Reutilization of Mango Byproducts: Study of the Effect of Extraction Solvent and Temperature on Their Antioxidant Properties

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Abstract: Mango biowastes, obtained after processing, contain large amounts of compounds with antioxidant activity that can be reused to reduce their environmental impact. The present study evaluates the effect of solvent (methanol, ethanol, acetone, water, methanol:water [1:1], ethanol:water [1:1], and acetone:water [1:1]), and temperature (25, 50, and 75 °C) on the efficiency of the extraction of antioxidants from mango peel and seed. Among the factors optimized, extraction solvent was the most important. The solvents that best obtained extracts with high antioxidant capacity were methanol, methanol:water, ethanol:water, and acetone:water (β-carotene test, antioxidant activity coefficient 173 to 926; thiobarbituric acid reactive substances test, inhibition ratio 15% to 89%; 2,2′-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid ABTS$^{\bullet+}$; and 2,2-diphenyl-1-picrylhydrazyl DPPH$^{\bullet}$ scavenging, 7 to 22 and 8 to 28 g trolox equivalent antioxidant capacity [TE] per 100 g mango biowaste on a dry matter basis [DW]). Similarly, the flavonoid (0.21 to 1.4 g (+)-catechin equivalents per 100 g DW), tannin (3.8 to 14 g tannic acid equivalents per 100 g DW), and proanthocyanidin (0.23 to 7.8 g leucoanthocyanidin equivalents per 100 g DW) content was highest in the peel extracts obtained with methanol, ethanol:water, or acetone:water and in the seed extracts obtained with methanol or acetone:water. From the perspective of food security, it is advisable to choose ethanol (which also has a notable antioxidant content), ethanol:water, or acetone:water, as they are all solvents that can be used in compliance with good manufacturing practice. In general, increasing temperature improves the capacity of the extracts obtained from mango peel and seed to inhibit lipid peroxidation; however, its effect on the extraction of phytochemical compounds or on the capacity of the extracts to scavenge free radicals was negligible in comparison to that of the solvent.

Keywords: antioxidant activity, extraction optimization, free radicals scavenging, *Mangifera indica* L. biowastes, phenolic compounds

Practical Application: There are many antioxidant compounds in mango peel and seed, and they could be used as a natural and very inexpensive alternative to synthetic food additives. However, the conditions in which the antioxidants are extracted must be optimized. This work proves that conditions such as extraction solvent or temperature have a crucial impact on obtaining extracts rich in antioxidants from mango biowastes.

Introduction

The main biowastes produced when processing mangos (*Mangifera indica* L.) are the peel and the seed, which represent approximately 35% to 60% of the fruit (Larrauri and others 1996). The disposal of mango biowaste is a growing problem due to increasing production of this material (estimated to be around 75000 mT worldwide). From an environmental perspective, it is vital to reuse the plant byproducts produced by the agro-food industry.

Mango peel and seed has very high antioxidant activity, a fact attributed to its high phytochemical content (Berardini and others 2005; Soong and Barlow 2006; Abdalla and others 2007; Ajila and others 2007; Barreto and others 2008). Therefore, mango byproducts have been studied as a safer natural alternative to synthetic food antioxidants in biscuits, buffalo ghee, vegetable oils, and potato chips (Puravankara and others 2000; Abdalla and others 2007).

Most of the developments made over the past few years in the evaluation of antioxidants from mango biowastes have focused on the identification of phytochemical compounds (Berardini and others 2004, 2005; Barreto and others 2008). However, research on sample preparation for analysis, which is the foundation for developing a quality, accurate, robust, and rugged analytical procedure to quantify mango biowaste antioxidants, has not been studied in great detail. The chemical characteristics of the solvent and the diverse structure and composition of the natural products ensure that each biowaste-solvent system behaves differently (González and González 2010; González-Montelongo and others 2010a, 2010b). Moreover, other factors, such as contact time and temperature, number of extraction cycles, solvent to plant material ratio, and extraction technique, significantly influence how

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efficiently active ingredients can be recovered from plant biowastes (González and González 2010).

Direct extraction using solvents is the most common technique employed to obtain extracts with high antioxidant activity from mango peel and seeds. Methanol or ethanol has been used as extraction solvents (Barreto and others 2008; Nithitanakool and others 2009); however mixtures of methanol, ethanol, acetone with water (between 50% and 95%) are the most widely used extracting agents for mango biowaste antioxidants (Kabuki and others 2000; Soong and Barlow 2004, 2006; Berardini and others 2005; Abdalla and others 2007; Ajila and others 2007). Extraction is typically conducted at room temperature (Kabuki and others 2000; Puravankara and others 2000; Berardini and others 2005; Barreto and others 2008). But in some cases, hot solvents at temperatures between 70 and 80 °C or heat reflux (Soong and Barlow 2006; Ajila and others 2007; Nithitanakool and others 2009) have been used to extract phytochemical compounds from mango peel and seed.

However, although extraction is a critical step in the analytical determination of antioxidants in mango biowastes, it has not been studied in detail. This work is a 1st attempt at establishing the optimal conditions for obtaining extracts with high antioxidant capacity for mango peel and seed. To this end, how variables such as type of solvent and temperature influence the extraction process of compounds with antioxidant properties from mango biowastes was studied.

Materials and Methods

Plant material

Mango (cv. 'Keitt') was obtained from the research fields of Instituto Canario de Investigaciones Agrarias in Tenerife (Canary Islands, Spain). Fruit (around 20 kg) was harvested at physiological maturity (mature-green stage; maturity indexes for harvesting: 180 to 240 d after full blooming of flowers and change in flesh color from white to yellow) and allowed to ripen, until the consumption stage, at 18 °C and 80% to 90% relative humidity. At full ripeness, lightness (L^*), hue angle ($h°$), and chromaticity (C^*) of mango peel or pulp were 66 ± 4 or 86 ± 3, 64 ± 20 or 87 ± 3, and 49 ± 8 or 78 ± 6, respectively. Fruit texture was measured as deformation (73 ± 7 °Durofel) and penetration force (53 ± 13 N). Total soluble solids, pH, and titratable acidity were 17 ± 3 °Brix, 4.3 ± 0.3, and 369 ± 77 mg citric acid per 100 g, respectively.

