and the determination coefficient (R^2) was used to determine how well the model fits the responses. The significance of the dependent variables was statistically analyzed by computing the F value at $p<0.05$. The extraction conditions were optimized for the maximum content of phenolic compounds (TPC), the maximum antioxidant activities (ABTS and DPPH) and ultrasonic density (UD). The responses were determined under the optimum extraction conditions. Finally, the experimental data was compared with the predicted values based on the standard errors to validate the model. Following this the adjusted determination coefficients (Adj. R^2) were obtained.

3. Results and discussion

3.1. Effect of the solvent composition on TPC and AA.

3.1.1. Conventional Solvent Extraction (CSE).

In Figure 1 the effects of the solvent concentrations on the TPC and AA obtained during conventional extraction are shown in two-dimensional simplex contour plots (Figure 1 A, C and E). Moreover, the fitted line plots of the experimental versus predicted values for the

response variables are depicted (Figure 1 B, D and F). From the simplex centroid mixture design, the special cubic regression model was established. This studied the responses as a function of the significant interactions effects between the proportions of the solvents.

The results obtained for the simplex contour plot of the total phenolic content (Figure 1A) showed that, the maximum response variable was located between the ethanol and acetone vertices. Thus, the ethanol-acetone blend showed the highest activity in the conventional extraction of TPC. The optimum position was also located more towards the ethanol vertex. The model (Eq. 5) showed that the regression coefficients for each linear effect had a significant ($p<0.05$) and positive effect on the increase of the TPC extracted.

The ethanol solvent obtained the highest value of the regression coefficient (116.88) in this term of the equation.

$$\hat{y}_{TPC} = 116.88x_1 + 47.25x_2 + 21.19x_3 + 483.54x_1x_2 - 54.53x_1x_3 - 4.85x_2x_3 - 630.30x_1x_2x_3 \quad (5)$$

Additionally, the model indicates that the binary interaction term from ethanol-acetone blends had a significant ($p<0.05$) and positive regression coefficient, while the other binary mixtures interactions and the cubic term of the model had little significance ($p<0.05$). From the special cubic regression model, the extraction conditions were optimized to obtain the maximum value of TPC, which corresponded to an ethanol-acetone blend with a maximum value of 205.08 mg GAE/100 g DM, with a proportion of solvents of 60 and 40%, respectively. The results may be attributed to the fact that the extraction was governed by the polarities of solvents and the synergistic interaction between them. Thus, they have an affinity with the biocomponents from the solid matrix, which make the solvent system selective in the extraction. In the case of Mango peel, the specific biocomponents are polyphenols, anthocyanins, carotenoids, flavonols, vitamin E and vitamin C. There is also the presence of ethyl gallate and glucosides, which are considered as polar and low molecular weight compounds. Ethanol is classified as a polar-protic solvent, as it contains

hydroxyl groups and is a hydrogen bond donor, resulting in preferential extraction of low molecular weight compounds, such as glycoside and non-glycoside phenolic compounds. Acetone is a polar-aprotic solvent, which has no available hydrogen atoms and, is considered an intermediate polarity-solvent. This is because it is able to solvate compounds with low and high molecular weight with protonatable functional groups, like phenolic compounds such as tannins, proanthocyanidins and flavonols. It was reported by Taghizadeh et al. (Taghizadeh et al., 2018) that ethanol was the most potent solvent in extracting the total phenolic compounds from pistachios kernel and hull, followed by acetone extracts; similar results were obtained by Mokrani et al. (Mokrani and Madani, 2016) in peach extracts. They attributed their results to the polarity of solvent and the solubility of phenolic compounds within them, concluding that there is no single solvent able to extract all phenolic compounds from vegetable samples. Furthermore, Wijekoon et al. (Wijekoon et al., 2011) reported that acetone mixtures have been one of the most effective solvents for extracting phenolics from Bunga kantan plant, followed by pure solvents. Other works (Nguyen et al., 2015; Rezaie et al., 2015) showed that a polar-protic solvent (ethanol) followed by a polar-aprotic solvent (acetone) were the most efficient solvents for extraction of antioxidant compounds (phenolics) than their aprotic counterparts (hexane solvent). Considering the influence of the solvent on the TPC extraction, the cubic regression model fitted to the experimental data was able to describe the effect of the extraction of TPC with different solvents (Figure 1B). This was confirmed by the high determination coefficient (R^2=0.946) and the adjusted determination coefficient (R^2=0.971). Therefore, the model can be used for predictive purposes for the extraction of total phenolic compounds using the solvents considered in this study.

