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ORIGINAL PAPER

Optimization of Factors Affecting Extraction of Antioxidants from Mango Seed

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Abstract A microwave-assisted extraction procedure was developed to obtain extracts rich in antioxidants from mango seed. Central composite design '2^5+star' and response surface methodology were used in order to optimise the extraction factors: the water content in the acetone/water mixture used as extractant, seed weight-to-solvent volume ratio, number of steps, extraction time and pH of water. The results suggest that the extractant composition and the seed weight-to-solvent volume ratio were statistically the most significant factors. The optimum values of the factors that influence the capacity to inhibit lipid peroxidation (evaluated with the β-carotene bleaching test), scavenge 2,2-diphenyl-1-picrylhydrazyl and 2,2′-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid-free radicals and obtain extracts with high phenolic compound content (tannins and proanthocyanidins) were three steps; the mixture acetone/water (50:50, *v*/*v*) as extractant, a seed weight-to-solvent volume ratio of 1:30 (*w*/*v*), an extraction time of 0 min in the microwave (the rest of the extraction process includes homogenisation and centrifugation time), and a pH of 8.0.

Keywords *Mangifera indica* L. biowastes · Microwave-assisted extraction · Response surface methodology · Lipid peroxidation inhibition · Free radicals scavenging · Phenolic compounds

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Introduction

The growing concern about food safety on the part of consumers, authorities and food industry producers (Commission of the European Communities 2000) has created a need to identify safer natural alternatives to synthetic additives. The food industry has already begun studying fruit biowastes as one of such alternative because plant biowastes contain many compounds that have antioxidant and antimicrobial capacity. The main biowastes of mango (*Mangifera indica* L.) processing are the peel and the seed, which represent approximately 35–60% of the fruit (Larrauri et al. 1996). Mango seed has been shown to be a good source of polyphenols, sesquiterpenoids, phytosterols and tocopherols, with high antioxidant activity (Kabuki et al. 2000; Puravankara et al. 2000; Berardini et al. 2004; Soong and Barlow 2004, 2006; Abdalla et al. 2007; Engels et al. 2009, 2010; Maisuthisakul and Gordon 2009).

Extraction is the most important step in recovering phytochemical antioxidants from plant biowastes, since its objective is to liberate these compounds from the structures where they are found (González and González 2010; González-Montelongo et al. 2010a; Dorta et al. 2011). Solvent extraction is the most common technique employed to obtain extracts with high antioxidant activity from mango seeds. Methanol or ethanol have been used as extraction solvents (Barreto et al. 2008; Nithitanakool et al. 2009); however, mixtures of ethanol or acetone with water (between 50% and 99.5%) are the most widely used extracting agents for antioxidants from mango seed (Kabuki et al. 2000; Berardini et al. 2004; Soong and Barlow 2004, 2006; Abdalla et al. 2007; Ribeiro et al. 2008; Engels et al. 2009; 2010; Maisuthisakul and Gordon 2009; Dorta et al. 2011). The recovery of bioactive compounds from biowastes is also influenced by the extraction time and the number of steps. Extraction times of a few minutes (less than 5 min; Abdalla et al. 2007) or hours (between 1 and

12 h; Kabuki et al. 2000; Berardini et al. 2004; Barreto et al. 2008; Ribeiro et al. 2008; Soong and Barlow 2004, 2006; Engels et al. 2009; 2010; Maisuthisakul and Gordon 2009; Dorta et al. 2011) have been used. Usually, a single extraction step is used (Kabuki et al. 2000; Soong and Barlow 2004, 2006; Abdalla et al. 2007; Dorta et al. 2011) but sometimes from two to three steps have been used (Berardini et al. 2004; Barreto et al. 2008; Ribeiro et al. 2008; Engels et al. 2009, 2010). Another aspect to take into consideration when extracting antioxidant compounds from biowastes is the plant material weight to solvent volume ratio. The most commonly reported ratios range between 1:1 and 1:10 (*w*/*v*; Kabuki et al. 2000; Abdalla et al. 2007; Engels et al. 2009; 2010; Maisuthisakul and Gordon 2009; Nithitanakool et al. 2009). However, higher ratios between 1:20 and 1:100 (*w*/*v*; Berardini et al. 2004; Ribeiro et al. 2008; Dorta et al. 2011) or between 1:125 and 1:250 (*w*/*v*; Soong and Barlow 2004, 2006) have also been used with promising results.

However, the procedures employed to extract phytochemicals from mango seed have only been optimised for the extraction of other types of plant material, not specifically for mango seed. Extraction procedures differ between different biowastes because of their different matrices, with unique properties in terms of structure and composition (related to species, varieties, ripening stages, etc.). Therefore, considerable caution should be exercised when using procedures that have been developed for specific plant tissue types and phytochemical extractions should be optimised for each biowaste (González and González 2010; González-Montelongo et al. 2010a). Recently, our research group recently studied how factors such as the type of solvent and temperature influence the extraction process of compounds with antioxidant properties from mango peel and seed (Dorta et al. 2011).

Microwave energy has been used to accelerate solvent extraction of antioxidant phytochemicals from plant material (Pan et al. 2008; Périno-Issartier et al. 2011; Routray and Orsat 2011). Microwave-assisted extraction (MAE) provides higher recoveries and requires considerably less time and smaller amounts of solvents compared to conventional extraction with solvents (González and González 2010). For example, the phenolic compounds from longan pericarp were extracted with 95% ethanol (plant material weight to solvent volume ratio 1:10, *w*/*v*) employing MAE, at 80 °C for 30 min (Pan et al. 2008). The phenolic content of extracts obtained from longan pericarp was similar when using either MAE or Soxhlet extraction. However, the antioxidant activity of microwave-assisted extracts was superior to that of Soxhlet extracts, had faster extraction times (2 h for Soxhlet and 30 min for MAE) and required less solvent. All of which are clear advantages to using this technique. Despite its advantages, it has rarely been used to extract phytochemicals from tropical biowastes and no information about the application of MAE for extracting bioactive compounds or for obtaining extracts with high antioxidant activity of mango seed was found.

Response surface methodology (RSM) is a useful technique to evaluate the effect of influential factors on one or more response variables, which are simultaneously modified. Moreover, the establishment of a mathematical model helps to locate the region where the extraction is optimised (Montgomery 1991). The choice of an experimental design mainly depends on the objectives of the experiment. Therefore, randomised block designs are used if the primary goal of the experiment is to verify if a factor considered to be important continues to be so in the presence of the other factors. Full or fractional factorial designs are used if the primary purpose of the experiment is to identify the few influential factors among all of the factors evaluated. However, if the experimental design is used to find improved or optimal process settings, central composite (CCD) or Box–Behnken designs are usually used.

CCD and RSM have been used to optimise the extraction of phytochemical compounds from tropical fruit biowastes. The optimisation of the number of steps (optimum obtained with 3), extraction time (homogenisation for 1 min and further centrifugation for 20 min) and temperature (25 °C) when extracting phenolics from banana peel (González-Montelongo et al. 2010a) has been carried out by experimental design. The effect of acetone concentration in water, extraction temperature and extraction time on phenolic content extracted from star fruit residue was also studied by using RSM (Yap et al. 2009). Acetone concentration was statistically the most significant factor and the optimal extraction conditions obtained were: 65% acetone concentration, 43 °C extraction temperature and 3.9 h extraction time. However, although CCD and RSM have been used for the optimisation of the microwave-assisted extraction of phytochemicals from plant materials, there are no applications in tropical fruit bio-wastes.

Optimising conditions to obtain extracts with high antioxidant activity from mango seed using microwave-assisted extraction is proposed for the first time in this study. The use of a CCD and RSM to optimise five factors (water content in the solvent mixture, seed weight-to-solvent volume ratio, number of steps, extraction time and water pH in the extractant) determines an optimal set of operational conditions.

Materials and Methods

Chemical and Reagents

Gallic acid, Folin–Ciocalteau reagent (2 N), linoleic acid, Tween 40, 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), 2,2′-azino-bis-(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS),

hydrogen peroxide (30%) and polyvinylpolypyrrolidone were purchased from Sigma (Madrid, Spain). (±) 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) and trans-β-carotene were supplied by Aldrich (Madrid, Spain). The concentration of the enzyme horseradish peroxidase (HRP) type VI [RZ ($A_{403\ nm}/A_{275\ nm}$)=2.8], obtained from Sigma, was determined by measuring the absorbance at 403 nm using an extinction coefficient of ε_{403nm}= 100 mM^{-1} cm^{-1} (Arnao et al. 2001). Tannic acid and ferric ammonium sulphate were obtained from Merck (Darmstadt, Germany) and hydrochloric acid and *n*-butanol from Panreac (Madrid, Spain). Acetone and chloroform were purchased of high-performance liquid chromatography grade from Scharlau Chemie (Barcelona, Spain). Deionised water of 18 MΩ cm resistivity purified with a milli-Q system (Millipore, Bedford, USA) was used.