After ripening, the peel or the seed (shell of fibrous endocarp, testa, and embryo) was manually separated by using a very sharp knife, cut (0.5 × 1 cm), frozen into liquid nitrogen to prevent the antioxidant compounds from oxidation, and freeze dried (Christ alpha-1–4 LSC freeze-dryer, Osterode, Germany) at 50 mPa and −40 °C. The dried mango peels and seeds (water losses, 51 ± 6% and 71 ± 2%, respectively) were ground to a fine powder by impact grinding with an IKA A11 mill (Staufen im Breisgau, Germany) and sieved for 15 min (sieve Mecánica Científica, 105611, Madrid, Spain). The samples were then granulometrically characterized as follows: peel: 39% > 500 μm, 12% between 355 and 500 μm, and 49% < 355 μm; seed: 48% > 500 μm, 9% between 355 and 500 μm, and 43% < 355 μm. The mango peel and seed were stored at –20 °C until the extractions were carried out.

Solvent extraction

The solvent extraction method to obtain extracts with high antioxidant capacity from mango peel and seed was optimized in this work. Mango peel or seed was extracted with different solvents (plant material-to-solvent ratio: 1:50 w:v): methanol, ethanol, acetone, water acidified with hydrochloric acid (pH 3.0), or mixtures (1:1 v:v) of the organic solvents and water. High-performance liquid chromatography grade organic solvents were purchased from Scharlau Chemie (Barcelona, Spain) and deionized water (resistivity: 18 MΩ cm) was obtained with a milli-Q system (Millipore, Bedford, Mass., U.S.A.). The mixture was homogenized with a Politron PT-6000 (Kinematica AG, Lucerne, Switzerland) high-speed blender at 325 × g for 1 min. The extractions (at least 3 independent processes) were carried out in a water bath at 25, 50, or 75 °C for 60 min in hermetically sealed tubes to avoid solvent loss. Extracts were centrifuged at 3000 × g for 20 min in a Jouan CR-312 centrifuge (Thermo Electron Corp., Madrid, Spain) and stored at –80 °C until the analyses were carried out (in less than 3 d).

Extract capacity to inhibit lipid peroxidation

The antioxidant properties of crude extracts were evaluated by using different methods to obtain information about their capacity to inhibit lipid peroxidation or to scavenge free radicals. All measurements were made on a Shimadzu UV-visible 160A double-beam spectrophotometer (Kyoto, Japan) equipped with a Hellma (Jamaica, N.Y., U.S.A.) cell (path length: 10^{-2} m). In each method, a control using the different solvents, instead of extracts, was used. The repeatability standard deviation of all the antioxidant activity procedures was <10%.

The β-carotene bleaching method is based on the capacity of antioxidants to decrease oxidative losses of β-carotene (Aldrich, Madrid, Spain) in a β-carotene/linoleic acid (Sigma, Madrid, Spain) emulsion (González-Montelongo and others 2010a). To induce autoxidation, the temperature was increased (50 °C) and oxygenated deionized water (generated by bubbling air into water for 60 min) was used. The loss in orange color intensity, due to the loss of β-carotene conjugated double bonds, was measured at 470 nm after incubation for 210 min. The antioxidant activity was expressed as antioxidant activity coefficient.

The capacity of extracts to react with secondary oxidation products formed in the advanced stages of this reaction was evaluated by using the modified assay of thiobarbituric acid reactive substances (TBARS) described by González-Montelongo and others (2010a). The method is based on the peroxidation of a liposome system (phosphatidyl-choline, Alfa Aesar, Karlsruhe, Germany) in chloroform (Scharlau Chimie) induced by iron chloride (Sigma) containing potassium chloride (Merck, Darmstadt, Germany) in the presence of the antioxidants. The production of TBARS was measured at 535 nm after incubation at 95 °C for 60 min and expressed as inhibition ratio (IP,%).

Extract capacity to scavenge free radicals

The scavenging activity against the 2,2′-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid $ABTS^{\bullet+}$ radical was determined by a method (Arnao and others 2001) based on enzymatic generation of the radical by reaction of the ABTS (Sigma) with horseradish peroxidase (type VI, RZ $[A_{403\,nm}/A_{275\,nm}] = 2.8$; Sigma) in sodium phosphate buffer pH 7.5, in the presence of hydrogen peroxide (Sigma). The assay temperature was controlled at 25 °C, and the inhibition by the biowaste antioxidants was measured at 730 nm after 6 min.

The capacity to scavenge the 2,2-diphenyl-1-picrylhydrazyl $DPPH^{\bullet}$ (Sigma) radical was monitored according the method described by Brand-Williams and others (1995) at 515 nm after 15 min.

In both methods, results were expressed as (González-Montelongo and others 2010a) gram TE (trolox [Aldrich] equivalent antioxidant capacity) per 100 g mango biowaste on a dry matter basis (DW). Calibration graphs ($r^2 > 0.960$), in each of the evaluated solvents, were constructed by plotting the absorbance against the trolox concentration at 7 concentration levels analyzed in triplicate (50 to 500 mg/L).

Phytochemical compound content

The Folin–Ciocalteu method for total phenolic compounds (González-Montelongo and others 2010a) was coupled with the use of polyvinylpolypyrrolidone (PVPP, Sigma) to measure tannins (FAO/IAEA 2000). The tannins precipitate when PVPP is added to the extracts (PVPP:phenolic compounds ratio: 100:1 w:w) and the pH of the extract is adjusted at 3.0. The supernatant only contains compounds other than tannins; therefore, they were quantified by calculating the difference between total phenolic compounds and the phenols in the supernatant (González and González 2010) and expressed as gram tannic acid (Merck) equivalents (TAEs) per 100 g DW. Calibration curves ($r^2 > 0.990$) were constructed by plotting the absorbance against the tannic acid amount at 7 levels analyzed in triplicate (0.90 to 25 μg). The repeatability of all the procedures used to evaluate phytochemical compounds was <5%.

Total flavonoids were determined by using a spectrophotometric method (Thoo and others 2010), with measurement at 510 nm, based on the formation of complexes between aluminum chloride (BDH, Barcelona, Spain) and the C-4 keto and the C-3 or C-5 hydroxyl groups of flavones and flavonols and the orthodihydroxyl groups in the A ring or B ring of flavonoids (González and González 2010). Results were expressed as gram catechin (Sigma) equivalents per 100 g DW, and linear calibration curves ($r^2 > 0.997$) were obtained at concentrations between 7.5 and 35 mg.

