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Original article

Optimisation of microwave-assisted extraction of prune (*Prunus domestica*) antioxidants by response surface methodology

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Summary Optimal conditions for microwave-assisted extraction (MAE) of total phenols (TP), epicatechin gallate and antioxidant activity from prune (*Prunus domestica*), rejected in transformation process of plum to prune, were determined by response surface methodology. The central composite design was used to study the effects of three independent variables: microwave power, irradiation time and solvent polarity on the TP, epicatechin gallate and antioxidant activity. Epicatechin gallate was identified as a major phenolic compound in prune extract by RP-HPLC. Microwave power and irradiation time significantly affected all responses ($P < 0.01$). The highest TP (598.89 mg GAE/100 g prune) was obtained using water as an extraction solvent at 500 W, during 115 s. However, the optimal conditions for epicatechin gallate extraction were ethanol 80%, 435 W and 120 s. MAE is more efficient than conventional extraction method to obtain TP from prune. The experimental values were reasonably close to the predicted values confirming the validity of the predicted models.

Keywords Antioxidant activity, epicatechin gallate, microwave-assisted extraction, prune, response surface methodology, total phenols.

Introduction

Phenolic compounds are a group of aromatic secondary plant metabolites widely spread throughout the plant kingdom (Athanasios *et al.*, 2007). Most of the beneficial characteristics of phenolic compounds have been ascribed to their antioxidant activity (Rice-Evans *et al.*, 1997). As antioxidants, polyphenols play an important role in the prevention of human pathologies. Moreover, they have many industrial applications, for example they can be used as natural colourants, preservative for foods and applied in the production of paints, paper and cosmetics (Hayat *et al.*, 2010). Therefore, the extraction and purification of phytochemicals from natural sources are highly desirable (Tabart *et al.*, 2007). Much interest has been focused on the search for plants rich in phenolic compounds and the development of extraction techniques able to obtain high phenolic compounds yields (Chirinos *et al.*, 2007; Alu'datt *et al.*, 2010).

Prunes are the dried fruits of certain cultivars of *Prunus domestica* L. (Rosaceae) and have been promoted as a healthy food (Tarhan, 2007). *In vitro* assays have shown that prunes have the highest antioxidative capacity among dried fruits (Kimura *et al.*, 2008). Kayano *et al.* (2003) reported that the antioxidant activity of prunes is very high in comparison with the antioxidant activities of other fruits and vegetables on the basis of the oxygen radical absorbance capacity (ORAC). Dried plum puree at concentrations of 3% or higher has been shown to be as effective as, synthetic antioxidants BHA and BHT, in retarding lipid oxidation in precooked pork patties (Nuñez de Gonzalez *et al.*, 2008).

The use of microwave to extract polyphenols from plants received a great deal of attention due to its advantages (reduction in extraction time and solvent volume) over traditional extraction methods. (Sparr Eskilsson & Björklund, 2000). Microwave-assisted extraction (MAE) is based upon the selective and rapid localised heating of moisture in the sample by microwaves. Due to the localised heating, pressure builds up within the cells of the sample, leading to a fast transfer of the compounds from the cells into the extracting solvent (Mandal & Mandal, 2010).

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The extraction conditions may not be generalised due to the diverse nature of natural antioxidants existing in different plant materials (Wettasinghe & Shahidi, 1999). For this, the optimisation of experimental conditions is very important. Response surface methodology (RSM) has been shown to be a powerful tool in optimising experimental conditions to maximise the response. RSM is a collection of statistical techniques for designing experiments, building models, evaluating the effects of factors and searching optimal conditions for desirable responses (Szydowska-Czerniak *et al*., 2011). It has been successfully used in optimising the extraction of phenolic compounds from fruits, vegetables and by-products (Wardhani *et al*., 2010; Pérez-Serradilla & Luque de Castro, 2011; Song *et al*., 2011).

In the transformation process of the plum to prune, the sorting step is very important to reject split, spoiled, badly or not calibrated dried fruit. The use of fruits rejected as a source of antioxidants would be very interesting.