Obtaining Mango Seeds

Mango (*M. indica* L., cv. 'Keitt') was obtained from fields located in Tenerife (Canary Islands, Spain). Fruit (around 20 kg) was harvested at physiological maturity stage (mature—green) and allowed to ripen (full-ripeness or consumption stage) at 18 °C and 80–90% relative humidity. The ripeness stage of the mangoes (n=21) was characterised by peel and pulp colour and texture, total soluble solids, pH and titratable acidity.

Peel and pulp colour were measured (CIELab colour space) in three different points of the fruit equator with a Minolta Chroma Meter model CR-300 (Ramsay, USA) colour difference metre, using chromatic attributes: lightness (L*), hue angle (h°) and chroma value (C*). The measuring area of the colourimeter has a diameter of 8 mm; the illuminant and the standard observer used for testing were D65 and 2°, respectively; a white calibration plate was used to calibrate the colourimeter. L*, h° and C* of mango peel at full ripeness ranged between 62 and 70, 44 and 84, and 41 and 57, respectively. The colour of mango pulp was also characterised by lightness ranging between 83 and 89, hue angle between 84 and 90 and chromaticity between 72 and 84.

Deformation force was measured using a Durofel DFT 100 (Agro Technologie, Forges Les Eaux, France) with a mobile tip of 25 mm^2, that gives a unit (° Durofel) for each 0.025 mm of fruit deformation. Penetration force was measured with a TA-HD-Plus texture analyzer supplied by Texture Technologies Co. (Godalming, UK). Mangoes were placed horizontally on the plate and compression was applied using a cylindrical probe (4.0 mm in diameter) at an assay speed of 2.0 mm/s. The range of deformation and penetration force was 66–80 °Durofel and 40–66 N, respectively.

Total soluble solids (TSS) were determined using an ATAGO ATC-1 hand refractometer (Tokyo, Japan) and pH was measured by a WTW pH metre (St Woburn, USA) at room temperature. After determination of pH, titratable acidity was measured with 0.1 N sodium hydroxide standard solution (Merck, Darmstadt, Germany) up to pH 8.1. TSS, pH and titratable acidity ranged between: 14–20 °Brix, 4.0–4.6 and 292–446 mg citric acid/100 g, respectively.

After ripening, the seed (shell of fibrous endocarp, testa and embryo) was manually separated (seed weight ranged between 2.1% and 6.3% of total mango weight), cut into small pieces (0.5×1 cm) and freeze-dried using a Christ alpha-1-4 LSC freeze-dryer (Osterode, Germany). The condenser temperature was −40 °C, the shelf temperature was set at 25 °C and the vacuum was 50 mPa for 5 days (Dorta et al. 2012). The dried mango seeds (water content, 49±5%; lightness, 76±1; hue angle, 91±1; chromaticity, 14±1) were ground to a fine powder by impact grinding with an IKA A11 mill (Staufen im Breisgau, Germany) and sieved by intense vibratory and rotary movements for 15 min (Mecánica Científica, 105611 sieve, Madrid, Spain), using nine sieves with diameters ranging between 2.8 mm and 50 μm. Thus, the sample granulometry was characterised: 48%> 500 μm, 9% between 355 and 500 μm and 43%<355 μm. The dried mango seed powder was stored at −20 °C until the extractions were carried out.

Microwave-Assisted Extraction

All extractions were carried out using microwave (ETHOS 1, Milestone SRL, Sorisole, Italy) at 50 °C and an initial potency of 500 W (Dorta et al. 2011). The temperature in the microwave extractor was continually controlled allowing the accurate and precise temperature control in all extraction vessels. The control system included a direct temperature control device (a sapphire fibre optic sensing element (ATC-FO sensor, MLS GmbH, Leutkirch, Germany) inserted in one of the extraction vessels) and a focused, high sensitivity infrared sensor, both interfaced to a microprocessor-controlled rotor positioning system. The extraction was carried out in closed vessels, with a built-in safety valve. An accurately weighed aliquot of freeze-dried seed powder (seed weight-to-solvent volume ratio varied depending on the particular experiment; ranging between 1:10–1:50 *w/v*) was homogenised, with a Politron PT-6000 (Kinematica AG, Lucerne, Switzerland) high speed blender at 1,250 rad/s for 1 min, with 20 ml of acetone:water (water content varied depending on the particular experiment; ranging between 5% and 95%). Water pH and extraction time (ranging between 3–8 and 0–120 min, respectively) were also modified depending on the particular experiment. Extracts were centrifuged at 525 rad/s for 20 min in a Jouan CR-312 centrifuge (Thermo Electron Corporation, Madrid, Spain). Therefore, the extraction time includes the time of the high-speed homogenisation (1 min), the time in the microwave (0–120 min) and the centrifugation time (20 min).

Depending on the experiment, this procedure was repeated between one and three times; the resulting supernatants were mixed together and acetone/water was added until a final volume of 60 ml was reached. The extracts obtained were stored at −80 °C until the assessments of antioxidant potential and bioactive compound content were carried out (in less than 3 days). Each extraction process was done in triplicate.

Experimental Design

A CCD '2^5+star' projected on a face-centred star design with two centre points was used to identify the relationship between five independent factors and the dependent variables or responses, as well as to determine the optimal conditions for the extraction process. The factors (water content in the solvent mixture, seed weight-to-solvent volume ratio, number of steps, extraction time and water pH) were set at three separate coded levels (Table 1). The design consisted of 44 randomised runs (n=132 experiments, because each extraction process was done in triplicate), with 32 factorial points, two centre points and 10 axial points. The antioxidant activity (evaluated using the β-carotene, DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ tests) and the phenolic compound (tannins and proanthocyanidins) content were chosen as the dependent variables because of their known dependency on the extraction process. Data from the CCD were approximated to a second-order polynomial equation and analysis of variance (ANOVA) was generated to determine individual linear, quadratic and interaction regression coefficients. The significances of polynomial relations were examined statistically by computing the F value at a probability (p) of 0.050. The percentage of contribution of each factor to the antioxidant activity of the extracts or to the phytochemical extraction was obtained from ANOVA and was calculated from the percentage of the standardised effect of the significance of each main effect or of each interaction and the total standardised effect of all main effects and interactions. Experiments to determine the adequacy of the model were done by using combinations of variables at different levels (within the experimental range).

Antioxidant Activity of Mango Seed Extracts

Multiple reaction characteristics and mechanisms as well as different phase localizations are usually involved in the assay of antioxidant capacity; for these reasons, no single assay will accurately reflect all of the radical sources or all antioxidants in a mixed or complex system such as a biological biowastes (Prior et al. 2005). In this work, the antioxidant activity of the extracts was evaluated by using different methods to obtain information about the capacity to can deactivate radicals by hydrogen atom transfer (HAT) and by single electron transfer (SET). HAT-based methods (such as β-carotene bleaching assay) measure the classical ability of an antioxidant to quench free radicals by hydrogen donation, and SET-based methods (such as DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays) detect the ability of a potential antioxidant to transfer one electron to reduce any compound, including metals, carbonyls, and radicals. SET and HAT mechanisms almost always occur together in all samples, with the balance determined by antioxidant structure and pH. All measurements were made on a Shimadzu UV-visible 160A double-beam spectrophotometer (Kyoto, Japan) equipped with a Hellma (Jamaica, USA) cell (path length 10^{-2} m).

The β-carotene bleaching method is based on the capacity of antioxidants to decrease oxidative losses of β-carotene in a β-carotene/linoleic acid system (Miller 1971). To induce autoxidation, the temperature was increased (50 °C) and it was used oxygenated deionised water, which was generated by bubbling air into water for 60 min. In these conditions, the β-carotene molecules lose their conjugated double bonds and the loss in orange colour intensity was measured at 470 nm after incubation for 210 min. The antioxidant activity was expressed as antioxidant activity coefficient (AAC; González-Montelongo et al. 2010a). The repeatability standard deviation of the procedure was always <10%.