The determination of condensed tannins (proanthocyanidins) is based on oxidative depolymerization of proanthocyanidins in butanol-HCl (95:5 v:v; Panreac, Madrid, Spain) (solvent:extract ratio, 5:1) (FAO/IAEA 2000). The presence of iron reagent (2% ferric ammonium sulfate [Merck] in 2 N HCl) increases the reproducibility and sensitivity of the assay. The mixture was incubated at 97 °C for 60 min; the proanthocyanidin content (gram leucoanthocyanidin equivalents per 100 g DW) was measured at 550 nm and calculated by the equation

$$Proanthocyanidins = \left(\frac{Abs_{550} \cdot 78 \cdot dilution\ factor}{DW(\%)} \right).$$

Statistical analysis

Data analysis was carried out with the Statgraphics-Plus software 5.1 (Statistical Graphics, Rockville, Md., U.S.A.). Three independent replications by each treatment were used in the statistical analysis. Grubbs' test was applied to detect outliers in the data set and analysis of variance was used to evaluate the effect of the different solvents and temperature extraction on antioxidant activity and phytochemical compound content in mango biowaste extracts. Fisher's least-significant-difference test, at the 5% significance level, was applied to experimental results to assess intrapair significant differences. Simple linear correlation analysis was used to measure the correlation between the phytochemical compounds with the biowaste antioxidant activity.

Results

Effect of temperature and solvent on antioxidant activity in mango peel extracts and on their phytochemical composition

All mango peel extracts prevented the bleaching of β-carotene in carotene/linoleic acid mixtures, although they exhibited varying degrees of antioxidant capacity (Table 1). When extraction was done at 25 °C, the extracts obtained with ethanol:water, acetone, and acetone:water were the most effective in inhibiting the lipid peroxidation (between 1.2 and 1.8 times higher than for the other evaluated solvents). In all mango peel extracts, the capacity to inhibit β-carotene bleaching improved by increasing extraction temperature (Table 1). This increase was dramatic in extracts obtained with simple solvents, specifically methanol and ethanol (around 4 times the activity obtained at 25 °C). In some cases, the temperature increase also improved the antioxidant capacity of the mango peel extracts when this capacity was evaluated with the TBARS method (Table 1). Except for aqueous extracts, whose antioxidant capacity was between 68% and 79% lower than that obtained with the other solvents, at 75 °C, the other mango peel extracts inhibited lipid peroxidation between 66% (ethanol:water) and 80% to 87% (methanol:water, methanol, and acetone:water) more than the control without antioxidants.

The extracts obtained with methanol:water, ethanol:water or acetone:water, and with methanol had the highest capacity to scavenge $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals (Figure 1). In general, the scavenging activity of mango peel extracts against both free radicals increased slightly when the extraction temperature was increased. When temperature was higher than 25 °C, the antiradical activity increased 1.5 times compared with extractions at 25 °C, especially in extractions with ethanol:water and ethanol (Figure 1). The lowest scavenging activity was observed in aqueous extracts.

Table 1–The effect of extraction solvent and temperature on the capacity of mango peel extracts to inhibit lipid peroxidation.

Extraction temperature	Extraction solvent	β-Carotene bleaching test, AAC	TBARS assay, IP
25 °C	Methanol	$201 \pm 28^{c/B}$	$47 \pm 1^{c/B}$
	Ethanol	$155 \pm 3^{d/C}$	$83 \pm 5^{a/A}$
	Acetone	$265 \pm 22^{ab/B}$	$61 \pm 1^{b/B}$
	Water	$219 \pm 9^{c/B}$	$27 \pm 5^{d/A}$
	Methanol:water (1:1)	$209 \pm 8^{c/B}$	$24 \pm 2^{d/B}$
	Ethanol:water (1:1)	$286 \pm 14^{a/A}$	$61 \pm 7^{b/A}$
	Acetone:water (1:1)	$256 \pm 6^{b/B}$	$50 \pm 1^{c/C}$
50 °C	Methanol	$822 \pm 104^{a/A}$	$85 \pm 1^{a/A}$
	Ethanol	$548 \pm 13^{b/B}$	$66 \pm 3^{b/B}$
	Acetone	$433 \pm 20^{c/A}$	$86 \pm 3^{a/A}$
	Water	$396 \pm 16^{c/A}$	$18 \pm 1^{d/B}$
	Methanol:water (1:1)	$372 \pm 18^{c/A}$	$84 \pm 2^{a/A}$
	Ethanol:water (1:1)	$361 \pm 32^{c/A}$	$63 \pm 1^{c/A}$
	Acetone:water (1:1)	$397 \pm 20^{c/A}$	$68 \pm 10^{b/B}$
75 °C	Methanol	$791 \pm 26^{a/A}$	$86 \pm 2^{a/A}$
	Ethanol	$670 \pm 88^{b/A}$	$75 \pm 9^{bc/AB}$
	Acetone	$495 \pm 25^{c/A}$	$69 \pm 7^{c/B}$
	Water	$440 \pm 90^{cd/A}$	$28 \pm 4^{e/A}$
	Methanol:water (1:1)	$375 \pm 18^{d/A}$	$80 \pm 2^{ab/A}$
	Ethanol:water (1:1)	$355 \pm 9^{d/A}$	$66 \pm 6^{d/A}$
	Acetone:water (1:1)	$393 \pm 14^{d/A}$	$87 \pm 2^{a/A}$

Values are the mean ± standard deviation of at least 3 determinations from independent extractions.
Different lower or upper case letters denote significant differences ($P < 0.050$) between extraction solvents (a to e) or between extraction temperatures (A to C) used to obtain the peel extract, respectively.
AAC = antioxidant activity coefficient; IP = inhibition ratio (%).

Mango peel extracts contained considerable amounts of flavonoids, tannins, and proanthocyanidins (Table 2). Tannins represented 88% to 94% of the total phytochemical compounds found in the extracts, except in those obtained with acetone, in which the tannin content represented 59% to 81% of the total evaluated compounds. Extracts obtained with methanol, ethanol:water, and acetone:water had the highest phenolic compound content (sum of flavonoids, tannins, and proanthocyanidins): 8.1 to 12 g/100 g DW. On the other hand, the lowest phytochemical compound content was observed for aqueous extracts (20% to 79% lower than that obtained with the rest of the solvents). Overall, the impact of extraction temperature on the phytochemical compound content of the extracts was low in comparison to that of the extraction solvent; nevertheless, it is interesting that increasing temperature favored the extraction of flavonoids with acetone:water, of tannins with acetone or acetone:water, and of proanthocyanidins with ethanol, methanol:water, or acetone:water. On the other hand, when extraction temperature increased, flavonoid and tannin content decreased in methanolic and aqueous extracts, respectively.