To our knowledge, there have been no previous studies published on the use of MAE to extract phenolic antioxidants from prune. The aim of the present work was to investigate the effects of three different independent variables, namely microwave power, irradiation time and ethanol fraction on the yields of total and the major phenolic compound and on the antioxidant activities of prune extracts. The comparison between the conventional extraction and MAE was also investigated.

Materials and methods

Reagents

The compounds 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, epicatechin gallate and Folin–Ciocalteu phenol reagent were purchased from Sigma-Aldrich (Steinheim, Germany). All solvents used were of analytical grade and purchased from Prolabo (CE).

Plant material

The prune samples were collected from Djelfa factory in Algeria. Prunes used in this work were rejected after the transformation process of plum to prune, before the step of packaging. Prunes were rejected because they were too small (not calibrated). The average weight of rejected prunes was 5.54 ± 0.76 g per prune.

Moisture

Sample was maintained in a stove at 105 °C until it reached constant weight. The result is average of three samples. Moisture of prune sample was 30.66 ± 0.58%.

Instrumentation

The instrument used for the extraction of phenolic compounds from prune was the domestic microwave oven (MW8123ST; Samsung, Selangor, Malaysia) modified in laboratory. The apparatus was equipped with a digital control system for irradiation time and microwave power (the latter linearly adjustable from 100 to 900 W). Its schematic diagram is shown in Fig. 1. The identification and quantification of a major phenolic compound in prune extract was carried out using a Shimadzu HPLC system (Shimadzu, Kyoto, Japan). Supelco RP- C8 column (250 × 4.6 mm, 5 μm) (Sigma-Aldrich Corporation, Bellefonte, PA, USA) was used for separation. The column thermostat was set at 30 °C. The flow rate of mobile phase was 1 mL min^{-1}. The binary mobile phase consisted of solvent (A) [2.5% methanol and 97.5% $HClO_4$ (0.1%)] and solvent (B) (100% methanol). The solvent gradient as a function of time was as follows: 0–11 min, 5–15% B; 11–15 min, 15–23% B; 15–19 min, 23–35% B; 19–31 min, 35–45% B; 31–36 min, 45–80% B; 36–40 min, 80% B; 40–40.1, 80–5% B; 40.1–45%, 5%. The absorbance was measured by a diode-array detector (Shimadzu). Measurements taken at a wavelength of 280 nm were evaluated using a LabSolution data processing system (Shimadzu). The major phenolic compound was identified by the retention time and the UV–vis spectra of standards and quantified from peak area at 280 nm and by

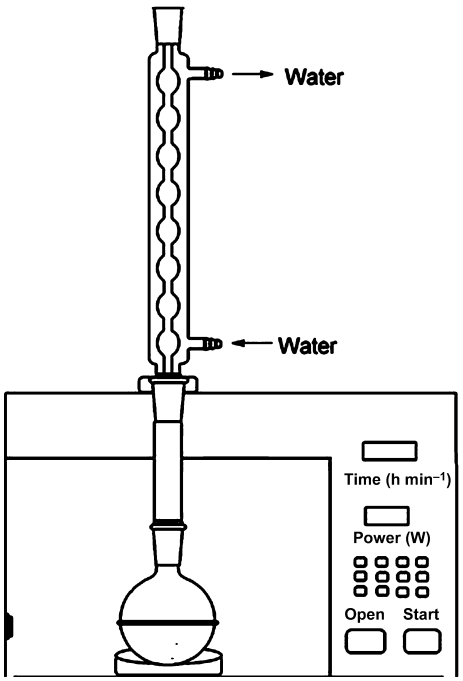

Figure 1 Schematic diagram of microwave equipment.

an external standard method using calibration curves. Concentration is expressed as a milligram per 100 gram of prune (mg/100 g prune).