The capacity to scavenge the DPPH$^{\bullet}$ radical was monitored according to a slightly modified version of the method used by Brand-Williams et al. (1995) at 515 nm after 15 min. The scavenging activity against the ABTS$^{\bullet+}$ radical was determined by a method (Arnao et al. 2001) based on enzymatic generation of the radical by reaction of the ABTS with the horseradish peroxidase in sodium phosphate buffer pH 7.5, in the presence of hydrogen peroxide. The assay temperature was controlled at 25 °C and the inhibition by the biowaste antioxidants was measured at 730 nm after 6 min. In both methods, a control with the addition of acetone/water (instead of extracts) was used. Results were expressed as grams of trolox equivalent antioxidant capacity/100 g mango seed on a dry matter basis (DW; González-Montelongo et al. 2010a). Calibration graphs (r^2>0.963) were constructed by plotting the absorbance against the trolox concentration at seven concentration levels (50–500 mg/l for DPPH$^{\bullet}$ test and 20–250 mg/l for ABTS$^{\bullet+}$ test) analysed in triplicate. The procedure's repeatability was <5%.

Bioactive Compounds Content

The Folin–Ciocalteu method, to determine the total content of phenolic compounds (González-Montelongo et al. 2010a), was coupled with the use of an insoluble matrix (polyvinylpolypyrrolidone, PVPP) to analyse tannins (FAO/IAEA 2000). The tannins precipitate when PVPP is added to extracts (PVPP/phenolic compounds ratio, 100:1 w/w) and the supernatant only contains other than tannins; therefore, tannins were quantified by calculating the difference between

Table 1 Uncoded and coded values of the factors studied in the different experiments of the central composite design

Experiments[a]	Uncoded and coded (between parentheses) values of factors									
	n_{ext}		w_{ext}		w/v_{ext}		t_{ext}[b]		pH_{ext}	
Factorial points										
1	1	(−1)	95	(+1)	1:10	(−1)	120	(+1)	8	(+1)
2	3	(+1)	95	(+1)	1:10	(−1)	0	(−1)	3	(−1)
4	3	(+1)	5	(−1)	1:50	(+1)	120	(+1)	3	(−1)
6	1	(−1)	95	(+1)	1:50	(+1)	120	(+1)	8	(+1)
7	3	(+1)	95	(+1)	1:50	(+1)	120	(+1)	8	(+1)
8	1	(−1)	95	(+1)	1:10	(−1)	120	(+1)	3	(−1)
9	1	(−1)	95	(+1)	1:50	(+1)	120	(+1)	3	(−1)
10	3	(+1)	5	(−1)	1:10	(−1)	0	(−1)	3	(−1)
11	3	(+1)	95	(+1)	1:10	(−1)	120	(+1)	8	(+1)
12	1	(−1)	5	(−1)	1:10	(−1)	0	(−1)	8	(+1)
13	1	(−1)	5	(−1)	1:10	(−1)	0	(−1)	3	(−1)
15	3	(+1)	5	(−1)	1:50	(+1)	0	(−1)	8	(+1)
16	1	(−1)	5	(−1)	1:10	(−1)	120	(+1)	3	(−1)
17	3	(+1)	95	(+1)	1:10	(−1)	0	(−1)	8	(+1)
18	1	(−1)	95	(+1)	1:50	(+1)	0	(−1)	3	(−1)
19	3	(+1)	95	(+1)	1:50	(+1)	120	(+1)	3	(−1)
23	3	(+1)	95	(+1)	1:10	(−1)	120	(+1)	3	(−1)
26	3	(+1)	5	(−1)	1:10	(−1)	120	(+1)	3	(−1)
27	1	(−1)	95	(+1)	1:10	(−1)	0	(−1)	8	(+1)
28	1	(−1)	5	(−1)	1:50	(+1)	120	(+1)	3	(−1)
29	1	(−1)	95	(+1)	1:10	(−1)	0	(−1)	3	(−1)
31	1	(−1)	5	(−1)	1:50	(+1)	120	(+1)	8	(+1)
32	3	(+1)	95	(+1)	1:50	(+1)	0	(−1)	3	(−1)
34	1	(−1)	5	(−1)	1:50	(+1)	0	(−1)	3	(−1)
35	3	(+1)	5	(−1)	1:50	(+1)	120	(+1)	8	(+1)
37	1	(−1)	5	(−1)	1:10	(−1)	120	(+1)	8	(+1)
38	3	(+1)	95	(+1)	1:50	(+1)	0	(−1)	8	(+1)
39	3	(+1)	5	(−1)	1:50	(+1)	0	(−1)	3	(−1)
40	3	(+1)	5	(−1)	1:10	(−1)	120	(+1)	8	(+1)
41	1	(−1)	5	(−1)	1:50	(+1)	0	(−1)	8	(+1)
42	1	(−1)	95	(+1)	1:50	(+1)	0	(−1)	8	(+1)
43	3	(+1)	5	(−1)	1:10	(−1)	0	(−1)	8	(+1)
Centre points										
14	2	(0)	50	(0)	1:30	(0)	60	(0)	5.5	(0)
36	2	(0)	50	(0)	1:30	(0)	60	(0)	5.5	(0)
Axial points										
3	2	(0)	5	(−1)	1:30	(0)	60	(0)	5.5	(0)
5	2	(0)	50	(0)	1:30	(0)	120	(+1)	5.5	(0)
20	2	(0)	50	(0)	1:30	(0)	60	(0)	3	(−1)
21	1	(−1)	50	(0)	1:30	(0)	60	(0)	5.5	(0)
22	2	(0)	50	(0)	1:30	(0)	0	(−1)	5.5	(0)
24	2	(0)	50	(0)	1:10	(−1)	60	(0)	5.5	(0)
25	2	(0)	95	(+1)	1:30	(0)	60	(0)	5.5	(0)
30	2	(0)	50	(0)	1:30	(0)	60	(0)	8	(+1)
33	3	(+1)	50	(0)	1:30	(0)	60	(0)	5.5	(0)
44	2	(0)	50	(0)	1:50	(+1)	60	(0)	5.5	(0)

n_{ext} number of steps, w_{ext} water content in extractant, w/v_{ext} seed weight-to-solvent volume ratio in extractions, t_{ext} extraction time, pH_{ext} water pH, t_{ext} extraction time in the microwave

[a]The numbers (1–44) indicate the randomised order in which the experiments were developed

[b]The rest of the extraction process includes, in all cases, 1 min (high-speed homogenisation) and 20 min (centrifugation)

total phenolic compounds and the phenols in the supernatant (FAO/IAEA 2000). They were expressed as grams of tannic acid equivalents (TAEs)/100 g DW mango seeds. Calibration curves (r^2>0.990) were constructed by plotting the absorbance against the tannic acid amount at seven levels analysed in triplicate (0.90–25 μg). The repeatability of the procedure was <5%.

The determination of condensed tannins (proanthocyanidins) is based on oxidative depolymerisation of proanthocyanidins in butanol-HCl (95:5 *v*/*v*, solvent/extract ratio, 5:1) (FAO/IAEA 2000). The presence of iron reagent (2% ferric ammonium sulphate 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 (grams of leucoanthocyanidin equivalents (LEs)/100 g DW) was measured at 550 nm. Condensed tannin content was calculated by the formula (FAO/IAEA 2000).

$$\text{Proanthocyanidins (g/100 g)} = \left[\frac{(\text{Abs}_{550} \cdot 78 \cdot \text{dilution factor})}{\text{DW (\%)}}\right]$$

Statistical Analysis

Statgraphics-Plus software 5.1 (Statistical Graphics, Rockville, MD, USA) was employed to generate design, ANOVA and to obtain the response surface plots. Simple linear correlation analysis was used to measure the correlation between the phenolic compound content and the antioxidant activity of the extracts.

Results and Discussion

Diagnostic checking of models on optimisation of the extraction parameters for obtaining high antioxidant mango seed extracts

In accordance with our previous experience in the treatment of mango seed, five factors that can potentially affect microwave-assisted extraction of mango seed to obtain extracts with high antioxidant activity were evaluated: number of steps, water content in the solvent mixture, seed weight-to-solvent volume ratio, extraction time and water pH. The minimum and maximum levels (Tables 2 and 3) given to each factor were chosen based on the experience of our research group and that of other authors in obtaining extracts with high antioxidant activity from mango seed (Kabuki et al. 2000; Puravankara et al. 2000; Berardini et al. 2004; Soong and Barlow 2004, 2006; Abdalla et al. 2007; Engels et al. 2009, 2010; Maisuthisakul and Gordon 2009; Dorta et al. 2011). Other factors implicated in the extraction were kept constant: type of extractant (a mixture of acetone in water), extraction temperature (50 °C), volume of extractant (20 ml) and final volume of the extract (60 ml).