Statistical analysis was carried out to determine any correlation between antioxidant capacity and the phytochemical compounds in mango peel extracts (Table 3). The correlations between phytochemical compounds and the inhibition of lipid peroxidation were relatively weak. Therefore, the inhibition of β-carotene bleaching was correlated with proanthocyanidins and the inhibition of TBARS formation with tannins and proanthocyanidins (Table 3). The correlation coefficients indicated a moderately strong relationship between the capacity of biowastes to scavenge $ABTS^{\bullet+}$

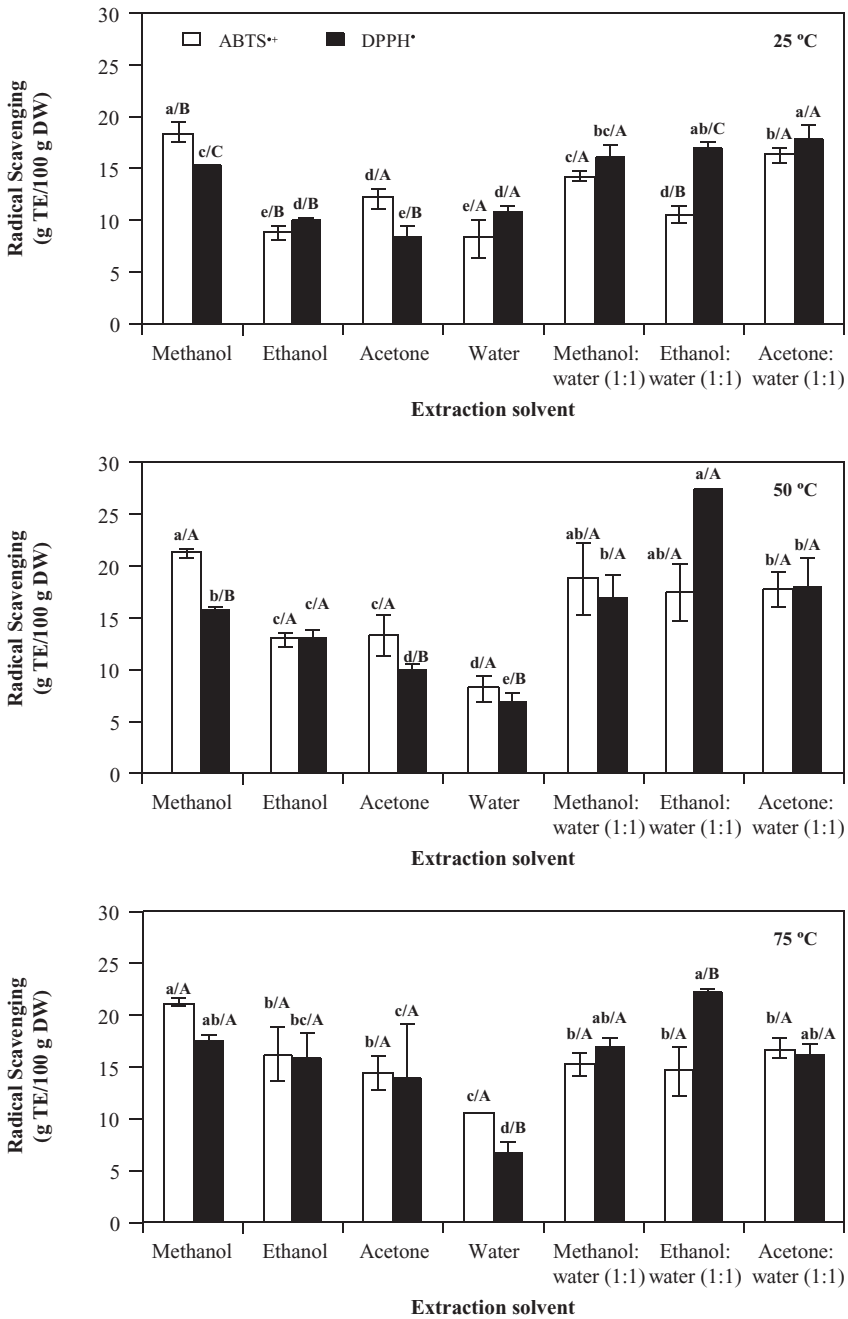

Figure 1–Effect of extraction solvent and temperature on the capacity of mango peel extracts to scavenge free radicals. TE = trolox equivalent antioxidant capacity. Each extraction process was done at least in triplicate. Different lower or upper case letters denote significant differences ($P < 0.050$) between extraction solvents (a to e) or temperatures (A to C) used to obtain the peel extract, respectively.

or DPPH$^{\bullet}$ radicals and proanthocyanidin content and of their capacity to scavenge ABTS$^{\bullet+}$ and tannin content. The capacity to scavenge DPPH$^{\bullet}$ radicals had relatively weak correlations with flavonoid content and tannin content (Table 3).

Effect of temperature and solvents on antioxidant activity in mango seed extracts and on their phytochemical composition

A high capacity to inhibit lipid peroxidation, evaluated with the β-carotene test, was found in all mango seed extracts (Table 4), although the extracts obtained using methanol and mixtures of methanol, ethanol, and acetone with water were the most efficient in inhibiting lipid peroxidation. Moreover, antioxidant capacity increased (1.6 to 2.4 times) when the extraction temperature was increased. When extraction was done at 25 °C, the extracts obtained with ethanol and acetone were the most effective in inhibiting the lipid peroxidation with the TBARS method (Table 4). However, the temperature increase improved the antioxidant capacity of the mango seed extracts obtained with methanol, ethanol:water, and acetone:water while decreasing that of extracts obtained with ethanol and acetone. The aqueous extracts had the lowest (1.5 to 4.0 times) capacity to prevent TBARS formation.

Mango seed extracts showed strong scavenging activity against ABTS$^{\bullet+}$ and DPPH$^{\bullet}$ radicals (Figure 2). The extracts obtained with methanol or acetone:water were the most effective in scavenging both free radicals. Moreover, high antioxidant activity for the DPPH$^{\bullet}$ assay was also found in extracts obtained with ethanol, methanol:water, or ethanol:water. The impact of extraction temperature on scavenging activity of mango seed extracts was practically negligible in comparison to that of the solvent. The lowest antioxidant activity against ABTS$^{\bullet+}$ and DPPH$^{\bullet}$ radicals was found with aqueous extracts.