Extraction procedures

Microwave-assisted extraction

The fruit samples were prepared for extraction by cutting into small pieces by a knife. Two grams of sample was placed in a round-bottom flask with 40 mL of solvent. The flask was placed in a microwave oven and connected to condenser. After irradiation, the flask was taken out and cooled in ice water bath (4 °C). The solution was filtered through Whatman filter paper No. 1 under vacuum. After concentrating using rota vapour at 40 °C, the extract was lyophilised. The extraction variables evaluated were microwave power (100, 300, 500 W), irradiation time (30, 90, 150 s) and ethanol fraction (0%, 40% and 80%).

Conventional extractions

Extraction by maceration, stirring and Soxhlet were done in order to compare with MAE. Two grams of sample was extracted with 40 mL of water for 5, 15, 30, 60 min by agitation and for 5, 30, 60, 120 min by maceration. In Soxhlet extraction, 2 g of sample was put in a cellulose thimble (30 × 77 mm, Whatman, Maidstone, UK) and placed in a Soxhlet device. One hundred millilitres of methanol 80% was used as the solvent, and the extraction was performed for 4 h. After each extraction, the mixture was filtered through Whatman filter paper No. 1. Each extraction was repeated three times.

Total phenols

Total phenols (TP) content was determined by the Folin–Ciocalteu method. One millilitre of Folin–Ciocalteu reagent (diluted ten times by water) was mixed with 100 μL of the prune extract. After 5 min, 1 mL of aqueous solution of sodium carbonate (6%) was added. The mixture was kept for 60 min at room temperature. Absorbance was measured at 755 nm. Ethanol solution of gallic acid was used as standard (Velioglu *et al*., 1998).

Antioxidant activity

The antioxidant activity of the extracts was estimated by the DPPH method, according to the procedure described by Yi *et al*. (2008). An aliquot of 1.5 mL of sample solution was mixed with 1.5 mL of ethanolic solution of DPPH (0.2 mM). The reaction mixture was incubated for 30 min in the dark at room temperature. The absorbance of the resulting solution was measured at 517 nm with a spectrophotometer. Ethanol instead of sample solution was used as a control. DPPH scavenging capacity of the tested samples was measured as a decrease in the absorbance and was calculated using the following equation:

$$\text{Scavenging activity}\,(\%) = \frac{A_c - A_s}{Ac} \times 100$$

where A_c and A_s are the absorbance at 517 nm of the control and sample, respectively.

Statistical analysis

Response surface methodology (RSM) was used to determine the optimal conditions for extraction. RSM was performed using the DESIGN EXPERT software (Version 8.0.1. Stat-Ease, Inc. Minneapolis, MN, USA) program. Central composite design (CCD) was used to investigate the effects of three independent variables (microwave power, irradiation time and ethanol fraction) at three levels on the dependent variables (TP, epicatechin gallate and antioxidant activity). CCD uses the method of least-squares regression to fit the data to a quadratic model. The quadratic model for each response was as follows:

$$Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_i^2 + \Sigma\Sigma\beta_{ij} X_i X_j$$

where Y is the predicted response, β_0 a constant, β_i the linear coefficient, β_{ii} the quadratic coefficient, β_{ij} the interaction coefficient of variables i and j, and X_i and X_j are independent variables. The software uses this quadratic model to build response surfaces. The adequacy of the model was determined by evaluating the lack of fit, coefficient of determination (R^2) and the Fisher's test value (F-value) obtained from the analysis of variance (ANOVA) that was generated by the software. Statistical significance of the model and model parameters were determined at the 5% probability level ($P = 0.05$). Three-dimensional response surface plots were generated by keeping one response variable at its optimal level and plotting this against two factors (independent variables). The codes used in the response surface analysis and the corresponding parameter values are given in Table 1.