Tables 2 and 3 show the design matrix, which include the factors that influence microwave-assisted extraction and the antioxidant activity measured by using different methods (inhibition of β-carotene bleaching, scavenging of $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ free radicals) and of bioactive compounds (tannins and proanthocyanidins), respectively. The sequential listing of the experimental design parameters represents the statistically randomised order in which the experiments were undertaken. The comparison of the experimental values with the predicted values revealed that the two sets of values, for each antioxidant activity assay and for each bioactive compound, were very close (Tables 2 and 3). The coefficients of determination (R^2) indicated that the model (predicted values) explained between 82% and 92% of the variability observed in antioxidant activity and in proanthocyanidin content (experimental values) and 61% of the variability observed in tannin content (experimental values). The standard error of the estimates had a standard deviation of the residuals between 0.11 (proanthocyanidins, g LEs/100 g DW) and 50 (β-carotene bleaching, AAC) and the Durbin–Watson statistic tests indicated that, since the *p* value was greater than 0.050 for all the determinations, there was no indication of serial autocorrelation in the residuals, based on the order in which the residuals occur in the dataset.

Effects of the Extraction Factors on the Antioxidant Activity of Mango Seed Extracts

Water Content in the Solvent Mixture

ANOVA was used to estimate the statistical significance of the factors and interactions between them that had the greatest effect on obtaining extracts with high antioxidant capacity (Fig. 1). Among the factors studied, the water content in the acetone/water mixture used as extractant had the greatest impact on scavenging $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals and on the extraction of bioactive compounds, accounting for 61–70% for the former and between 36 (tannins) for the latter. This factor had a more limited influence on the inhibition of β-carotene bleaching (around 18%). Some of the response-surface graphs, selected from amongst those obtained using the experimental model, is shown in Fig. 2. In these graphics, the variable with the greatest effect on the extraction, composition of the extractant, is shown along with the seed weight-to-solvent volume ratio. The capacity to inhibit β-carotene bleaching, to scavenge free radicals and to extract phenolic compounds (tannins and proanthocyanidins) increased when the water content in the extractant decreased, being the optimum water content in the extractant between 5% and 50% (high acetone content; Fig. 2). This conclusion is similar for all the parameters studied (Table 3).

Table 2 Design matrix in the central composite design and experimental (EV) and predicted (PV) values obtained for antioxidant activity in extracts from mango seed

Experiments[a]	Inhibition of β-carotene bleaching		Scavenging radical activity			
	AAC		$DPPH^{\bullet}$		$ABTS^{\bullet+}$	
			(g TE/100 g)		(g TE/100 g)	
	EV	PV	EV	PV	EV	PV
1	279±20	257	1.7±0.1	2.5	1.9±0.1	3.9
2	470±9	422	6.7±0.3	6.7	6.8±0.7	8.9
3	347±9	379	9.9±1.2	11	15±2	15
4	221±18	269	16±1	14	23±4	18
5	408±12	371	17±1	16	19±1	17
6	231±7	239	8.3±1.0	8.2	8.6±1.2	7.7
7	321±11	290	13±2	12	13±1	11
8	106±17	180	1.7±0.1	1.2	2.8±0.5	1.0
9	108±3	132	5.5±0.2	5.0	5.6±0.3	5.4
10	513±37	517	16±1	16	20±1	22
11	432±27	391	4.3±0.4	5.3	4.9±0.2	5.2
12	398±32	433	15±1	14	17±1	19
13	460±14	462	13±1	14	18±1	18
14	384±12	400	15±1	14	19±1	16
15	278±29	178	19±1	19	21±1	20
16	381±9	303	8.1±1.7	6.6	12±1	8.9
17	330±23	361	7.3±0.7	4.9	6.5±0.6	3.0
18	184±31	129	7.9±0.1	7.1	7.9±0.7	8.4
19	246±20	215	8.2±0.8	9.1	12±3	13
20	452±30	425	15±1	14	17±1	18
21	402±2	428	13±1	13	16±1	15
22	340±10	362	17±2	18	18±1	19.8
23	289±25	345	3.9±0.2	4.7	3.9±0.1	6.4
24	402±2	385	9.9±0.4	9.5	12±1	11
25	332±21	285	4.1±0.7	3.3	4.4±0.9	4.7
26	441±13	427	11±1	11	16±1	15
27	343±12	297	3.6±0.2	4.4	4.9±0.6	3.9
28	278±27	227	4.9±1.0	9.0	2.3±0.4	11
29	303±41	326	5.1±0.5	5.5	5.6±0.5	5.6
30	436±35	448	15±1	15	17±1	17
31	290±5	333	15±1	14	22±2	16
32	152±19	143	8.4±0.3	8.9	17±1	14
33	522±26	481	16±1	16	16±1	18
34	177±3	237	16±1	14	21±2	18
35	340±2	343	17±1	18	15±1	20
36	357±16	400	12±1	14	14±1	16
37	376±14	380	9.3±0.9	9.2	12±1	15
38	210±38	112	8.3±0.9	9.2	4.5±0.2	7.0
39	187±13	210	17±1	17	22±2	23
40	428±29	471	12±1	13	17±1	17
41	285±26	237	17±1	17	19±2	19
42	128±16	130	7.6±0.6	7.9	3.9±0.2	6.0
43	461±25	455	16±1	16	21±3	19
44	220±22	223	12±1	13	12±1	14
R^2	0.855		0.917		0.823	
Standard error of the estimate	50		1.5		3.0	
Durbin–Watson statistic	1.9 (p=0.231)		1.7 (p=0.076)		1.7 (p=0.077)	

Values based on freeze-dried mango seed and expressed as the mean ± standard deviation (n=3)

AAC antioxidant activity coefficient, *TE* trolox equivalent capacity, *EV* experimental values, *PV* predicted values

[a]The numbers (1–44) indicate the randomised order in which the experiments were developed

Table 3 Design matrix in the central composite design and experimental (EV) and predicted (PV) values obtained for phenolic compounds quantified in extracts from mango seed

Experiments[a]	Tannins (g TAEs/100 g)		Proanthocyanidins (g LEs/100 g)	
	EV	PV	EV	PV
1	0.64±0.02	0.38	0.053±0.010	0.019
2	3.6±0.1	3.4	0.17±0.01	0.16
3	14±2	8.4	0.61±0.10	0.58
4	4.1±0.9	4.3	0.40±0.01	0.38
5	8.1±0.3	6.0	0.82±0.07	0.59
6	3.1±0.1	3.3	n.c.	0.028
7	4.5±0.4	3.5	0.062±0.014	0.081
8	0.84±0.11	0.10	0.012±0.005	−0.044
9	2.5±0.1	2.8	n.c.	0.047
10	4.6±0.1	5.2	1.1±0.1	1.1
11	1.6±0.1	1.3	0.074±0.012	0.16
12	2.2±0.4	3.6	1.0±0.1	1.0
13	3.6±0.5	3.7	1.1±0.1	0.97
14	6.0±0.6	6.9	0.61±0.06	0.62
15	2.2±0.5	3.6	0.41±0.01	0.43
16	1.4±0.3	2.3	0.51±0.05	0.65
17	2.6±0.1	2.6	0.16±0.01	0.24
18	3.2±0.3	2.5	0.17±0.04	0.18
19	3.6±0.3	3.7	0.013±0.002	0.047
20	6.4±0.8	6.1	0.53±0.04	0.65
21	5.9±0.6	5.5	0.70±0.14	0.59
22	5.3±0.7	6.6	0.54±0.04	0.73
23	1.7±0.1	1.7	0.066±0.006	0.049
24	4.8±0.3	4.3	0.99±0.13	0.76
25	2.0±0.4	7.0	0.039±0.006	0.032
26	3.3±0.7	3.5	0.88±0.06	0.90
27	1.8±0.3	1.5	0.25±0.01	0.24
28	3.8±0.6	3.8	0.32±0.04	0.23
29	2.0±0.2	1.5	0.15±0.02	0.22
30	6.4±0.4	6.0	0.83±0.09	0.68
31	3.5±0.3	4.3	0.24±0.01	0.21
32	4.5±0.4	3.7	0.17±0.01	0.033
33	6.7±0.3	6.3	0.59±0.05	0.67
34	2.5±0.6	3.5	0.42±0.04	0.42
35	3.7±0.8	4.0	0.39±0.01	0.42
36	4.7±0.3	6.9	0.48±0.03	0.62
37	2.8±0.2	2.6	0.54±0.09	0.72
38	3.4±0.2	3.1	0.13±0.01	0.028
39	4.1±0.1	4.2	0.42±0.03	0.43
40	1.4±0.1	3.0	1.1±0.1	1.0
41	4.6±0.5	3.6	0.37±0.02	0.37
42	3.1±0.3	2.6	0.12±0.02	0.12
43	6.0±0.4	4.4	1.2±0.1	1.2
44	5.6±1.1	5.3	0.25±0.01	0.44
R^2	0.606		0.913	
Standard error of the estimate	1.6		0.11	
Durbin–Watson statistic	2.0 (p=0.444)		2.1 (p=0.295)	