Most of the mango seed extracts were rich in tannins and proanthocyanidins (Table 5), representing 42% to 93% and 5% to 49%, respectively, of the total phytochemical compounds assayed (flavonoids, tannins, and proanthocyanidins). The highest total phytochemical compound content was found in extracts obtained

Table 2–Phytochemical compounds in mango peel extracts obtained using different solvents and temperatures.

Extraction temperature	Extraction solvent	Total flavonoids[a]	Tannins[b]	Proanthocyanidins[c]
25 °C	Methanol	$0.70 \pm 0.06^{a/A}$	$9.4 \pm 0.7^{a/A}$	$0.58 \pm 0.01^{a/A}$
	Ethanol	$0.50 \pm 0.05^{bc/A}$	$6.0 \pm 0.1^{d/A}$	$0.32 \pm 0.02^{bc/B}$
	Acetone	$0.55 \pm 0.05^{b/A}$	$1.1 \pm 0.5^{f/B}$	$0.20 \pm 0.05^{d/A}$
	Water	$0.18 \pm 0.01^{e/A}$	$3.4 \pm 0.4^{e/A}$	$0.24 \pm 0.03^{d/A}$
	Methanol:water (1:1)	$0.34 \pm 0.02^{d/A}$	$4.1 \pm 0.5^{e/A}$	$0.23 \pm 0.01^{d/AB}$
	Ethanol:water (1:1)	$0.46 \pm 0.09^{bc/A}$	$8.1 \pm 0.8^{b/A}$	$0.30 \pm 0.06^{c/A}$
	Acetone:water (1:1)	$0.43 \pm 0.04^{cd/B}$	$7.1 \pm 0.5^{c/B}$	$0.38 \pm 0.03^{b/B}$
50 °C	Methanol	$0.38 \pm 0.04^{c/B}$	$7.7 \pm 1.2^{b/A}$	$0.54 \pm 0.02^{a/A}$
	Ethanol	$0.42 \pm 0.04^{bc/A}$	$6.0 \pm 0.4^{c/A}$	$0.31 \pm 0.02^{c/B}$
	Acetone	$0.49 \pm 0.10^{ab/A}$	$2.7 \pm 0.6^{d/A}$	$0.25 \pm 0.02^{d/A}$
	Water	$0.17 \pm 0.05^{d/A}$	$3.8 \pm 0.7^{d/A}$	$0.12 \pm 0.02^{f/B}$
	Methanol:water (1:1)	$0.34 \pm 0.04^{c/A}$	$6.0 \pm 1.2^{c/A}$	$0.18 \pm 0.04^{e/B}$
	Ethanol:water (1:1)	$0.38 \pm 0.05^{c/B}$	$7.4 \pm 1.4^{b/A}$	$0.31 \pm 0.01^{c/A}$
	Acetone:water (1:1)	$0.54 \pm 0.02^{a/A}$	$10 \pm 1^{a/A}$	$0.43 \pm 0.02^{b/B}$
75 °C	Methanol	$0.37 \pm 0.03^{b/B}$	$7.8 \pm 0.6^{b/A}$	$0.54 \pm 0.02^{a/A}$
	Ethanol	$0.53 \pm 0.04^{a/A}$	$7.5 \pm 1.6^{bc/A}$	$0.43 \pm 0.02^{b/A}$
	Acetone	$0.51 \pm 0.05^{a/A}$	$3.3 \pm 0.3^{d/A}$	$0.27 \pm 0.04^{d/A}$
	Water	$0.17 \pm 0.04^{c/A}$	$2.1 \pm 0.1^{d/B}$	$0.22 \pm 0.04^{e/A}$
	Methanol:water (1:1)	$0.39 \pm 0.01^{b/A}$	$5.7 \pm 0.7^{c/A}$	$0.32 \pm 0.07^{c/A}$
	Ethanol:water (1:1)	$0.25 \pm 0.04^{c/C}$	$8.0 \pm 1.8^{b/A}$	$0.28 \pm 0.05^{cd/A}$
	Acetone:water (1:1)	$0.56 \pm 0.07^{a/A}$	$11 \pm 2^{a/A}$	$0.52 \pm 0.05^{a/A}$

Values are the mean ± standard deviation of at least 3 determinations from independent extractions.
Different lower or upper case letters denote significant differences ($P < 0.050$) between extraction solvents (a-f) or between extraction temperatures (A-C) used to obtain the peel extract, respectively.
[a]Expressed as g (+)-catechin equivalents/100 g DW (dry matter basis) plant material.
[b]Expressed as g tannic acid equivalents/100 g DW plant material.
[c]Expressed as g leucoanthocyanidin equivalents/100 g DW plant material.

Table 3–Correlation (simple regression analysis) between antioxidant activity and phytochemical compounds.

Antioxidant activity/ phytochemical compounds	Total flavonoids		Tannins		Proanthocyanidins	
	P-value[a]	*r*[b]	*P*-value[a]	*r*[b]	*P*-value[a]	*r*[b]
Mango peel						
β-Carotene bleaching test	0.664*	0.056*	0.430*	0.101*	0.002**	0.395*
TBARS assay	0.095*	−0.212*	0.003**	0.365*	0.002**	0.384*
DPPH$^{\bullet}$ test	0.026***	0.268*	0.039***	0.262*	0.000**	0.502*
ABTS$^{\bullet+}$ test	0.613*	−0.065*	0.000**	0.472*	0.000**	0.611*
Mango seed						
β-Carotene bleaching test	0.387*	−0.111*	0.012***	0.314*	0.736*	−0.044*
TBARS assay	0.005**	0.349*	0.005**	0.349*	0.009**	0.325*
DPPH$^{\bullet}$ test	0.000**	0.650*	0.000**	0.624*	0.000**	0.539*
ABTS$^{\bullet+}$ test	0.001**	0.415*	0.000**	0.568*	0.175*	0.175*

[a]Significance level:*$P > 0.050$, **$P < 0.010$, and ***P between 0.050 and 0.010.
[b]r = Pearson correlation coefficient; r between 0.250 and 0.450 indicates a relatively weak correlation between the variables, r between 0.450 and 0.900 indicates a moderately strong correlation, r between 0.900 and 1.000 indicates a strong correlation.

with acetone:water (15 to 21 g/100 g DW) and with methanol (12 to 14 g/100 g) and the lowest in aqueous extracts (around 2.0 g/100 g). Although the effect of temperature extraction was not as important as that of the solvent, it is important to highlight that tannin content increased 50% to 70% in extracts obtained with ethanol, acetone, or ethanol:water when the extraction temperature was increased from 25 to 75 °C (Table 5).