Results and discussion

Fitting the model

There are many factors that affect extraction efficiency of MAE (Hayat *et al*., 2010). In this study, three

Table 1 The coded values and corresponding actual values of the optimisation parameters used in the response surface analysis used in the response surface analysis

Code	Microwave power (W)	Irradiation time (s)	Ethanol fraction (%)
−1	100	30	0
0	300	75	40
1	500	120	80

independent variables (microwave power, irradiation time and ethanol fraction) that affect MAE were optimised using CCD. The extraction efficiency of the microwave process was estimated by measuring TP, epicatechin gallate and the antioxidant activity of prune extract. We chose to measure epicatechin gallate as an indicator of extraction efficiency, because HPLC analysis demonstrated that it was the major phenolic compound in prune extract (Fig. 2). Following the experimental design by CCD, 20 runs of the extraction including six replicates of the centre point were conducted (Table 2). During the optimisation of the MAE process from 100 g of prune, we obtained between 48.92 and 596.42 mg GAE of TP from 4.19 to 119.42 mg of epicatechin gallate, and from 25.85% to 90.75% antioxidant activity. The correlation between TP and antioxidant activity and between epicatechin gallate and antioxidant activity was statistically significant ($P < 0.05$), with coefficient of correlation (r) values of 0.83 and 0.63, respectively.

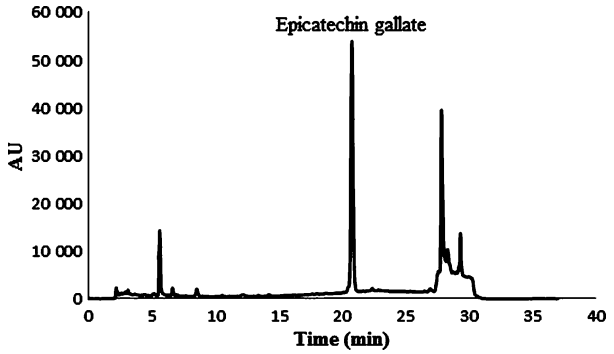

Figure 2 HPLC chromatogram of ethanolic prune extract with diode-array detection at λ = 280 nm.

The results of ANOVA for the quadratic model based on TP, epicatechin gallate and antioxidant activity are summarised in Table 3. Statistical analysis indicated that the proposed model was adequate, possessing no significant lack of fit ($P > 0.05$) and with satisfactory values of the R^2 for all responses. The R^2 values for TP, epicatechin gallate and antioxidant activity were 0.83, 0.84 and 0.81 respectively. The coefficient of determination (R^2) indicates that the model accurately represents the relationship between the parameters chosen (Wang *et al.*, 2008). The probability (P) values of all regression models were <0.05. A low probability value indicates a high significance of the model (Chan *et al.*, 2009). Coefficient of variation (CV) describes the extent to which the data were dispersed. In general, a small value of CV gives a better reproducibility, and a high CV indicates that variation in the mean value is high and does not satisfactorily develop an adequate response model (Liyana-Pathirana &

Table 2 Three-factor, three levels central composite design used for response surface methodology (RSM) and experimental data of the investigated responses of prune extracts

	Factor A microwave power (W)	Factor B irradiation time (s)	Factor C ethanol fraction (%)	Response 1 total phenols (mg GAE/100 g prune)	Response 2 epicatechin gallate (mg/100 g prune)	Response 3 DPPH scavenging activity (%)
1	300	75	40	356.42	44.18	65.28
2	100	120	80	131.42	75.12	65.85
3	100	75	40	76.42	35.01	30.00
4	500	75	40	398.92	54.16	90.75
5	300	75	40	288.92	92.21	86.23
6	300	75	40	241.42	82.46	90.00
7	300	75	80	206.42	80.33	65.09
8	300	75	0	508.92	49.28	89.62
9	300	75	40	376.42	89.96	92.45
10	500	120	0	596.42	59.93	90.75
11	100	120	0	118.92	19.98	49.06
12	500	120	80	243.92	119.42	73.40
13	500	30	0	116.42	35.40	34.15
14	300	30	40	71.42	41.78	34.53
15	100	30	0	58.92	4.09	25.85
16	500	30	80	133.92	85.83	38.49
17	100	30	80	48.92	4.13	28.49
18	300	120	40	293.92	79.05	77.55
19	300	75	40	183.92	57.30	62.83
20	300	75	40	21.92	66.99	80.38

Table 3 ANOVA for the effect of microwave power, irradiation time and ethanol fraction on total phenols, epicatechin gallate and antioxidant activity using a quadratic response surface model