TAEs tannic acid equivalents, *LEs* leucoanthocyanidin equivalents, *n.c.* non-quantifiable data, *EV* experimental values, *PV* predicted values

Values based on freeze-dried mango seed and expressed as the mean ± standard deviation (n=3)

[a]The numbers (1–44) indicate the randomised order in which the experiments were developed

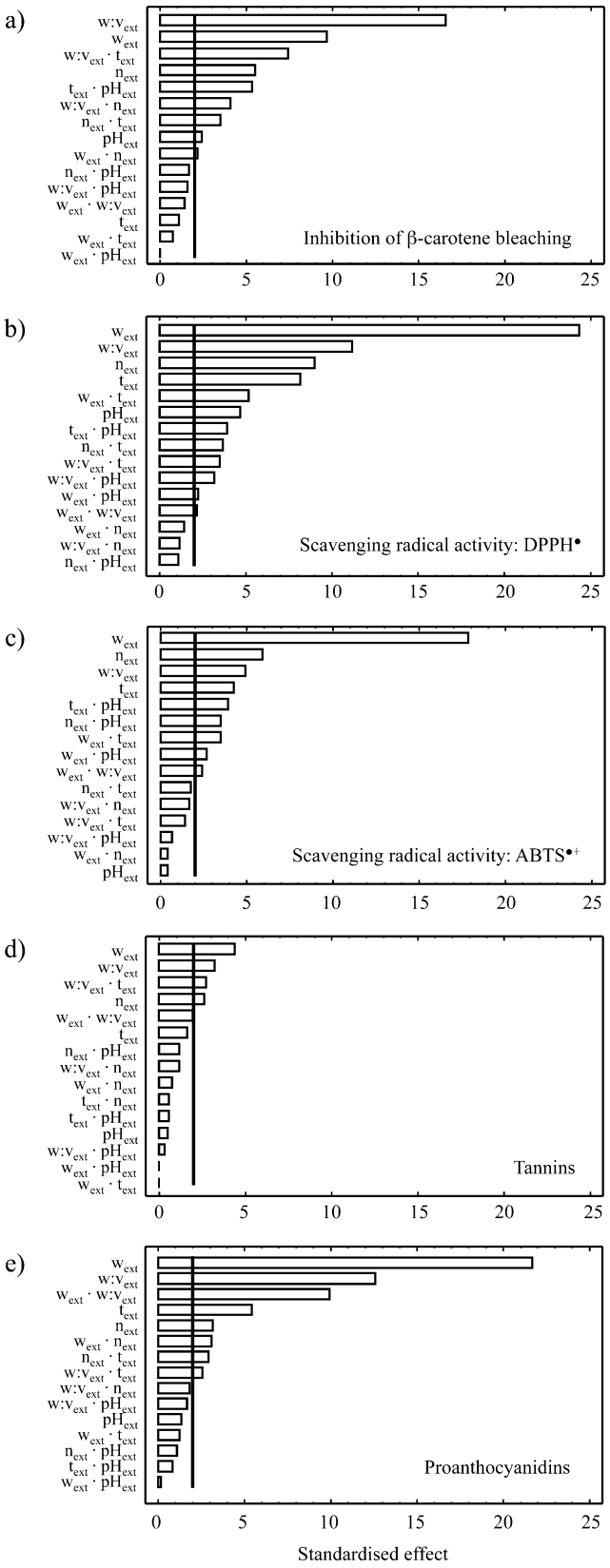

Fig. 1 Pareto charts for the standardised main and interactions in the central composite design for the (**a**) inhibition of β-carotene bleaching, (**b**) scavenging of DPPH$^{\bullet}$ free radical, (**c**) scavenging of ABTS$^{\bullet+}$ free radical, (**d**) tannin content and (**e**) proanthocyanidin content. *Vertical line* indicates the statistical significance of the effects

The mixtures of acetone with water, containing a high percentage of organic solvent, have been reported as one of or the most effective solvents for extracting phenolic compounds from protein matrices, such as mango seed (Ribeiro et al. 2008; Dorta et al. 2011), since they appear to degrade the polyphenol–protein complexes (Kallithraka et al. 1995). Additionally, Dorta et al. (2011) described that using an acetone/water mixture was more efficient than acetone or water in obtaining extracts from mango seed with high antioxidant activity. Similar results were obtained by Yap et al. (2009) in star fruit biowastes, for which the water concentration in the acetone/water mixture was statistically the most significant factor ($p<0.010$) in the extraction of phenolic compounds, between the studied. These authors found that either a high proportion of water in the solvent mixture or acetone (100%) did not contribute to obtaining a high recovery of phenolic compounds in the extracts.

Seed Weight-to-Solvent Volume Ratio in Extractions

The seed weight-to-solvent volume ratio in the extractions had the second greatest impact on how efficiently bioactive compounds, which are responsible for antioxidant activity, were extracted from mango seed (Fig. 1). This is due to the high impact that this factor (52%) had on the extract's capacity to prevent β-carotene bleaching. Moreover, this factor was statistically significant for the capacity to scavenge free radicals (factor contribution 5.1–13%) and for the phenolic compound extraction, being especially relevant for the extraction of tannins and proanthocyanidins accounting 19% and 20%, respectively. The capacity to inhibit lipid peroxidation (Fig. 2a) and to extract tannins (Fig. 2c) and proanthocyanidins (Fig. 2d) improved dramatically (90%, 55% and 95%, respectively) by decreasing the seed weight-to-solvent volume ratio from 1:50 to 1:30. However, the increase in the capacity to inhibit lipid peroxidation was much lower (5%) when the ratio decreased from 1:30 to 1:10 and this decrease caused a very negative impact on the extraction of tannins, decreasing their extraction a 45%. Thus, the optimum seed weight-to-solvent volume ratio can be fixed at 1:30 (*v*/*v*). It is important highlight that the interaction between the water content in the solvent mixture and the seed weight-to-solvent volume ratio influenced the proanthocyanidin extraction (accounting for 12%), being the