As in the case of mango peel extracts, the correlations between phytochemical compounds and lipid peroxidation inhibition were limited and relatively weak (Table 3). The inhibition of β-carotene bleaching was correlated with tannins and the inhibition of TBARS formation with flavonoids, tannins, and proanthocyanidins. On the other hand, the correlation coefficients indicated a moderately strong relationship between flavonoid, tannin, and proanthocyanidin contents and the capacity of mango seed extracts to scavenge DPPH$^{\bullet}$ free radicals and between tannin content and the capacity to scavenge ABTS$^{\bullet+}$ radicals (Table 3). All were positive correlations; therefore, antioxidant activity increased as phytochemical compounds increased.

Discussion

Mango peel and seed contain considerable amounts of phytochemical compounds with remarkable antioxidant activity. However, this work proves that extraction is a critical step in the separation of phytochemical compounds from these biowastes; specifically, extraction solvent and temperature are factors to be taken into account when the aim is to obtain extracts with high antioxidant capacity. The polarity of the solvent and of the different

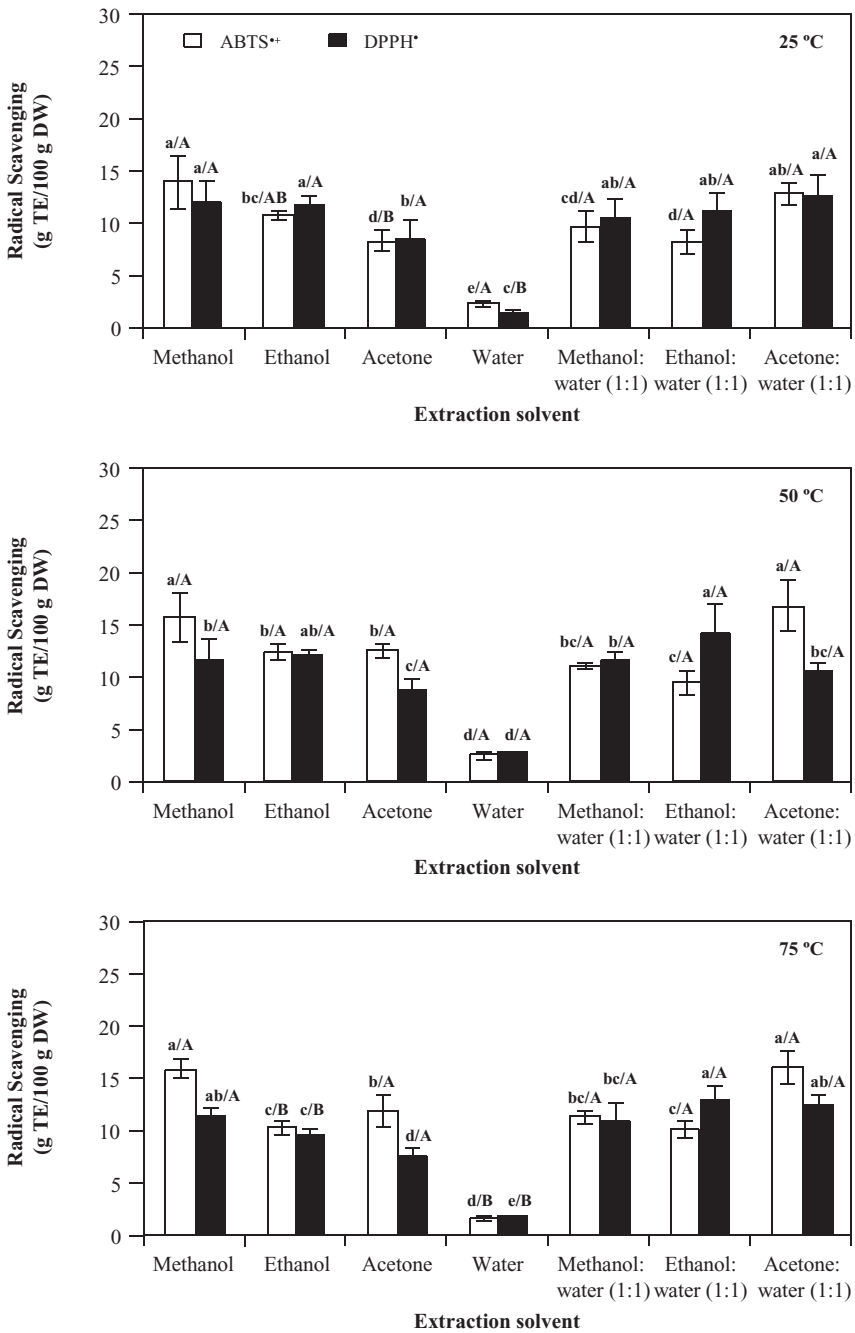

Figure 2–Effect of extraction solvent and temperature on the capacity of mango seed extracts to scavenge free radicals. TE = trolox equivalent antioxidant capacity. Each extraction process was done at least in triplicate. Different lower or upper case letters denote significant differences ($P < 0.050$) between extraction solvents (a to e) or temperatures (A to B) used to obtain the seed extract, respectively.

phenolic compounds affect extraction efficiency and the activity of the obtained extracts (González and González 2010) and, in general, highly hydroxylated aglycone forms of phenolic compounds are soluble in alcohols such as methanol or ethanol and their mixtures with water. Less polar solvents such as ethyl acetate, acetone, and chloroform are used for the less polar and highly methoxylated aglycone forms that are common in fruit peel (Lafka and others 2007). Furthermore, with dried materials, low-polarity solvents, and ethyl acetate simply leach the sample whereas alcoholic solvents rupture cell membranes and enhance the extraction of endocellular materials (Robards 2003). The phytochemicals contained in the extracts obtained by using a polar solvent, for example, water, methanol, ethanol, could be partitioned into a less polar solvent, for example, ethyl acetate, chloroform, diethyl ether, n-hexane. *n*-Hexane (Berardini and others 2005) has been also used to remove lipids from plant material prior to extracting antioxidant compounds from them or from the extracts. The most suitable solvents for obtaining mango peel and seed extracts, with high antioxidant capacity were methanol, methanol:water, ethanol:water, and acetone:water. Similarly, the phytochemical compound content (flavonoids, tannins, and proanthocyanidins) was highest in peel extracts obtained with methanol, ethanol:water, or acetone:water and in seed extracts obtained with methanol or acetone:water. The most widely reported extracting solvents for mango biowaste antioxidants are mixtures (between 50% and 95%) of methanol, ethanol, and acetone with water (Kabuki and others 2000; Soong and Barlow 2004, 2006; Berardini and others 2005; Abdalla and others 2007; Ajila and others 2007). Among these, the acetone:water mixture has been reported as one of or the most effective solvent for extracting phenolic compounds from fruit by-products (Shui and Leong 2006; González-Montelongo and others 2010b), specifically in the extraction of polyphenols from protein matrices, since they appear to degrade polyphenol-protein complexes (Kallithraka and others 1995). This could explain why it is so efficient when extracting antioxidants from mango seed, as this material has a high protein content (6.7 g/100 g DW) (Abdalla and others 2007).