	Total phenols		Epicatechin gallate		Antioxidant activity	
Source	***F*-value**	***P*-value**	***F*-value**	***P*-value**	***F*-value**	***P*-value**
Model	5.26	0.0080	5.83	0.0055	4.69	0.0121
A-Microwave power (W)	14.40	0.0035	16.73	0.0022	7.73	0.0194
B-Irradiation time (s)	11.80	0.0064	11.87	0.0063	17.87	0.0018
C-Ethanol fraction (%)	5.22	0.0455	13.74	0.0041	0.15	0.7029
AB	3.24	0.1021	0.37	0.5570	0.56	0.4707
AC	1.84	0.2045	1.34	0.2744	0.62	0.4500
BC	1.95	0.1925	1.84	0.2050	0.03	0.8586
A^2	1.08	0.3230	3.36	0.0969	2.29	0.1611
B^2	4.31	0.0645	0.07	0.7975	4.03	0.0726
C^2	1.50	0.2490	0.03	0.8673	0.17	0.6861
Lack of fit	1.61	0.3075	0.51	0.7615	1.65	0.2982
R^2	0.83		0.84		0.81	
CV	0.38		0.28		0.23	

CV, coefficient of variation

Shahidi, 2005). The CV for TP, epicatechin gallate and antioxidant activity was within the acceptable range.

The software generated the following regression equation, which demonstrates the empirical relationship between microwave power (A), irradiation time (B), ethanol fraction (C), TP (Y_1), epicatechin gallate (Y_2) and antioxidant activity (Y_3) in terms of coded units:

$$Y_1 = +283.71 + 105.50A + 95.50B - 63.50C + 55.94AB - 42.19AC - 43.44BC - 55.11A^2 - 110.11B^2 + 64.89C^2$$

$$Y_2 = +15494.88 + 4892.69A + 4120.88B + 4434.71C - 812.67AB + 1546.77AC + 1813.34BC - 4179.37A^2 - 601.08B^2 + 391.03C^2$$

$$Y_3 = +77.20 + 12.83A + 19.51B - 1.81C + 3.87AB - 4.06AC - 0.94BC - 13.32A^2 - 17.66B^2 + 3.66C^2$$

The significance of each coefficient was determined using the *F*-test and *P*-value and is shown in Table 3. The corresponding variables would be more significant if the absolute *F*-value becomes greater and the *P*-value becomes smaller (Wang *et al*., 2007). The results indicate that linear parameters studied influence significantly the responses ($P < 0.05$). Microwave power was the most significant factor affecting the extraction of TP and epicatechin gallate from prune, followed by irradiation time for extraction of TP and by ethanol concentration for extraction of epicatechin gallate. However, the most significant parameter affecting the antioxidant activity was irradiation time (Table 3).

Analysis of response surface

The relationship between independent and dependent variables is illustrated in a three-dimensional representation of the response surface (Fig. 3). One factor was fixed as the optimal value calculated from the CCD experiment, and the effect of the two other factors on the response was shown by three-dimensional response surface plot.

The effects of microwave power (A) and irradiation time (B) on the TP of the prune extracts are reflected in Fig. 3. As A and B increase, the TP sharply increased, achieving saturated value when the extraction was performed for 115 s at 500 W. The experimental results demonstrate that an increase in the microwave power from 100 to 500 W over a period of 115 s improves TP yield up to 66.88%.

The extraction yield of epicatechin gallate was increased significantly with the enhancement of the microwave power up to 430 W and then stabilised from 430 to 500 W (Fig. 3). Therefore, the increased irradiation time within the ranges tested in this study led to an increase in the yield of epicatechin gallate. An increase in the microwave power from 100 to 430 W over a period of 120 s improves epicatechin gallate yield by 42%.