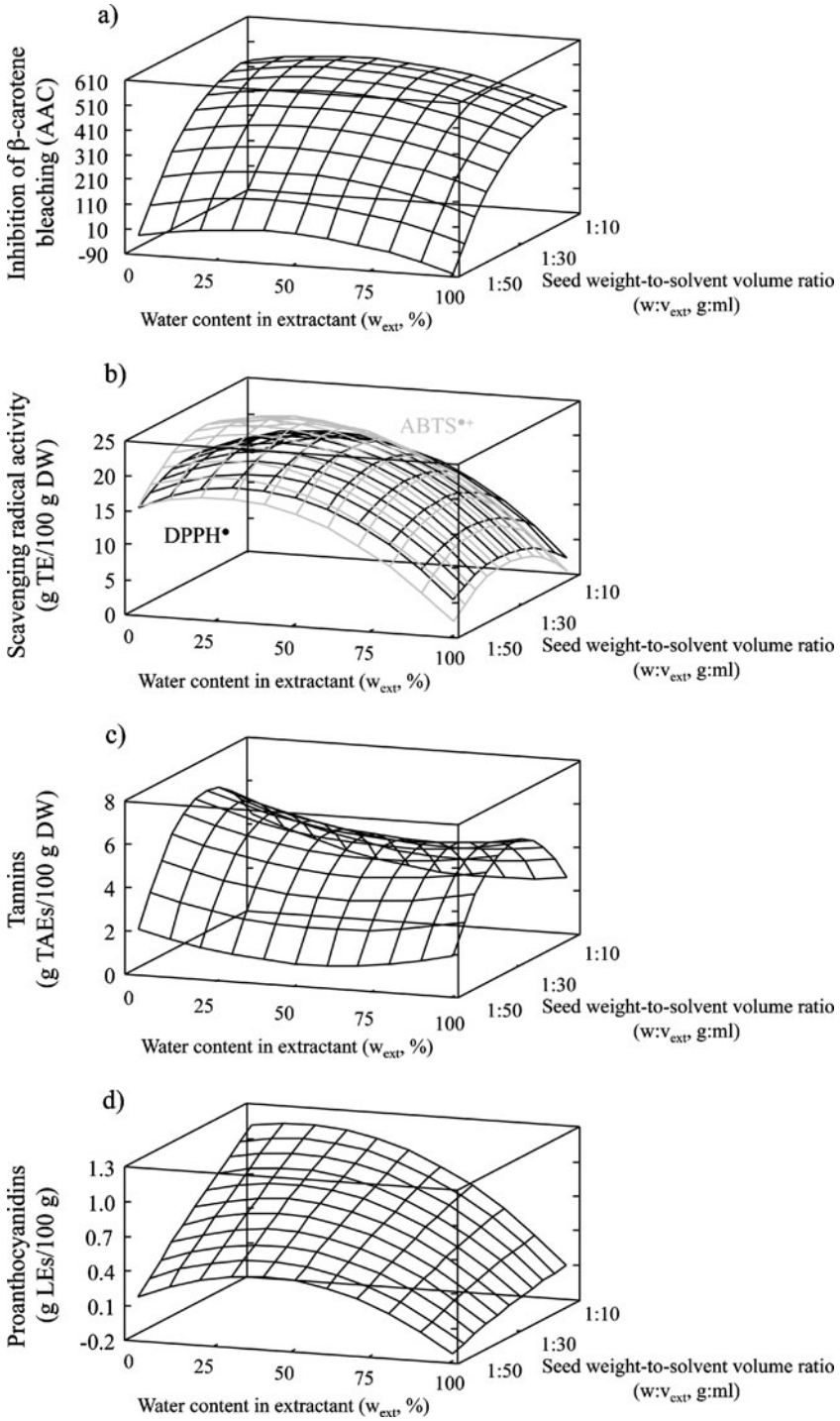

Fig. 2 Estimated response surfaces obtained by plotting the water content in extractant (w_{ext}, %) and seed weight-to-solvent volume ratio (w/v_{ext}, g/ml) for inhibition of β-carotene bleaching (**a**), scavenging activity (**b**), tannins content (**c**) and proanthocyanidins content (**d**) when fixing extraction time at 0 min, water pH in extractant at 8.0 and number of steps at 3

optimum extraction achieved at low water content and low ratio between seed weight and solvent volume.

Pinelo et al. (2005) have reported that solid-to-solvent ratio, optimised between 1:1 and 1:5 (*w*/*v*), played an important role in the efficient extraction of phenolic compounds from grape pomace and in the antiradical activity of extracts. The highest efficiency extraction was obtained when a high solid-to-solvent ratio (1:1, *w*/*v*) was employed.

Number of Steps

The number of steps influenced the extraction of bioactive compounds and the obtaining of extracts capable to inhibit β-carotene bleaching and to scavenge free radicals, with a 95% confidence level (Fig. 1). However, except for tannins (factor contribution, 13%), the impact of this factor on antioxidant activity and on phytochemical extraction was

practically negligible (1.2–8.3%) in comparison to the water content in the solvent mixture and to the seed weight-to-solvent volume ratio. The extraction efficiency improved generally by increasing the number of steps. This effect was also noticed in Fig. 3 which represent extraction time versus number of steps. This fact suggested that 3 was the optimal number of steps.

The number of steps is not a factor frequently studied for its relationship with the antioxidant activity of the extracts and with its bioactive compounds content, although it has been proven that is a crucial factor in the extraction processes (González-Montelongo et al. 2010a). González-Montelongo et al. (2010a) suggested that the optimum number of steps to obtain extracts with high antioxidant capacity and with high phenolic compound content from banana peel was 3. Xu et al. (2008) described that a second extraction with water from citrus peels was necessary since a considerable amount of phenolic compounds (with the consequent increase in antioxidant activity) were extracted from this residue by doing so. In the obtaining of antioxidant compounds (gallotannins, gallates, flavonols, xanthones, anthocyanins or benzophenone derivates) from mango biowastes (bark, kernel, seed or peel), two to three extraction steps are usually used (Berardini et al. 2004; Soong and Barlow 2004, 2006; Barreto et al. 2008; Ribeiro et al. 2008; Engels et al. 2009, 2010).

Extraction Time

Extraction time significantly affected obtaining extracts that scavenge $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals and extracting proanthocyanidins, while it had not impact in the capacity of the extracts to prevent β-carotene bleaching and in the tannin content in the extracts (Fig. 1). Moreover, the impact of this factor on scavenging activity and on proanthocyanidins content was very low (4–7%). Zero minutes were the optimum in the microwave (therefore, the extraction process took place during homogenisation and centrifugation time) to obtain extracts with high antioxidant activity (Fig. 3). It has been proved that, to obtain extracts with high antioxidant activity and to extract phytochemicals from mango seeds, it is enough with homogenising the samples for 1 min. Therefore, the use of a microwave-assisted extractor is not necessary with the associate reduction of costs, rapidity and simplicity. The interaction between seed weight-to-solvent volume ratio and extraction time influenced the capacity of the extracts to prevent β-carotene bleaching (accounting for 10%) and the content of tannins in the extracts (14%; Fig. 1). Despite this, interaction also affected the capacity to scavenge $DPPH^{\bullet}$ radical and the proanthocyanidins content in the extracts, its impact was very low (around 1%). Similarly, although the interaction between water content in the extractant and extraction time affected the capacity of extracts to scavenge free radicals, its effect was negligible (3%). Moreover, the results of this work demonstrate that any degradation of phytochemical compounds occurs in the experimental conditions evaluated, even at prolonged extraction periods.

Similarly, the impact of extraction time on obtaining extracts, from banana peel, that inhibit β-carotene bleaching and that scavenge $DPPH^{\bullet}$ radicals was very low (5–8%; González-Montelongo et al. 2010a), being the optimal time in the water bath 0 min (taking place the extraction during the homogenisation and the centrifugation). Xu et al. (2008) and Pinelo et al. (2005) also described that prolonging the extraction time did not improve antioxidant activity in citrus peel or grape pomace extracts, respectively. Yap et al. (2009) reported that increasing extraction time from 1.5 to 4.5 h led to a reduction of phenolic compounds in the 66% acetone extracts obtained from star fruit residues. Spigno et al. (2007) concluded that obtaining extracts rich in phenolics with high antioxidant activity from grape marc by-products could be more energy efficient using a high temperature (60 °C) for a short time (5 h), rather than a lower temperature (45 °C) for a longer time (15 h).

Water pH in the Extractant

The pH of the water in the solvent mixture can determine the degree of solubility of water-soluble compounds and also influence the possible solubilisation of the hydrolysable fraction of the phytochemical compounds. Nevertheless, amongst the factors studied, the water pH had the less important effect on the antioxidant activity and the bioactive compounds content of the extracts (Fig. 1). The water pH was statistically significant for the capacity to prevent lipid peroxidation (factor contribution 1.0%) and to scavenge $DPPH^{\bullet}$ radicals (2.2%); therefore, its effect can be considered as negligible. A slight increase in the scavenging of $DPPH^{\bullet}$ radicals with water pH (from 3.0 to 8.0) was found, thus, a pH of 8.0 was selected as the optimal for extracting antioxidants from mango seed.

Mylonaki et al. (2008) optimised the extraction of phenolic compounds from olive leaf and obtained that ethanol concentration in the extractant and extraction time mostly affected the extraction yield, while the impact of water pH on the extraction was low. However, it has been hypothesised that increasing water pH in the mixtures of water and organic solvents used as extractants might enhance polyphenol solubility by promoting the dissociation of the most acidic phenolic groups, making the polyphenols more polar (Mylonaki et al. 2008). An increase (around 50%) in the recovery of phenolic compounds was described by González-Montelongo et al. (2010b) when the water pH in the acetone/water extractant was higher than 4.0.