The AA of mango peel extracts obtained with different proportions of solvents was determined and the results of the simplex centroid plots for ABTS and DPPH are shown in Figures 1C and 1E, respectively. For the ABTS results (Figure 1C), the zone with the highest

AA of phenolic compounds extracted was located in the side of triangle ethanol-hexane, with the highest activity towards the ethanol vertex. On the other hand, the DPPH results (Figure 1E) showed the highest interaction activities in the sides of triangle corresponding to ethanol-hexane and acetone-hexane. The side of acetone-hexane, specifically towards the acetone vertex was found to have the highest activity. According to the simplex centroid plots, the quantitative relationships between the AA and the factors were defined by Eq (6) for ABTS and Eq (7) for DPPH.

$$y_{ABTS} = 20.47x_1 + 19.31x_2 + 13.77x_3 - 4.0x_1x_2 + 8.28x_1x_3 + 6.82x_2x_3 - 13.49x_1x_2x_3 \quad (6)$$

$$y_{DPPH} = 27.81x_1 + 27.35x_2 + 13.48x_3 - 1.65x_1x_2 + 12.82x_1x_3 + 30.69x_2x_3 - 36.98x_1x_2x_3 \quad (7)$$

All variables of the linear term in ABTS showed significant ($p<0.05$) and positive regression coefficients, with the highest value for ethanol (20.47). The binary blends were significant ($p<0.05$), however, only ethanol-hexane and acetone-hexane showed positive regression coefficients (8.28 and 6.82, respectively). The cubic term was not significant ($p>0.05$). From ABTS, the optimal value reached for antioxidant activity was 20.55 mg TROLOX/100 g DM in the ethanol-hexane blend with a solvent proportion of 90% ethanol and 10% hexane. The determination coefficient and the adjusted determination coefficient (Figure 1D) for the special cubic regression model described by Eq. (6) were R^2=0.955 and R^2=0.934, respectively. The equation obtained for DPPH (Eq. 7) showed that the linear term had significant ($p<0.05$) and positive values for the regression coefficients, while the interaction in binary blends was only significant and positive for the acetone-hexane blend (30.69). No significant interaction was observed in the cubic term. From the established model, the maximum extraction was found to occur with an acetone-hexane blend with solvent

proportions of 70% and 30%, respectively. These solvent proportions obtained the maximum value of AA, which was 29.63 mg TROLOX/100 g DM. The model showed a determination coefficient value of R^2=0.944 and adjusted determination coefficient of R^2=0.733 (Figure 1F). Although both methods measure the antioxidant activity, differences were observed in the results. This could be because the ABTS method measures the antioxidant activity of hydrophilic and lipophilic compounds, while the DPPH method could only be measuring the lipophilic compounds. This is a limitation when attempting to interpret the role of the hydrophilic antioxidants (Arnao, 2001; Gülçin, 2012; Karadag et al., 2009).

3.1.2. Ultrasonic-assisted extraction (UAE)

Ultrasonic-assisted extraction was evaluated using the simplex centroid mixture design in a similar way to the CSE. Two-dimensional simplex contour plots (Figure 2) were obtained to show the interactions of the factors with the response variables.

The simplex centroid plot for TPC (Figure 2A) showed that the maximum interaction of the phenolic content extracted with ultrasound was located between the ethanol and acetone vertex, with a slight tendency towards of ethanol vertex. The regression coefficients (Eq. 8) from the model in the linear term and between the binary blends were significant ($p<0.05$) and positive.

$$y_{TPC} = 1035.17x_1 + 491.67x_2 + 80.82x_3 + 2813.52x_1x_2 - 857.13x_1x_3 - 400.03x_2x_3 - 1453.40x_1x_2x_3 \quad (8)$$

Thus, pure ethanol (1035.17) and the ethanol-acetone blend (2813.52) obtained the highest regression coefficients. No significant effect was obtained for the cubic term. The maximum value of phenolic compounds obtained during UAE was calculated from the model as 1493.01 mg GAE/100 g DM from the binary blend with 60% ethanol and 40% acetone. The determination coefficient (R^2=0.949) and the adjusted value (R^2=0.980) between the

