

MICROWAVE-ASSISTED EXTRACTION OF PHENOLIC ANTIOXIDANTS FROM SEMIARID PLANTS

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Received 2013-11-18; Revised 2013-12-29; Accepted 2014-03-22

ABSTRACT

Microwave-Assisted Extraction (MAE) kinetic in three temperatures was investigated for extraction of antioxidant phenolic compounds from *Jatropha dioica*, *Flourensia cernua*, *Eucalyptus camaldulensis* and *Turnera diffusa* which are plants of semiarid regions of Mexico. The two-site kinetic model and saturation simple equation were used to describe the experimental data. The fit of the data demonstrated that saturation simple equation was better and could explain the data with a slight loss of goodness of fit. The results showed that MAE optimum conditions were extraction temperature of 60°C and extraction time in the range from 1 to 6 min. The MAE was more efficient than the conventional Heat Reflux Extraction (HRE). Maximal antioxidant potential was observed in *E. camaldulensis* extracts. Interestingly differences in phenolic composition and antioxidant activity were observed in the extracts obtained by MAE and HRE for all plants used. Quercetin and catechin were found to be the predominant phenolic compounds. The MAE procedure resulted in suitable alternative to obtain bioactive phenolic compounds from semiarid plants due to this technology is higher efficient than conventional methods increasing yield in shorter times.

Keywords: Plant-Extracts, Polyphenols, Antioxidants, Green Chemistry, Modeling Kinetics

1. INTRODUCTION

The topic about research on antioxidants has become a topic of increasing interest and it has increased considerably during the past 10 years (Prior *et al.*, 2005) and is tendency continuously increasing up to the present day. Antioxidants naturals are gaining importance due to their benefits for human health where several studies have established an inverse correlation between the intake fruits and vegetables antioxidants and the occurrence of diseases such as inflammation, cardiovascular disease and cancer and to prevent the deterioration of fats and other constituents of foods (Moure *et al.*, 2001; Huang *et al.*, 2005; Routray and Orsat, 2011). In addition to antioxidant activity, several studies demonstrated the antimicrobial activity making them a good alternative to antibiotics and chemical preservatives (Oliveira *et al.*, 2008).

Recently, synthetic antioxidants have shown serious side effects. Therefore, exists an interest towards research on vegetable sources for identifying new antioxidants that could be used in the substitution of natural substances antioxidants instead of the synthetic ones (Chan *et al.*, 2011; Périno *et al.*, 2011; Li *et al.*, 2012).

Vegetable materials contain many compounds with antioxidant activities which are obtained in form of plants extracts. However, polyphenols are the major plant compounds with antioxidant activity and other several activities associated with healthy properties ascribed to their antioxidant activity and free radical scavenging abilities (Kähkönen *et al.*, 2001; Huang *et al.*, 2005; Bakkali *et al.*, 2008).

Extraction represents the primary step in getting crude extract from plants. Plant extract have been obtained commonly by conventional solvent extraction.

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Conventional extraction techniques include soaking, maceration, water percolation, Soxhlet extraction. Despite of this, conventional extraction methods have been associated with high solvent requirements of toxic organic solvents, usually required long extraction time which increased risk of degradation of thermo-labile constituents and resulting in low yields of extraction (Ballard *et al.*, 2010; Chan *et al.*, 2011; Wu *et al.*, 2012).

Moreover, with the increasing energy prices, the drive to reduce CO₂ emissions and eliminate the hazardous substances used in or generated by a method into the context of green chemistry, food industries are challenged to find or development new technologies in order to reduce energy consumption, product/process safety and control and for cost reduction and increased quality as well as functionality (Keith *et al.*, 2007; Yan *et al.*, 2010; Périno *et al.*, 2011).

Over the past decade, various novel extraction techniques have been introduced and investigated, most of which were claimed to be better in terms of sustainability due to its efficiency and decrease extraction time and solvent consumption.