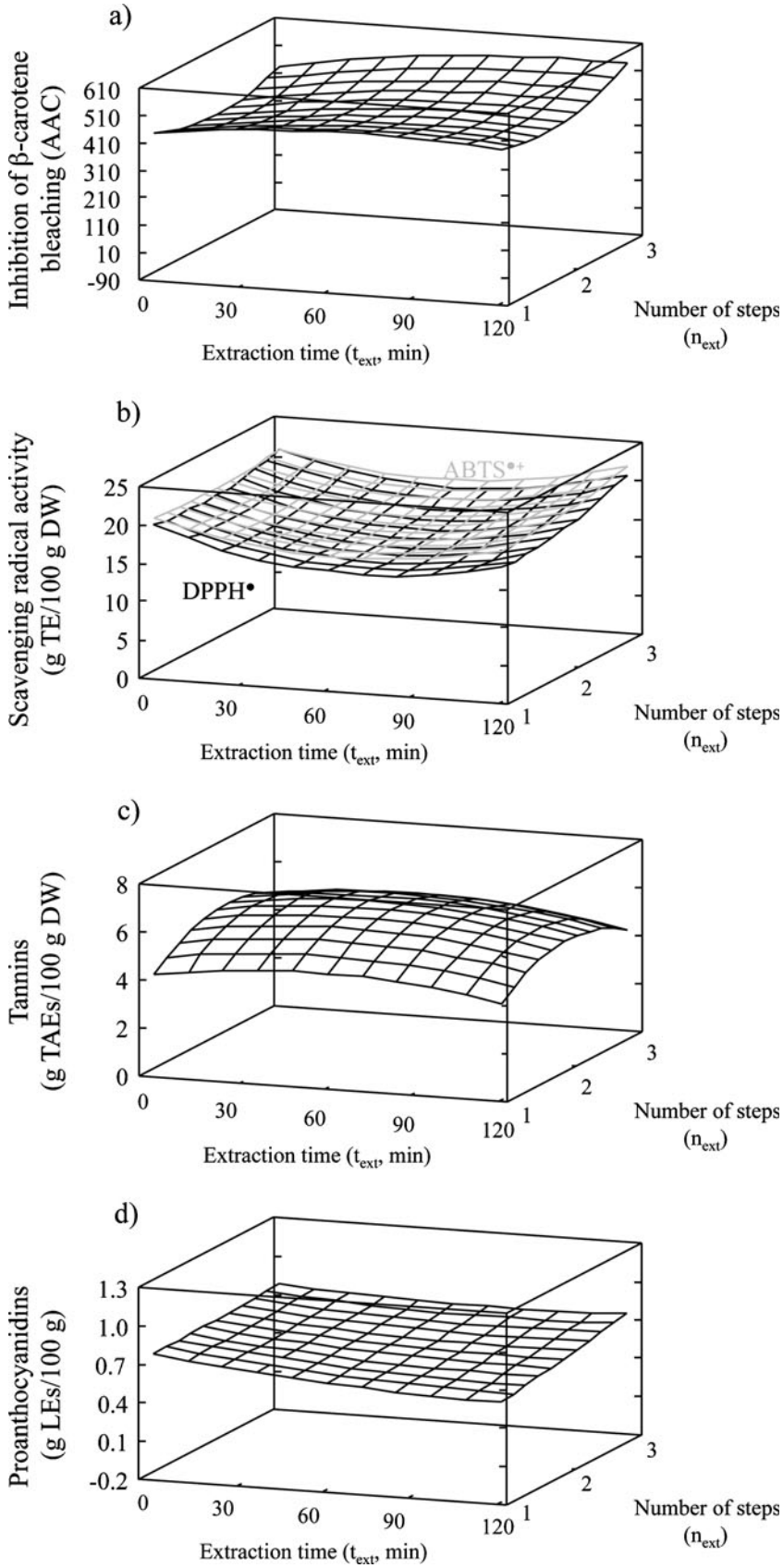

Fig. 3 Estimated response surfaces obtained by plotting the extraction time (t_{ext}, min) and number of steps (n_{ext}) for inhibition of β-carotene bleaching (**a**), scavenging activity (**b**), tannins content (**c**) and proanthocyanidins content (**d**) when fixing water content in extractant at 50%, water pH in extractant at 8.0 and seed weight-to-volume ratio at 1:10

The Optimal Extraction Conditions to Maximise Antioxidant Activity of Mango Seed Extracts

Using three extractions, a water content of 50% in the acetone/water mixture used as extractant, a seed weight-to-solvent volume ratio of 1:30, an extraction time of 0 min in the microwave (the rest of extraction process includes homogenisation and centrifugation time) and a water pH of 8.0 led to the highest antioxidant activity, measured as the capacity to inhibit lipid peroxidation and to scavenge $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals. These conditions also contribute to obtain a high phenolic compound content in mango seed extracts. To determine the accuracy of the extraction procedure, extraction using optimal conditions and random (non-optimal) conditions were carried out in triplicate (Table 4). The experimental values for antioxidant capacity and phenolic compound content, in the optimal conditions, were quite close to the optimised values for these optimal conditions which confirmed that the model was valid. Moreover, the values obtained for both types of conditions indicate that the extraction of biowaste using non-optimal extraction conditions (for example, conditions that were not optimised for this specific plant material) can waste part of the antioxidant potential of these biowastes. For example, important discrepancies were found between the phenolic compound content reported by Abdalla et al. (2007) in their extraction with methanol/water (19:1, *v*/*v*) from mango seed (total phenolic compounds, 0.11 g gallic acid equivalents/100 g DW; tannins, 0.023 g TAEs/100 g DW) and those obtained in this work. They could probably be related, among other factors, to the different extraction conditions used to obtain these compounds from this plant matrix. Soong and Barlow (2006) evaluated three extraction types in the obtaining of 50% methanolic extracts with high antioxidant activity from mango seed: (1) water bath extraction at 70 °C for 1 h, (2) water bath extraction (70 °C for 1 h) and hydrolysis at 35 °C for 16 h, and (3) water bath extraction (70 °C for 1 h) and hydrolysis at 85 °C for 2 h. The capacity to scavenge $ABTS^{\bullet+}$ radicals was affected remarkably by the extraction conditions, increasing 20% with increasing severity of hydrolysis.

With all the data obtained for the different extraction conditions evaluated (n=132), statistical analysis based on regression lines was carried out to determine if there was any correlation between antioxidant capacity and the phenolic compounds in the extracts. The statistical relationship between the capacity of the extracts to scavenge $DPPH^{\bullet}$ and $ABTS^{\bullet+}$radicals and proanthocyanidin content in them was moderately strong (p=0.000, r>0.563) and between the antiradical capacity and the tannin content was relatively weak (p=0.000, r>0.423). These correlations illustrate that these antioxidant compounds greatly affect the ability of the mango seed to scavenge free radicals. The correlation coefficients indicated a moderately strong relationship between the capacity to inhibit lipid peroxidation and the proanthocyanidin content (p=0.000, r=0.650) and a relatively weak relationship between this antioxidant activity and the tannin content (p=0.002, r=0.270). Therefore, it is possible that other non-phenolic compounds in the mango seed extracts affect the ability to prevent lipid peroxidation, probably fibre which antioxidant capacity has been previously reported (Larrauri et al. 1996).

Conclusions

Mango seed is a good and non-expensive source of phenolic compounds that can be used as food additives by the food industry. The economic feasibility of the extraction procedure

Table 4 Confirmatory trials of the optimal conditions to obtain mango seed extracts with high antioxidant activity

Response parameter	Optimal extraction conditions[a] Three steps, 50% water, 1:30 seed:solvent, 0 min, pH 8	Random (non-optimal) extraction conditions[a] one step, 95% water, 1:10 seed:solvent, 120 min, pH 3
Inhibition of β-carotene bleaching[b]		
AAC	400±6	107±11
Scavenging radical activity[b]		
$DPPH^{\bullet}$ (g TE/100 g)	18±2	1.7±0.1
$ABTS^{\bullet+}$ (g TE/100 g)	22±1	2.2±0.4
Phenolics compounds content[b]		
Tannins (g TAEs/100 g)	8.3±1.6	0.17±0.02
Proanthocyanidins (g LEs/100 g)	0.39±0.02	0.049±0.009

AAC antioxidant activity coefficient, *TE* trolox equivalent antioxidant capacity, *TAEs* tannic acid equivalents, *LEs* leucoanthocyanidin equivalents

[a] The extraction time (0 or 120 min) indicated corresponds to the time in the microwave; the rest of the extraction process includes, in all cases, 1 min (high-speed homogenisation) and 20 min (centrifugation)

[b] Values based on freeze-dried mango seed and expressed as the mean±standard deviation (n=3)

depends on finding the optimal extraction conditions to maximise the efficiency of the process. In this work, RSM was successfully employed to optimise the conditions to obtain extracts with high antioxidant activity from mango seed. The most important factor amongst the optimised conditions is the extractant composition (the water content in the mixture acetone/water), affecting the extract's capacity to scavenge $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals and the phenolic compounds content in the extracts. The seed weight-to-solvent volume ratio used in the extractions has an important effect on the capacity to prevent lipid peroxidation. Optimum values of the factors that influence the antioxidant capacity and the phenolic compound content in the extracts obtained from mango seed are: three steps, the mixture acetone/water (50:50, *v*/*v*) as extractant, a seed weight-to-solvent volume ratio of 1:30 (*w*/*v*), an extraction time of 0 min in the microwave (the rest of the extraction process includes homogenisation and centrifugation time) and a pH of 8.0. This work proves that using experimental design helps to visualise relationships between responses and extraction conditions, while also giving a clear idea of interactions between different extraction conditions.