296 experimental data and predictive values (Figure 2B) indicates that the response data can be
297 properly represented by the model.
298 Rezaie et al. (Rezaie et al., 2015) found a direct relationship between the phenolic
299 compounds extracted with ultrasound and the solvent polarity. The polar protic solvents
300 obtained the highest content of total phenolic extracted, followed by polar aprotic and non-
301 polar solvents. This result was explained by the understanding that ethanol has a selective
302 behavior to extract glycosidic and non-glycosidic phenolic compounds, while acetone can
303 generally only extract non-glycosidic phenolics. Similar results were obtained in the present
304 study, but a larger increase of total phenolic extracted content was observed when an
305 interaction between solvents occurred, whereas the aforementioned authors evaluated only
306 pure solvents on the ultrasonic extraction. Also, those authors mentioned that, when
307 employing ultrasound waves, the physical properties of solvents (vapor pressure) had an
308 influence on the ultrasonic cavitation, which increased the rate of swelling of plant materials
309 to improve the contact surface between the solvent and plant matrix.
310 A high interaction was observed on the ethanol-hexane and acetone-hexane vertex for
311 simplex contour plots of antioxidant activity obtained through ABTS (Figure 2C) and DPPH
312 (Figure 2E) assays. Nevertheless, the ABTS model showed (Eq. 9) a significant ($p<0.05$)
313 positive effect for pure solvents and a significant ($p<0.05$) negative effect for the ethanol-
314 acetone blend. The other binary and ternary interactions showed no significant ($p>0.05$)
315 effects.
316 $$y_{ABTS} = 21.08x_1 + 20.75x_2 + 16.95x_3 - 42.49x_1x_2 + 9.05x_1x_3 + 7.85x_2x_3 + 47.14x_1x_2x_3$$
317 (9)
318 Therefore, from the positive and significant interactions, the maximum value for ABTS was
319 determined. This was found to be a solvent composed of 100% ethanol, which resulted in
320 the maximum value of antioxidant activity of 21.1 mg TROLOX/100 g DM. Comparing with
321 the literature, the effect of ethanolic extracts obtained with ultrasound on the antioxidant

capacity has been reported on date-seeds, where the ethanol concentration of 60% was found to be the most suitable to scavenge ABTS free radicals (Liu et al., 2018).

The DPPH model (Eq. 10) showed that the linear and the binary interaction terms had significant ($p<0.05$) effects on antioxidant activity. Only the ethanol-acetone blend showed a negative interaction and also, the cubic term showed no significant effect.

$$y_{DPPH} = 25.43x_1 + 26.11x_2 + 20.62x_3 - 9.42x_1x_2 + 11.55x_1x_3 + 8.01x_2x_3 - 0.97x_1x_2x_3 \quad (10)$$

The optimum value obtained in the antioxidant activity determined by DPPH was 26.41 mg TROLOX/100 g DM with a solvent blend of 70% acetone and 30% hexane. It was previously reported by Lim et al. (Lim et al., 2019) that polar protic solvents (ethanol) showed strong DPPH radical scavenging activities and also, these authors reported that similar activity was observed for polar aprotic (acetone) solvent; however, non-polar solvent (hexane) exhibited a weak radical scavenging activity. Nevertheless, the results obtained in the present work, suggested that the antioxidant activity was favored by the interaction between acetone and hexane.

For ABTS and DPPH assays, the high coefficients of determination (R^2_{ABTS}=0.940; R^2_{DPPH}=0.956) indicate that models can be used for predictive purposes of the antioxidant activity of extracts obtained with ultrasonication.

Several works (Moreira and de Souza Dias, 2018; Rezaie et al., 2015; Sumere et al., 2018) have reported that the efficiency of ultrasonic extraction with different solvents and the antioxidant activity of extracts obtained are associated to a combination of different factors. These include temperature, particle size, cavitation phenomena, solvent viscosity, dielectric constant, the solubility of compounds in the solvent, mass transfer phenomena or degradation of compounds.