This result is in agreement with the study done by Ballard *et al*. (2010) who have found that an increase in the microwave power from 95 W to 855 W and an irradiation time of 30 s improves TP yield by 53.9%. The accelerated extraction of TP and epicatechin gallate by increasing microwave power can be correlated with the direct effects of microwave energy on phytomolecules by ionic conduction and dipole rotation, which produce power dissipated in a volumetric fashion inside the solvent and plant material that then generates molecular movement and heating. It is known that the temperature of the extraction medium increases with increased microwave power. These increased temperatures result in improved extraction efficiencies, as desorption of analytes from active sites in the matrix will increase. Additionally, solvents have higher capacity to solubilise analytes at higher temperatures, while surface tension and solvent viscosity decrease with temperature, which improves sample wetting and matrix penetration (Sparr Eskilsson & Björklund, 2000).

In this work, different mixtures of ethanol–water were tested. Ethanol was used as a solvent because it

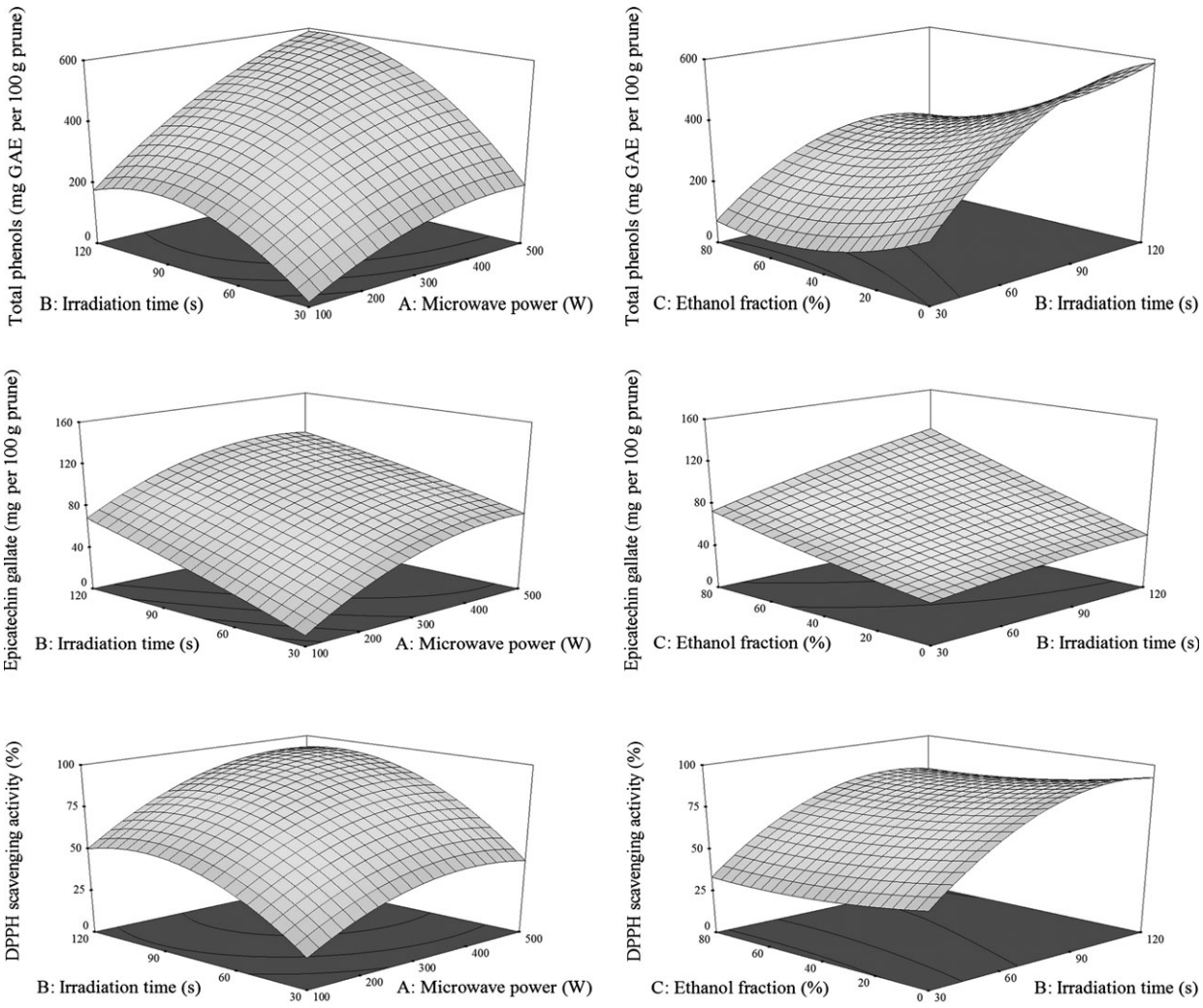

Figure 3 Response surface of the independent variables on total phenols, epicatechin gallate and antioxidant activity of prune extracts.