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References

Abdalla, A. E. M., Darwish, S., Ayad, E. H. E., & El-Hamahmy, R. M. (2007). Egyptian mango by-product 1. Compositional quality of mango seed kernel. *Food Chemistry, 103*(4), 1134–1140.

Arnao, M. B., Cano, A., & Acosta, M. (2001). The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chemistry, 73*, 239–244.

Barreto, J. C., Trevisan, M. T. S., Hull, W. E., Erben, G., de Brito, E. S., Pfundstein, B., Würtele, G., Spiegelhalder, B., & Owen, R. W. (2008). Characterization and quantitation of polyphenolic compounds in bark, kernel, leaves, and peel of mango (*Mangifera indica* L.). *Journal of Agricultural and Food Chemistry, 56*, 5599–5610.

Berardini, N., Carle, R., & Schieber, A. (2004). Characterization of gallotannins and benzophenone derivatives from mango (*Mangifera indica* L. cv. Tommy Atkins) peels, pulp and kernels by high-performance liquid chromatography/electrospray ionization mass spectrometry. *Rapid Communications in Mass Spectrometry, 18*, 2208–2216.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT- Food Science and Technology, 28*(1), 25–30.

Commission of the European Communities. (2000). *White Paper on Food Safety COM (1999) 719 Final*. Brussels: Commission of the European Communities.

Dorta, E., Lobo, M. G., & González, M. (2011). Reutilization of mango by-products: Study of the effect of extraction solvent and temperature on their antioxidant properties. *Journal of Food Science*. doi:10.1111/j.1750-3841.2011.02477.x.

Dorta, E., Lobo, M. G., & González, M. (2012). Using drying treatments to stabilise mango peel and seed: effect on antioxidant activity. *LWT- Food Science and Technology, 45*, 261–268.

Engels, C., Knödler, M., Zhao, Y. Y., Carle, R., Gänzle, M. G., & Schieber, A. (2009). Antimicrobial activity of gallotannins isolated from mango (*Mangifera indica* L.) kernels. *Journal of Agricultural and Food Chemistry, 57*, 7712–7718.

Engels, C., Gänzle, M. G., & Schieber, A. (2010). Fractionation of gallotannins from mango (*Mangifera indica* L.) kernels by high-speed counter-current chromatography and determination of their antibacterial activity. *Journal of Agricultural and Food Chemistry, 58*, 775–780.

FAO/IAEA. (2000). *Quantification of tannins in tree foliage*. Vienna: Joint FAO/IAEA Division of Nuclear Techniques in food and Agriculture.

González, M., & González, V. (2010). Sample preparation of tropical and subtropical fruit biowastes to determine antioxidant phytochemicals. *Analytical Methods, 2*, 1842–1866.

González-Montelongo, R., Lobo, M. G., & González, M. (2010a). The effect of extraction temperature, time and number of steps on the antioxidant capacity of methanolic banana peel extracts. *Separation and Purification Technology, 71*, 347–355.

González-Montelongo, R., Lobo, M. G., & González, M. (2010b). Antioxidant activity in banana peel extracts: testing extraction conditions and related bioactive compounds. *Food Chemistry, 119*(3), 1030–1039.

Kabuki, T., Nakajima, H., Arai, M., Ueda, S., Kuwabara, Y., & Dosako, S. (2000). Characterization of novel antimicrobial compounds from mango (*Mangifera indica* L.) kernel seeds. *Food Chemistry, 71*, 61–66.

Kallithraka, S., García-Viguera, C., Bridle, P., & Bakker, J. (1995). Survey of solvents for the extraction of grape seed phenolics. *Phytochemical Analysis, 6*, 265–267.

Larrauri, J. A., Rupérez, P., Borroto, B., & Saura-Calixto, F. (1996). Mango peels as a new tropical fibre: preparation and characterization. *LWT- Food Science and Technology, 29*(8), 729–733.

Maisuthisakul, P., & Gordon, M. H. (2009). Antioxidant and tyrosinase inhibitory activity of mango seed kernel by product. *Food Chemistry, 117*, 332–342.

Miller, H. E. (1971). A simplified method for the evaluation of antioxidants. *Journal of the American Oil Chemists' Society, 48*(2), 91.

Montgomery, D. C. (1991). *Design and analysis of experiments*. New York: Wiley.

Mylonaki, S., Kiassos, E., Makris, D. P., & Kefalas, P. (2008). Optimisation of the extraction of olive (*Olea europaea)* leaf phenolics using water/ethanol-based solvent systems and response surface methodology. *Analytical and Bioanalytical Chemistry, 392*, 977–985.

Nithitanakool, S., Pithayanukul, P., Bavovada, R., & Saparpakorn, P. (2009). Molecular docking studies and anti-tyrosinase activity of Thai mango seed kernel extract. *Molecules, 14*(1), 257–265.

Pan, Y., Wang, K., Huang, S., Wang, H., Mu, X., He, C., Ji, X., Zhang, J., & Huang, F. (2008). Antioxidant activity of microwave-assisted extract of longan (*Dimocarpus Longan* Lour.) peel. *Food Chemistry, 106*, 1264–1270.

Périno-Issartier, S., Zill-e-Huma, Z. H., Abert-Vian, M., & Chemat, F. (2011). Solvent free microwave-assisted extraction of antioxidants from sea buckthorn (*Hippophae rhamnoides*) food by-products. *Food and Bioprocess Technology, 4*, 1020–1028.

Pinelo, M., Rubilar, M., Jerez, M., Sineiro, J., & Núñez, M. J. (2005). Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. *Journal of Agricultural and Food Chemistry, 53*, 2111–2117.

Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods

and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*, 4290–4302.

Puravankara, D., Boghra, V., & Sharma, R. S. (2000). Effect of antioxidant principles isolated from mango (*Mangifera indica* L.) seed kernels on oxidative stability of buffalo ghee (butter-fat). *Journal of the Science of Food and Agriculture, 80*(4), 522–526.

Ribeiro, S. M. R., Barbosa, L. C. A., Queiroz, J. H., Knödler, M., & Schieber, A. (2008). Phenolic compounds and antioxidant capacity of Brazilian mango (*Mangifera indica* L.*)* varieties. *Food Chemistry, 110*(3), 620–626.

Routray, W., & Orsat, V. (2011). Microwave-assisted extraction of flavonoids: a review. *Food and Bioprocess Technology*. doi:10.1007/s11947-011-0573-z.

Soong, Y. Y., & Barlow, P. J. (2004). Antioxidant activity and phenolic content of selected fruit seeds. *Food Chemistry, 88*(3), 411–417.

Soong, Y. Y., & Barlow, P. J. (2006). Quantification of gallic acid and ellagic acid from longan (*Dimocarpus longan* Lour.) seed and mango (*Mangifera indica* L.) kernel and their effects on antioxidant activity. *Food Chemistry, 97*, 524–530.

Spigno, G., Tramelli, L., & De Faveri, D. M. (2007). Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. *Journal of Food Engineering, 81*, 200–208.

Xu, G. H., Chen, J. C., Liu, D. H., Zhang, Y. H., Jiang, P., & Ye, X. Q. (2008). Minerals, phenolics compounds, and antioxidant capacity of citrus peel extract by hot water. *Journal of Food Science, 73*(1), C11–C18.

Yap, C. F., Ho, C. W., Wan-Aida, W. M., Chan, S. W., Lee, C. Y., & Leong, Y. S. (2009). Optimization of extraction conditions of total phenolic compounds from star fruit (*Averrhoa carambola* L.) residues. *Sains Malaysiana, 38*(4), 511–520.