3.2. Ultrasonic effects on the extraction.

The simplex centroid mixture design and the experimental results of TPC and AA obtained with CSE (0% US electric power) and UAE (100% US electric power) are summarized in Table 1. Regardless of solvent composition, the TPC results show that a higher content was found for UAE compared to CSE. The average increase was 630% when the ultrasound was applied. The highest intensification effect of ultrasound was obtained for the extraction with an ethanol-acetone ratio of 1:1 (50-50%); in that case, an increase of 639% of TPC was observed with UAE (1483.98±56.86 mg GAE/100 g DM) when compared to CSE (200.69±16.69 mg GAE/100 g DM). These results were in agreement with those reported by He et al. (He et al., 2016) who showed that the UEA of anthocyanins and phenolic compounds from Blueberry Wine Pomace resulted in higher yileds when compared to a CSE method. Their results showed an increase of 148% for anthocyanins and 223% for phenolic compounds when compared to the CSE method. Song et al. (Song et al., 2014) found that the UAE yielded 26.4% more flavonoids from pine needles, than CSE. Both works reported that the UAE was more efficient than the CSE method due to both a shortened extraction time and an increased yield. This was attributed to UAE promoting the penetration of the solvent into the sample matrix and increasing the mass transfer rates. Therefore, UAE proves to be effective for increasing the extraction yield of phenolic compounds in many vegetal matrices. This intensification on solid-liquid extraction could be explained due to cavitation (violent collapse and implosion of gas bubbles in the liquid solvent) and micro stirring, which causes cell tissues disruption and improves the extraction efficiency (Tiwari, 2015). Chemat et al. (Chemat et al., 2017) explained that mass transfer in ultrasonic extraction is improved by the presence of different effects of cavitation, such as the fragmentation, erosion, sonocapillary effect, sonoporation, local shear stress and detexturation. The fragmentation is carried out by the effect of the inter-particle collisions and shockwaves created from cavitation with a reduction of the particle size and therefore, the increase of the surface area. Erosion is the damage on the surface of plant structures,

enhancing the accessibility of solvent to the sample, improving the extraction and solubilization. The sonocapillary effect is the increase of depth and velocity of penetration of solvent into canals and pores by cavitation. It has a positive impact on desorption and diffusion of a solute from a plant structure. Sonoporation is related to the cell membrane pores and perforations of the membrane, which improve the permeability. The local shear stress is created by the oscillation and collapse of the cavitation bubbles within the solvent and at the vicinity of the solid materials. Shear forces are generated within the liquid, resulting in streaming and acoustic micro-streaming effects. Finally, detexturation is the disruption and destruction of cell structures. These authors mentioned that during the ultrasonic extraction, a combination of all these physical effects probably occurs, enhancing the mass transfer and the extraction performance resulting from the presence of ultrasound.

Additionally, the AA results with ABTS showed (Table 1) a similar behavior to TPC, since for all pure solvents and most of their mixtures the UAE obtained a significantly ($p<0.05$) higher activity than CSE (average of 6%). A Pearson correlation between phenolic content results and antioxidant activity effects on ABTS for CSE ($r=0.452$) and UAE ($r=0.105$) revealed a weak significant ($p<0.05$) correlation. Nonetheless, the AA of phenolic compounds present in extracts cannot be predicted only on the basis of its total phenolic content. It should also be determined by specific phenolic compounds present in the extract (Kähkönen et al., 1999). These results are in agreement with the observations made by Meneses et al. (Meneses et al., 2013) who utilized a simple regression analysis, between the correlation of phenolic compounds obtained from Brewer's spent grains and AA. They found a weak significant correlation ($R^2=0.20$) when TPC was evaluated, however a strong correlation was observed for a specific phenol (flavonoids), which they believed contributed significantly to the overall AA. On the other hand, the DPPH results (Table 1) showed that CSE obtained higher values of AA than UAE in most cases. The results showed that the

ethanol-acetone blend obtained a decrease of 14.2%. In counterpart, the AA obtained for blends with high proportions of hexane showed an increase of 34.5% when the ultrasound was applied. It should also be noted that the DPPH assay has some drawbacks which limit its application (Arnao, 2001; Gülçin, 2012; Karadag et al., 2009). These are because DPPH radicals are less reactive than ABTS radicals and DPPH methods could be considering only the lipophilic compounds of the extract and also, the decrease in activity could be due to the UAE effect on these types of compounds decreasing their AA. Also, the Pearson correlation between TPC and AA from CSE and UAE revealed a weak significant ($p<0.05$) effect on DPPH ($r=0.377$ and $r=0.174$, respectively). Nevertheless, in general, the results indicated that the extracts from mango manila peels had an adequate capacity to scavenge DPPH and ABTS free radicals.