The low antioxidant capacity and content of phytochemical compounds detected in aqueous extracts obtained from peel and seed extracts could be related to the unpolar character of the cell walls of the plant material; this characteristic allows solvents that are less polar than water (methanol, ethanol and acetone) to extract the antioxidant compounds from cells more easily. Similar results

Table 4–The effect of extraction solvent and temperature on the capacity of mango seed extracts to inhibit lipid peroxidation.

Extraction temperature	Extraction solvent	β-Carotene bleaching test, AAC	TBARS assay, IP
25 °C	Methanol	$218 \pm 19^{a/B}$	$44 \pm 10^{c/B}$
	Ethanol	$192 \pm 1^{b/C}$	$87 \pm 1^{a/A}$
	Acetone	$133 \pm 10^{d/C}$	$85 \pm 4^{a/A}$
	Water	$161 \pm 19^{c/C}$	$27 \pm 2^{d/A}$
	Methanol:water (1:1)	$237 \pm 3^{a/C}$	$18 \pm 3^{d/C}$
	Ethanol:water (1:1)	$223 \pm 18^{a/B}$	$48 \pm 7^{c/B}$
	Acetone:water (1:1)	$229 \pm 9^{a/B}$	$73 \pm 4^{b/B}$
50 °C	Methanol	$444 \pm 50^{ab/A}$	$78 \pm 9^{a/A}$
	Ethanol	$293 \pm 12^{c/B}$	$60 \pm 3^{bc/B}$
	Acetone	$321 \pm 32^{c/A}$	$67 \pm 2^{b/B}$
	Water	$472 \pm 19^{a/A}$	$23 \pm 4^{d/A}$
	Methanol:water (1:1)	$457 \pm 31^{a/A}$	$53 \pm 3^{c/B}$
	Ethanol:water (1:1)	$459 \pm 4^{a/A}$	$80 \pm 4^{a/A}$
	Acetone:water (1:1)	$394 \pm 48^{b/A}$	$85 \pm 1^{a/A}$
75 °C	Methanol	$523 \pm 45^{a/A}$	$68 \pm 3^{c/A}$
	Ethanol	$361 \pm 6^{c/A}$	$52 \pm 1^{c/C}$
	Acetone	$265 \pm 3^{d/B}$	$58 \pm 1^{d/C}$
	Water	$331 \pm 46^{c/B}$	$20 \pm 3^{f/A}$
	Methanol:water (1:1)	$368 \pm 4^{c/B}$	$66 \pm 1^{c/A}$
	Ethanol:water (1:1)	$467 \pm 6^{b/A}$	$82 \pm 4^{a/A}$
	Acetone:water (1:1)	$439 \pm 14^{b/A}$	$72 \pm 5^{b/B}$

Values are the mean ± standard deviation of at least 3 determinations from independent extractions.
Different lower or upper case letters denote significant differences ($P < 0.050$) between extraction solvents (a to f) or between extraction temperatures (A to C) used to obtain the seed extract, respectively.
AAC = antioxidant activity coefficient; IP = inhibition ratio (%).

Table 5–Phytochemical compounds in mango seed extracts obtained using different solvents and temperatures.

Extraction temperature	Extraction solvent	Total flavonoids[a]	Tannins[b]	Proanthocyanidins[c]
25 °C	Methanol	$0.93 \pm 0.06^{b/A}$	$11 \pm 2^{b/A}$	$2.3 \pm 0.1^{c/A}$
	Ethanol	$0.63 \pm 0.13^{cd/B}$	$3.5 \pm 0.4^{de/B}$	$1.6 \pm 0.4^{d/A}$
	Acetone	$0.54 \pm 0.12^{d/B}$	$2.1 \pm 0.8^{ef/B}$	$1.4 \pm 0.1^{d/A}$
	Water	$0.075 \pm 0.009^{e/A}$	$1.5 \pm 0.1^{f/A}$	$0.091 \pm 0.001^{e/B}$
	Methanol:water (1:1)	$0.70 \pm 0.05^{c/A}$	$6.8 \pm 0.4^{c/A}$	$1.8 \pm 0.1^{cd/A}$
	Ethanol:water (1:1)	$0.95 \pm 0.01^{b/A}$	$4.3 \pm 0.5^{d/B}$	$5.1 \pm 0.4^{b/A}$
	Acetone:water (1:1)	$1.3 \pm 0.1^{a/A}$	$13 \pm 1^{a/A}$	$7.0 \pm 0.8^{a/A}$
50 °C	Methanol	$0.48 \pm 0.04^{b/C}$	$9.9 \pm 0.4^{a/A}$	$1.5 \pm 0.1^{b/C}$
	Ethanol	$0.50 \pm 0.08^{b/B}$	$5.2 \pm 0.9^{bc/B}$	$1.8 \pm 0.3^{b/A}$
	Acetone	$0.38 \pm 0.06^{c/C}$	$3.9 \pm 1.0^{c/B}$	$0.95 \pm 0.01^{c/B}$
	Water	$0.079 \pm 0.02^{d/A}$	$1.7 \pm 0.3^{d/A}$	$0.047 \pm 0.005^{c/B}$
	Methanol:water (1:1)	$0.47 \pm 0.02^{bc/B}$	$6.5 \pm 0.5^{b/A}$	$1.4 \pm 0.2^{b/A}$
	Ethanol:water (1:1)	$0.42 \pm 0.10^{bc/B}$	$6.1 \pm 0.7^{b/AB}$	$1.9 \pm 0.3^{b/C}$
	Acetone:water (1:1)	$0.93 \pm 0.04^{a/B}$	$11 \pm 1^{a/B}$	$3.8 \pm 0.6^{a/B}$
75 °C	Methanol	$0.72 \pm 0.04^{c/B}$	$11 \pm 1^{a/A}$	$1.7 \pm 0.2^{c/B}$
	Ethanol	$0.85 \pm 0.11^{ab/A}$	$9.6 \pm 1.2^{ab/A}$	$1.7 \pm 0.1^{c/A}$
	Acetone	$0.75 \pm 0.01^{bc/A}$	$6.9 \pm 1.2^{c/A}$	$1.4 \pm 0.1^{c/A}$
	Water	$0.057 \pm 0.007^{d/A}$	$1.4 \pm 0.1^{d/A}$	$0.28 \pm 0.04^{d/A}$
	Methanol:water (1:1)	$0.71 \pm 0.13^{c/A}$	$6.9 \pm 0.6^{c/A}$	$1.6 \pm 0.3^{c/A}$
	Ethanol:water (1:1)	$0.97 \pm 0.02^{a/A}$	$8.3 \pm 1.8^{bc/A}$	$2.7 \pm 0.3^{b/AB}$
	Acetone:water (1:1)	$0.66 \pm 0.05^{c/C}$	$10 \pm 1^{a/B}$	$4.4 \pm 0.7^{a/B}$