Interest in Microwave-Assisted Extraction (MAE) has increased significantly over the past 5-10 years in particular medicinal plant research, as a result of its inherent advantages as special heating mechanism, moderate capital cost and its good performance under atmospheric conditions (Ballard *et al.*, 2010; Chan *et al.*, 2011). In addition, MAE technique possesses many advantages compared to other methods for the extraction of compounds such as bioactive compounds (Sanchez-Aldana *et al.*, 2013) saving in processing time and solvent, higher extraction rate, better products with lower cost, reduced energy consumption (up to 85-fold savings) and waste generation (Hao *et al.*, 2002; Yan *et al.*, 2010; Yemis and Mazza, 2012). Therefore, microwave-assisted extraction is an interesting, potential and powerful alternative to conventional liquid solvent extraction methods, especially in the case of plant material (Hao *et al.*, 2002).

Evaluating extractions variables, there are some tools based on experimental studies called mathematical models. Mathematical models provide information that know-how helps the designers and researchers find the best design parameters and the most effective process conditions to optimize purposes and are gaining more attention in food systems. It is important to develop models for the extraction process where different extraction operating parameters are optimized for process economics (Ho *et al.*, 2008). One of them is kinetic model which offers excellent basis for curve

fitting as a function of process conditions of both the extraction medium and the extracting plant material is a useful tool for an objective, fast and economic assessment of any processes (Karacabey *et al.*, 2011).

In Mexico have been reported several plant species with several beneficial properties mainly due to have developed ability to grow under extreme climatic conditions. Its development a pool of chemical compounds produced as defense mechanism environmental. Actually, extracts of this kind of plants have been used effectively in test as inhibitors pathogenic bacterial and fungal (Castillo *et al.*, 2008; Jasso de Rodriguez *et al.*, 2011) and antioxidants properties have been explored poorly. Besides, there is a lack of knowledge about phytochemicals compounds presents in these plants. Examples of these plants are *J. dioica* (Dragon's blood), *F. cernua* (Tar bush), *T. diffusa* (damiana) and *E. camaldulensis* (Eucalyptus) which are plants of semiarid region of Mexico.

Recently, our research has focused on developing novel processes that will be both economically and substantially acceptable such as MAE in the isolation of the biological active compounds from vegetal materials. To the best of our knowledge, there is no report published on the use of MAE to extract phenolic antioxidants from plants used. Thus, the objectives of the current study were to investigate the effects of MAE on the extraction efficiency and recovery of polyphenolic antioxidant compounds from *J. dioica*, *F. cernua*, *T. diffusa* and *E. camaldulensis* plants and fit the experimental data using empirical models in order to explain and provide useful information for future optimizations. A comparison was also drawn with results obtained using conventional heat reflux extraction.

2. MATERIALS AND METHODS

2.1. Chemicals and Standards

Pyrogallol (PG), Gallic Acid (GA), Resorcinol (RS), Chlorogenic Acid (CHA), Methyl Gallate (MG), Coumaric Acid (CUA), Catechin (CAT), 2-Hydroxycinnamic Acid (HA), Ellagic Acid (EA), Quercetin (QE), Cinnamic Acid (CA), 2,20-diphenyl-1-picrylhydrazil (DPPH) free radical, linoleic acid, Folin-Ciocalteu (FC) reagent were purchased from Sigma-Aldrich. HPLC-grade Acetonitrile (ACN), acetic acid, methanol and ethanol were purchased from Labscan.

2.2. Sample Materials

The plants used in this study were *J. dioica* (stem and root), *F. cernua* (stem and leaves), *T. diffusa* (stem and

leaves) and *E. camaldulensis* (leaves) collected near Saltillo City, Coahuila, Mexico from January to June 2011. The samples were dried for 48 h at 60°C in an oven (LABNET International, Inc.) and then they were ground and pass through a 0.6-0.8 mm screen. The fine and dried powder was stored in sealed hermetically bags and under darkness until use in the MAE.