3.3. Effect of the solvent type on the ultrasonic density

The efficiency of an extraction process strongly depends on the nature of the matrix plant and the type of extractable compounds. Also, when ultrasound is applied, the increase of the extraction yield of these compounds has been attributed to the acoustic cavitation, which increases mass transfer (Chemat et al., 2017; Sumere et al., 2018). The acoustic cavitation (bubble collapse) is directly correlated to the pressure amplitude of the sound wave and consequently to ultrasonic intensity (Li et al., 2004). However, the acoustic cavitation is also affected by the physical and chemical properties of the solvent and it is necessary to understand how these solvent properties interaction with the ultrasound. Therefore, in order to quantify the contribution of the individual effects of ultrasonic density and solvent on the extraction process of the TPC, the net increase of the phenolic compounds extraction was evaluated between the UAE and CSE. These net increases were calculated by deducting the values from the CSE experiments from those of the UAE experiments for each different solution blend studied in this work. These results are presented in Figure 3.

The simplex contour plot (Figure 3A) showed that the increment of TPC extraction when the ultrasound was applied was not the same for any ratio of solvents. The largest increment on the extraction was located between the side of the ethanol and acetone vertices, with the largest increase towards the ethanol vertex. The model (Eq. 11) indicates that a significant and positive incremental effect exists and therefore, an increase when the extraction was carried out with ultrasonic application in pure solvents (linear term), where the highest increase corresponded to ethanol (918.29). Although the values for the binary blends were significant, only the ethanol-acetone (2329.97) blend had a high and synergistic behavior, while the ternary blends did not have a significant ($p<0.05$) increase on extraction during the process. The optimum proportion was the binary blend with 60% ethanol and 40% acetone, which increased the TPC extraction by 1287.93 mg GAE/100 g DM. A high determination coefficient (R^2=0.947) was obtained for these results, and the experiment data were in a good agreement with the predictive values (Figure 3B), confirming the viability and adequacy of the predicted model.

$$y_{TPC} = 918.29x_1 + 444.41x_2 + 59.63x_3 + 2329.97x_1x_2 - 802.61x_1x_3 - 395.16x_2x_3 - 823.17x_1x_2x_3 \quad (11)$$

The results suggest that not only the chemical effects (affinity) of solvent are present, but also, the improvement on the ultrasonic assisted extraction is due to the influence of the physical properties of solvent on the ultrasonic density. The ultrasonic effect on extraction is linked to the magnitude of the cavitation phenomenon, which is determined by the energy or intensity of the elastic wave (ultrasonic intensity). That is, the greater the intensity, the larger the cavitation effect (Li et al., 2004). The intensity of the ultrasonic wave is the energy flowing per unit area and time, and is related with the maximum acoustic pressure, which is given by the density of the medium and the speed of sound into the medium. In this sense, the impact of cavitation on extraction processes is a function of the ultrasonic energy, as known as a power or intensity conveyed into the medium, usually expressed in Wcm^{-2}. ~~or i~~It

449 can also be expressed as ultrasonic density (Wcm^{-3}). The intensity of ultrasound could
450 decrease due to the presence of the absorption phenomena such as viscous or frictional
451 interactions between molecules of the medium; therefore, the absorption of the ultrasonic
452 wave depends on the density and viscosity of the medium (Lupacchini et al., 2017). In this
453 regard, in the present study, a significant ($p<0.05$) correlation ($R^2=0.81$) between the
454 ultrasonic density and the TPC (Figure 4) was found, showing that, the higher the ultrasonic
455 density, the higher the TPC extracted.
456 Figure 5 depicts the two-dimensional simplex contour plot relating the type of solvent and
457 the ultrasonic density value. It can be observed that the ethanol-acetone blend showed the
458 highest ultrasonic density. From the adjusted model (Eq. 12), it is possible to observe that
459 the interaction of these solvents had a significant ($p<0.05$) and synergistic effect on the
460 ultrasonic density and the maximum value of ultrasonic density obtained was 0.217 Wm^{-3}
461 for the ethanol-acetone blend (90-10%).