has been widely used to extract phenolic compounds from botanical materials (Pompeu *et al*., 2009; Ballard *et al*., 2010). In addition, it is less toxic and can be easily recovered (Wang *et al*., 2008). The independent variable solvent had a significant effect ($P < 0.05$) on the TP and epicatechin gallate recovery from prune. However, it did not have a significant effect on the antioxidant activity (Table 3). When ethanol concentration was increased from 0% to 80% (v/v), and optimal conditions of microwave power and irradiation time were applied, the yield of epicatechin gallate increased from 57.33 to 121.85 mg/100 g of prune. On the other hand, TP decreased from 590.77 to 304.33 mg GAE/100 g of prune. The correlation between TP and epicatechin gallate was very low ($r = 0.36$). This result indicates that water is the best solvent for TP extraction. However, with specific phenolic compounds such as epicatechin gallate, ethanol 80% gives the best yield. Epicatechin gallate belongs to the flavan-3-ols family. Unlike most other flavonoids, the flavans are present as free aglycones or as polymers of aglycones (Vermerris & Nicholson, 2006), which may explain their reduced polarity. The mechanism of extraction appears different when the ethanol concentration changes. When water is used as a solvent, the tissues swell and cells explode under the effect of microwaves. Wang *et al*. (2008) reported that water molecules have a high dipole moment and absorb microwave energy strongly, leading to efficient heating of the sample. The selective interaction between microwave and the internal free molecules of water leads to a rapid increase in temperature. Such a system undergoes a dramatic expansion, with subsequent destruction of cell walls. However, there was no

rupture of prune cell walls after extraction with solvent containing high concentrations of ethanol. The prune tissues become rigid and consequently the phenolic compounds diffuse. Indeed, we observed an increase in the hardening of the sample tissue when ethanol concentration increases. This hardening is enhanced by microwaves and prolongation of the extraction time. When the extraction conditions were ethanol 80%, 500 W of microwave power and 120 s of extraction time, the prune sample looks like cork at the end of the extraction. Ethanol is known to induce tissue dehydration. However, we observed in this study that the use of microwaves enhances this deshydratation.

Optimal extraction conditions

Using a quadratic model to describe the experimental data, we optimised three experimental variables for maximal extraction of phenolic compounds, epicatechin gallate and antioxidant activity from prune extract. The results of the optimisation are summarised in Table 4. When comparing the optimal conditions based on TP to those obtained for epicatechin gallate, it was found that the optimums of microwave power and irradiation time were closely related. However, there was a difference in the concentration of ethanol required for optimal extraction of TP and epicatechin gallate. The optimal ethanol fractions were 0 and 80% for TP and epicatechin gallate, respectively.

Validation of the models

The optimised conditions obtained by RSM were used to validate the predictive model of extraction for TP, epicatechin gallate and antioxidant activity from prune. Table 4 shows that experimental values are reasonably close to the predicted values confirming the validity and the adequacy of the predicted models. The experimental data were within 95% confidence interval of predicted values.