2.3. MAE Procedure

Phenolic compounds were extracted from plants using pressurized MAE system (Ethos Synth, Milestone, Bregamo, Italia). Aqueous ethanol was used as safe and efficient solvent for the extraction of phenolic compounds (Wu *et al.*, 2012) and ethanol is used in the food industry (Li *et al.*, 2012). In the study MAE procedure was carried out as following: 4 g of material vegetal dried powder was suspended in 120 mL of ethanol (50% for *J. dioica* and 35% for *F. cernua*, *T. diffusa* and *E. camaldulensis* according to previous studies) in a 240 mL Teflon extraction vessel. The vessel were placed at the center of the microwave apparatus and heated to desired temperature in 1 min (control time) and after the kinetics was realized at 3, 6 and 9 min. Microprocessor EasyWAVE 4 software interface allows direct continuous monitoring and control of parameter evaluated. The temperature in the vessel was directly measured using a thermocouple type thermometer. The mixture was stirred with a magnetic stirrer bar for homogeneous heating. After microwave heating, the mixture in the extraction vessel was allowed to cool down (1 min at 20-24°C) using Criolab apparatus and immediately filtered using gauze. After, the extracts were centrifuged at 3000 rpm during 10 min, filtered using filter paper fine pore and dried in an oven at 60°C for 24 h. Samples were extracted in triplicate for each plant. Three kinetics extractions were followed for the three irradiation temperature used (50, 60 and 70°C).

2.4. Heat-Reflux Extraction (HRE)

With the purpose of compare the extraction efficiency of MAE, heat-reflux extraction was performed and considered as conventional method. Previous studies show the suitable extraction conditions of these materials under reflux-heat. Hence, in the present study, 20 mL ethanol-aqueous (35%, v/v) was added to 5 g dried powder of the materials vegetal (*F. cernua*, *T. diffusa* and *E. camaldulensis*) and extracted under refluxing at 60°C for 2 h of extraction. For *J. dioica*, a solid-to-solvent ratio of 1:15 (g/mL) and ethanol-aqueous (70%, v/v) were used. The extracts were centrifuged, filtered and dried in an oven at 60°C for 24 h. The extracts were

stored in eppendorf tubes in refrigeration until use. The extractions were realized in triplicate. For all the determinations, the extracts were re-suspended in water (1 mg of dried extract/mL).

2.5. Total Phenolic Content (TPC)

TPC in the obtained extracts were estimated by colorimetric assay according at methodology reported by Makkar (1999) with some modifications. First, 800 µL of sample was mixed with 800 µL of Folin and Ciocalteu's reagent. After 5 min, 800 µL of Na₂CO₃ (0.01M) was added and kept for 5 min. Finally the solution was diluted with 5 mL of distilled water and the absorbance was read at 790 nm (Varian 50 Bio Spectrophotometer). Gallic acid was used for constructing the standard curve (0-300 mgL⁻¹). The results are expressed as mg gallic acid equivalents/g of material vegetal.

2.6. Antioxidant Activity

Antioxidant activity was measured only for the extracts that presented high amount of polyphenolic compounds in each vegetal material by MAE and for all extracts obtained by conventional extractions. Antioxidant activity was measured for two methods, DPPH free radical-scavenging activity and inhibition of the oxidation of linoleic acid.

2.6.1. DPPH (1,1-Diphenyl-2-Picryl-Hydrazil) Radical Scavenging Activity

The antioxidant capacity of the obtained extracts was evaluated as the DPPH free radical-scavenging activity according to a method reported by (Bondet *et al.*, 1997; Molyneux, 2004) with some modifications: 193 µL of the methanolic solution of free radical DPPH (60 µM) was added to 7 µL of the sample extract and let stand for 30 min at room temperature before the absorbance was measured at 517 nm using a spectrophotometer microplate reader (Epoch, BioTek, Instruments, Inc.) controlled with the Gen5 Data Analysis software interface. The free radical-scavenging activity of the extracts was expressed as the percentage of DPPH reduced and was calculated according to the equation:

$$\text{Inhibition of DPPH (\%)} = \frac{(A_c - A_s)}{A_c} * 100$$

where, Ac is the absorbance of the control (7 µL of water instead of sample) and As is the absorbance of the sample.