462 $$y_{DU} = 0.216x_1 + 0.172x_2 + 0.142x_3 + 0.056x_1x_2 - 0.195x_1x_3 - 0.106x_2x_3 - 0.136x_1x_2x_3$$
463 (12)

464 The coefficient of determination (R^2) of the model is 0.997 and a good agreement ($R^2=0.953$)
465 of predictive values suggests that the model adequately fits the experimental data (Figure
466 5B). As noted by other authors (Chivate and Pandit, 1995; Li et al., 2004) in binary mixtures
467 of solvents, the physical properties of solvents are the key factors that impact the ultrasonic
468 energy. In this sense, solvent viscosity is considered one of the most important physical
469 properties that affect the extractability of biocomponents from a solid matrix using UAE.
470 When viscosity is low, the cavitation bubbles are more easily produced, since the molecular
471 forces of solvent can be more easily exceeded and this increases the diffusivity through the
472 pore of sample to leach out the biocomponents (Rezaie et al., 2015; Wijekoon et al., 2011).
473 For solvents with high viscosity, the power dissipated is higher, but the onset of cavitation is
474 longer, this affects the cavitation behavior and has a negative impact on the extraction yield

(Lupacchini et al., 2017). As can be seen in Table 2, ethanol has the higher viscosity value (1.07 cP) and would therefore have a lower effect on the ultrasonic intensity. However, in the present work, the optimal blend consists of 90% ethanol so therefore ethanol viscosity is not a determining factor that stimulates the extraction of the phenolic compounds. Together with viscosity, vapor pressure is also an important physical property that affects the cavitation activity in solvents and that must be considered. It has been reported (Table 2) (Lupacchini et al., 2017; Rezaie et al., 2015) that ethanol has a lower vapor pressure (44 mmHg) than acetone (180 mmHg) and hexane (124 mmHg). According to the literature (Rezaie et al., 2015), for ultrasonic assisted extraction, a solvent with low vapor pressure is preferred, since the collapse of the cavitation bubble is more intense, which enhances the effects of cavitation (fragmentation, erosion, sonocapillary effect, sonoporation, local shear stress and detexturation). Surface tension is another important physical property that must be taken into account. The formation of the liquid/gas interface is essential for cavitation and solvents with low surface tension should show higher dissipated powers (Lupacchini et al., 2017). Ethanol has been reported as a solvent with medium values for surface tension (22.3 $Dyn \cdot cm^{-1}$) (Table 2). Therefore, in spite that ethanol has the highest value of viscosity, the lowest value of vapor pressure and intermediate surface tension, it achieved the greatest ultrasonic density in the medium. Moreover, although acetone has a high vapor pressure (180 mmHg) and surface tension (23.3 $Dyn \cdot cm^{-1}$) compared to ethanol, its low viscosity improves cavitation, increasing the ultrasonic intensity. This could explain the synergistic behavior of the ethanol-acetone blend on the ultrasonic intensity found in the present work and therefore, the improvement in extraction. In contrasts, hexane has a low viscosity (0.3 cP) and surface tension (18.4 $Dyn \cdot cm^{-1}$) but a high vapor pressure (124 mmHg) which shows lower effectiveness in increasing the ultrasonic density.

A linear correlation (Pearson correlation coefficient) was carried out between the TPC and the physicochemical properties for each blend of solvents. The results showed that the

surface tension ~~property~~ obtained the highest value of coefficient (r=0.73), followed by viscosity (r=0.5). However, the correlation between the vapor pressure and the TPC could not be observed (r=-0.24). When these results were related to the surface tension values reported in Table 2, it was observed that the ethanol-acetone blend obtained the highest surface tension values (22.57 Dyn·cm^{-1}), so it was inferred that this physicochemical property was directly influenced by this type of solvent mixture, increasing the amount of ~~obtaining of~~ phenolic compounds obtained during ultrasonic assisted extraction.

Although for ethanol-acetone blends, UAE increases the values of TPC extraction and ultrasonic intensity, the AA showed (Figure 3C and 3E) an opposite behavior, where the pure hexane solvent showed the maximum value of increase. From the ABTS model (Eq. 13), the pure solvents, the ethanol-acetone blend and the cubic interaction term had significant ($p<0.05$) and positive effects on AA and these conditions were optimized obtaining the maximum value reached of 3.18 mg TROLOX/100 g DM, for pure hexane solvent. Meanwhile the DPPH model (Eq. 14) obtained significant interactions for the ethanol and hexane pure solvents, ethanol-hexane and acetone-hexane blend and the cubic terms. However the only positive linear terms were pure hexane and the interaction between the three solvents. Therefore, considering only the significant positive interactions, the maximum value of antioxidant increase was 7.18 mg TROLOX/100 g DM.