Values are the mean ± standard deviation of at least 3 determinations from independent extractions.
Different lower or upper case letters denote significant differences ($P < 0.050$) between extraction solvents (a to f) or between extraction temperatures (A to C) used to obtain the seed extract, respectively.
[a]Expressed as g (+)-catechin equivalents/100 g DW plant material.
[b]Expressed as g tannic acid equivalents/100 g DW (dry matter basis) plant material.
[c]Expressed as g leucoanthocyanidin equivalents/100 g DW plant material.

have been described for grape by-products (Lapornik and others 2005) and murta leaves (Rubilar and others 2006), where water was not an adequate medium for extraction, while mixtures with alcohols were more efficient solvents. Another explanation for the decrease could be ascribed to the polyphenol oxidase enzyme, which degrades polyphenols in aqueous extracts (brown hue observed in these extracts), whereas in other solvents the enzyme is inactive (González-Montelongo and others 2010b).

The use of organic solvents in the manufacturing process of food ingredients is strictly regulated in the European Union (2009). Although mango peel and seed extracts obtained with methanol (not a food-grade solvent) have high antioxidant activity, the conditions under which methanol can be used are strict and maximum residue values permitted in food and food ingredients are limited. Therefore, from the perspective of food security, it would be preferable to choose solvents such as ethanol (also with notable antioxidant activity and phytochemical compound content), ethanol:water or acetone:water, as they can all be used in compliance with good manufacturing practice. Similar decisions have been taken by Lapornik and others (2005), although they described that methanol had better characteristics than ethanol to extract polyphenols from red and black currant marc and from grape marc, in the end ethanol was selected because it was better suited for use in the food industry.

The results obtained in this work show that, in general, temperature favors the capacity of mango peel and seed extracts to inhibit lipid peroxidation; however, the effect on the extraction of phytochemical compounds or on the capacity of the extracts to scavenge free radicals was practically negligible in comparison to that of the solvent. High temperatures have been reported to improve the efficiency of extraction, due to the enhanced diffusion rate and solubility of phytochemicals in solvents. However, elevated temperatures could also affect the activity of the extracts due to the degradation of the phytochemical compounds (Yap and others 2009; Dorta and others 2012), losses due to volatilization, or antioxidant compounds reacting with other components of the plant material, thus impeding extraction (González and González 2010). This behavior was similar to the findings of González-Montelongo and others (2010a) in the extraction of banana peel with methanol, temperature had a high impact on the extract's capacity to prevent lipid peroxidation. However, the effect of changing extraction temperature was not statistically significant for scavenging $ABTS^{\bullet+}$ or $DPPH^{\bullet}$ radicals or extracting phenolic compounds. This could indicate that it is more difficult to extract the phytochemical compounds that inhibit lipid peroxidation at low temperatures (possibly because of their lower solubility in the solvents) than phytochemical compounds that scavenge radicals. The optimization of the antioxidant extraction from murta leaves (Rubilar and others 2006) and from apple pomace (Wijngaard and Brunton 2010) showed that increasing temperature improved extraction; however, temperature could not be increased indefinitely, because the stability of phenolic compounds decreased. It has also been reported that raising the extraction temperature from 40 to 100 °C does not yield a higher content of phenolic compounds and stronger antioxidant capacity in extracts obtained from citrus peel (Xu and others 2008).

In relation to the antioxidant activity and phenolic compound content described for mango biowaste extracts in this work, data were similar than what was reported for mango peel by Ajila and others (2007) (extraction with ethanol:water or acetone:water [4:1, v:v] at room temperature; 5.5 to 11 g GAEs per 100 g DW) and for mango seed by Maisuthisakul and Gordon (2009) (extraction with 95% ethanol, refluxing for 3 h; 20-g ascorbic acid equivalent antioxidant capacity (AE) per 100 g DW; and 6.8 g TAEs/100 g DW) or Soong and Barlow (2004, 2006) (extraction with ethanol:water [1:1, v:v], at 70 °C for 1 h; 14 to 35 g AE/100 g DW; and 12 g GAEs/100 g DW). However, important discrepancies were found between the phytochemical content reported by Berardini and others (2005) from mango peel (extraction with acetone:water [4:1, v:v], at room temperature for 3 h; flavonol-*O*-glycosides; and xanthone-*C*-glycosides 0.020 to 0.50 g/100 g DW) or by Abdalla and others (2007) from mango seed (extraction with methanol:water [19:1, v:v], at 4 °C for 4 min; 0.11 g GAEs/100 g DW; and 0.023 g TAEs/100 g DW) and the content obtained in this work. The similarities and discrepancies could be related, among other factors, to similar or dissimilar extraction conditions used to obtain these compounds from the plant matrices. However, it is necessary to be careful when comparing data described by different authors, because the phytochemical composition of mango biowastes can vary depending on cultivar or other preharvest factors such as climate (temperatures, rainfalls, and light hours), soil type, and fertilization (González and González 2010).

Conclusions

The results of this work confirm that optimizing extraction conditions is a critical step in obtaining extracts rich in antioxidants from mango byproducts. The most important factor among the optimized conditions is the extraction solvent. The most suitable solvents to obtain extracts with high antioxidant capacity and high phytochemical compound content from mango peel are ethanol and ethanol:water (1:1); from mango seed the most suitable is acetone:water (1:1). Extraction temperature also contributes to extraction efficiency; specifically, temperatures between 50 and 75 °C favor the capacity of the extracts obtained from mango peel and seed to inhibit lipid peroxidation.

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