Comparison of TP and antioxidant activity using MAE and conventional methods

The efficiency of TP extraction and antioxidant activity using MAE was compared with classical methods such as solid/liquid extraction (maceration, stirring) and Soxhlet. The conditions of different techniques and their results are summarised in Table 5. Preliminary studies (not shown in this work) were done in all extraction techniques to determine the best solvent for extraction. Table 5 displays this in terms of TP yields, with the best results being obtained by MAE, which gave a significantly higher value ($P < 0.05$). Moreover, the use of microwaves in the extraction of phenolic compounds from prune increased significantly the antioxidant activity ($P < 0.05$) and dramatically reduced extraction time to only 2 min. The extraction time of MAE was far less than that of conventional methods. The MAE method is able to extract nearly 23% more TP from prune in 1/60 of the time required for solid/liquid stirring extraction (Table 5). Many studies reported in the literature have shown that applying MAE to many materials can significantly reduce extraction time compared with conventional extraction methods (Proestos & Komaitis, 2008; Spigno & De Faveri, 2009).

Conclusion

In the present study, RSM was successfully used to study the influence of the microwave power, extraction time and solvent polarity on TP, a major phenolic compound and antioxidant capacity of extracts obtained from prune by MAE. We have found by RP-HPLC that epicatechin gallate was a major phenolic compound in prune extract. The parameters tested influenced significantly the responses. The second-order polynomial model can be applied to optimise the parameters of prune extraction to obtain an extract with high TP and antioxidant capacity. Maximal values of TP and epicatechin gallate from prune were 598.89 mg GAE/100 g prune and 118.42 mg/100 g prune, respectively. The highest TP was obtained using water as an extraction solvent at 500 W, during 115 s. However, the optimal conditions for epicatechin gallate extraction were ethanol 80%, 435 W and 120 s. The objective of extracting phenolic compounds from their plant sources is to release these compounds from the vacuolar structures where they are found, either by rupturing plant tissue or by a diffusion process (Aspé & Fernández, 2011). In the current study, we observed

Table 4 Optimum conditions for the microwave-assisted extraction (MAE) of total phenols (mg EAG/100 g prune), epicatechin gallate (mg/100 g prune) and antioxidant activity (%) from prune

	Microwave power (W)	Irradiation time (s)	Ethanol fraction (%)	Prediction	Experimental
Total phenols	500	115	0	590.89	598.89 ± 87.91
Epicatechin gallate	435	120	80	121.85	118.42 ± 16.72
Antioxidant activity	450	104	0	95	90.55 ± 14.65

All values represent means ± SD ($n = 3$).

Table 5 Comparison of total phenols using microwave-assisted extraction (MEA) and conventional extraction methods

Extraction methods	Extraction time	Extraction solvent	Total phenols (mg GAE/100 g prune)	Antioxidant activity (%)
MAE	2 min	Water	579.12 ± 34.50^{a}	94.75 ± 10.45^{a}
Maceration	5 min	Water	14.36 ± 2.26^{j}	13.50 ± 2.89
	30 min	Water	46.93 ± 9.90^{h}	18.65 ± 2.24^{f}
	60 min	Water	93.89 ± 4.87^{g}	25.65 ± 2.78^{e}
	150 min	Water	221.15 ± 25.18^{d}	54.35 ± 3.98^{c}
	24 h	Water	496.49 ± 44.96^{b}	86.05 ± 7.98^{a}
Stirring	5 min	Water	28.82 ± 7.05^{i}	14.35 ± 2.88
	15 min	Water	93.65 ± 18.19^{g}	27.78 ± 3.87^{e}
	30 min	Water	141.40 ± 10.95^{e}	33.87 ± 4.08^{d}
	60 min	Water	228.22 ± 33.88^{d}	58.44 ± 6.57^{c}
	120 min	Water	443.73 ± 10.76^{c}	79.98 ± 9.65^{b}
Soxhlet	4 h	Ethanol 80%	55.55 ± 7.83^{h}	22.89 ± 5.54^{ef}
	24 h	Ethanol 80%	113.83 ± 12.89^{f}	35.86 ± 1.87^{d}

All values represent means ± SD ($n = 3$). Different letters following the values in the columns for each extraction method indicate significant differences according to LSD ($P < 0.05$).

that MAE with water as an extraction solvent released the phenolic compounds by rupturing plant tissue. However, when solvent with high ethanol concentration was used, the phenol compounds diffused. Finally, this study confirms that MAE is more efficient than conventional extraction method to obtain TP from prune.

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