$$y_{ABTS} = 0.62x_1 + 1.44x_2 + 3.18x_3 - 38.49x_1x_2 + 0.76x_1x_3 + 1.03x_2x_3 + 60.63x_1x_2x_3 \quad (13)$$

$$y_{DPPH} = -2.28x_1 - 1.38x_2 + 7.18x_3 - 8.01x_1x_2 - 16.72x_1x_3 - 23.21x_2x_3 + 88.30x_1x_2x_3 \quad (14)$$

Yusof et al. (Yusof et al., 2016) reported that the application of ultrasound drives the generation of highly reactive radicals, due to bubble collapse during cavitation. This results in sonochemical reactions that generate radicals and molecular products. Phenolic compounds allow the scavenging or prevention of free radical generation, which is achieved

by an efficient antioxidative defense system (Sridhar and Charles, 2019). However, considering the primary radicals on their molecules, H· is a strong reducing agent and OH· is a strong oxidizing agent, which could be used for various redox reactions and for this reason, each cavitation bubble could be considered as an electrochemical cell (Yusof et al., 2016). Also these molecules can be combining to give hydrogen peroxide and react, or they can also react with other substances to induce secondary reduction and oxidation reactions (Cravotto and Cintas, 2006). Therefore, the phenolic compounds are degraded and the strong oxidizing agents generated could be used for the degradation of other organic compounds, decreasing the AA.

In general, the results obtained in the present work indicated that, when considering conventional extraction, the greatest recovery of phenolic compounds and antioxidant activity was obtained for an ethanol-acetone solvent (Figure 1), due to the affinity and interaction among the solvent, the solute and the solid matrix. When ultrasound was utilised, mixtures of ethanol-acetone also provided the largest recovery of phenolic compounds (Figure 2). However, the highest antioxidant capacity was found for blends containing hexane. In fact, for mixtures of only ethanol-acetone, there is a decrease in the extraction of AA when ultrasound was utilized (Figures 3C and E). Therefore, it seems that for ethanol-acetone mixtures the large UI reached (Figure 5), improves extraction of phenolic compounds, but negatively affects the AA of the extracts. This negative effect could be associated with sonochemical reactions taking place due to acoustic cavitation, which would reduce the antioxidant activity of the phenolic compounds, even with respect to conventional extraction. These results indicate that the solvent composition affects the achieved UI and therefore the extraction processes and should be taken in account when developing ultrasonic assisted extraction processes.

4. Conclusions

552 Results demonstrated that ethanol-acetone blends significantly increased the recovery of
553 phenolic compounds from Mango peels during CSE and UAE. Furthermore, a significant
554 increase was found for the recovery of TPC when ultrasound was utilized, compared to the
555 conventional extraction. A significant correlation existed between the UI and TPC.
556 Therefore, a high UI achieved in the solvent resulted in an increase in the amount of phenolic
557 compounds extracted. However, for solvent blends which reached the maximum UI
558 (ethanol-acetone), the AA was negatively affected, probably due to sonochemical reactions,
559 which reduced the AA of phenolic compounds with respect to CSE.

560 The results showed that solvent composition affects not only the solvent-solute interaction
561 but also the ultrasonic intensity reached in the extraction medium. Large ultrasonic
562 intensities can affect the extraction capacity. Therefore, interactions between the type of
563 solvent-ultrasonic intensity must be considered to design more effective ultrasonic-assisted
564 extraction processes.

565 **Conflict of interest**

566 No conflicts of interest, financial or otherwise, are declared by the authors.

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Figure Captions

Figure 1. Simplex contour plots of the special cubic regression model and fitted line plots showing the effects of the solvent on total phenolic content (TPC) (A, B) and antioxidant activity evaluated with ABTS (C, D) and DPPH (E, F) assays of the extracts of mango manila peels obtained by conventional extraction.

Figure 2. Simplex contour plots of the special cubic regression model and fitted line plots showing the effects of the solvent on total phenolic content (TPC) (A, B) and antioxidant activity evaluated with ABTS (C, D) and DPPH (E, F) assays of the extracts of mango manila peels obtained by ultrasonic-assisted extraction.

Figure 3. Simplex contour plot of the special cubic regression model and fitted line plot showing the increment of ultrasonic-assisted extraction with different solvent on total phenolic content (TPC) (A, B) and antioxidant activity evaluated with ABTS (C, D) and DPPH (E, F) assays of the extracts of mango manila peels

Figure 4. Pearson's Correlation ($p<0.05$) between the ultrasonic intensity and the total phenolic compounds. Means ± standard deviation ($n = 3$).

Figure 5. Simplex contour plot of the special cubic regression model (A) and fitted line plot (B) for the effect of different combinations of solvents on ultrasonic intensity.

Table 1. Simplex-centroid mixture design of solvents and the effect of ultrasonic application on the extraction of phenolic compounds and antioxidants from mango peels.

Extracts Solvent proportions			US Electric power	Response function		
				TPC	AA (ABTS)	AA (DPPH)
X_1	X_2	X_3	%	mg GAE/100 g DM	mg TROLOX/100 g DM	
1	0	0	0	115.60±2.35[a]	20.43±0.08[a]	27.81±0.01[b]
			100	1030.69±43.50[b]	20.99±0.02[b]	25.48±0.10[a]
0	1	0	0	47.94±2.80[a]	19.39±0.24[a]	27.45±0.02[b]
			100	504.74±21.04[b]	20.93±0.03[b]	26.09±0.13[a]
0	0	1	0	22.30±0.05[a]	13.70±0.73[a]	13.24±0.80[a]
			100	73.92±1.45[b]	16.84±0.53[b]	20.59±0.48[b]
½	½	0	0	200.69±16.69[a]	18.99±0.22[a]	27.36±0.03[b]
			100	1483.98±56.86[b]	20.57±0.08[b]	23.47±0.15[a]
½	0	½	0	53.99±2.33[a]	18.97±0.10[a]	26.69±1.39[a]
			100	320.95±13.78[b]	20.87±0.07[b]	25.96±0.06[a]
0	½	½	0	36.62±4.32[a]	18.27±0.10[a]	27.81±0.14[b]
			100	198.57±48.35[b]	20.95±0.03[b]	25.28±0.67[a]
⅓	⅓	⅓	0	85.17±3.03[a]	17.99±0.03[a]	22.26±0.03[a]
			100	700.61±59.84[b]	17.87±0.11[a]	25.23±0.54[b]
¼	¼	½	0	45.93±1.89[a]	18.82±0.06[a]	27.80±0.05[b]
			100	437.09±50.83[b]	20.94±0.22[b]	25.19±0.19[a]
½	¼	¼	0	123.86±15.73[a]	19.50±0.03[b]	27.46±0.23[b]
			100	857.14±27.68[b]	18.68±0.85[a]	24.73±0.16[a]
¼	½	¼	0	89.44±5.63[a]	18.13±0.08[b]	27.63±0.07[b]
			100	609.45±42.91[b]	16.33±0.06[a]	25.22±0.22[a]

X_1 ethanol; X_2 acetone; X_3 hexane. The results are showed as the means (n=3) ± standard deviation. Different letters indicate significant differences, by the Tukey's test ($p<0.05$), between the conventional extraction (0%) and ultrasonic assisted extraction (100%), for each solvent.

Table 2. Physicochemical properties of the solvents used for the extraction. Phenolic compounds from mango peels. Determined at 20°C.

Solvent	Viscosity (cP)	Vapor pressure (mmHg)	Surface tensión (Dyn/cm)
Ethanol*	1.07	44	22.3
Acetone*	0.31	180	23.3
Hexane*	0.30	124	18.4
Ethanol-Acetone	0.76	153.95	22.57
Ethanol-Hexane	0.9	115.35	19.95
Acetone-Hexane	0.31	187.15	20.40
Ethanol-Acetone-Hexane (1:1:2)**	0.41	153.83	20.91
Ethanol-Acetone-Hexane (2:1:1)**	0.53	133.46	19.86
Ethanol-Acetone-Hexane (1:2:1)**	0.39	172.3	21.36
Ethanol-Acetone-Hexane (1:1:1)**	0.44	153.16	20.7

*Rezaie et al., 2015, Lupacchini et al., 2017

** Calculated values (vapor pressure from Raoult's Law, viscosity calculated with the Kendall and Monroe method, surface tension from the method of Winterfeld, Scriven and Davis).