2.6.2. Lipid Oxidation Inhibition Assay

The antioxidant activity was estimated according to Martínez *et al.* (2011). The method is based on the

spectrophotometric assay of primary products of linoleic acid peroxidation (dienes) which may be inhibited or stimulated by compounds presents in the extracts (Starzynska *et al.*, 2008). Firsts, the linoleic acid solution was prepared by diluting 0.56 g of linoleic acid and 1.5 g of Tween 20 in 8 mL of 96% ethanol. Each plant extract (50 μ L) was mixed with linoleic acid solution (100 μ L) and 1.5 mL of 0.02 M acetate buffer (pH 4.0). Controls contained 50 μ L of distilled water. The samples were homogenized in vortex and incubated at 37°C for 1 min. After 1 min, 750 μ L of 50 M FeCl₂ solution (0.0994 g FeCl₂ and 0.168 g EDTA diluted to 1 L with distilled water) were added to induce oxidation of linoleic acid. Two aliquots (250 μ L) were taken, at 1 and 24 h. Each aliquot was processing in the moment as following: Aliquot were added to 1 mL of 0.1 M NaOH in 10% ethanol to stop the oxidation process and diluted with 2.5 mL of 10% ethanol. The absorbance was measured at 232 nm against 10% ethanol blank. Percent inhibition of linoleic acid oxidation was calculated with the following equation:

$$\text{Lipid oxidation inhibition (\%)} = \frac{(A - B)}{A} * 100$$

where, A is the difference between the absorbance of the control sample (distilled water) after 24 h and 1 h of incubation and B is the difference between the absorbance of each extract sample after 24 h and 1 h of incubation.

2.7. HPLC Analysis

The extracts obtained under same conditions of the extracts used in the antioxidant activity were injected in the HPLC system for the characterization of the phenolic compounds presents. Qualitative analysis was carried out in a Varian Pro-Star 330 system equipped with a DAD detector (Varian Inc., USA). The monitoring wavelength was 280 nm. Fractionation of the samples was performed on an Pursuit XR_s C18 column (5 μ m, 150 \times 4.6 mm, Varian Inc.) at 30°C with flow rate of 1 mL/min. Acetic acid (3%) and acetonitrile were used as mobile phase, A and B respectively. The injection volume was 10 μ L (sample previously filtered through a 0.22 μ m nylon membrane). Gradient used was: Initial, 0% B; 0-1 min, 3% B; 1-3 min, 10% B; 3-8 min, 20% B; 8-13 min, 30% B; 13-18 min, 50% B. The column was then washed and reconditioned. All the solutions standard of the different polyphenolic compounds used were injected in the same manner (Ruiz-Martínez *et al.*, 2011).

2.8. Data Analysis

Nonlinear regression analysis and the fit MAE experimental data were performed using Microsoft Excel

software. MAE experimental data were fitted by minimization of the sum of squares error to the proposed models. The goodness of fits was assessed using determination coefficient (R²).

2.8.1. Mathematical Models

MAE overall experimental data were fitted with three empirical mathematical models: Two-site kinetic model (Karacabey *et al.*, 2011) and saturation simple equation in order to determine the best model describing the behavior of microwave-assisted extraction of TPC in semiarid region plants studied.

In two-site kinetic model the equation followed was:

$$C_t = C_{eq} [1 - [F \cdot e^{-k_1 \cdot t}] - [(1 - F) \cdot e^{-k_2 \cdot t}]]$$

Where:

- C_t = The solute a concentration in liquid phase any time t (mg solute A/g vegetal material)
- C_{eq} = The equilibrium solute a concentration (mg solute A/g vegetal material)
- F = The fraction of the solute released quickly
- (1-F) = The fraction of the solute released slowly
- k₁ = The first-order rate constant describing the fast released fraction (min⁻¹) and
- k₂ = The first-order rate constant describing the slow released fraction (min⁻¹)

Saturation simple equation was considered to describe the extraction kinetics of a target compound into a liquid solution. Then, the equation was used as:

$$C_t = (C_{eq} * t) / (k + t)$$

Where:

- C_t = The solute a concentration in liquid phase any time t (mg solute A/g vegetal material)
- C_{eq} = The equilibrium solute a concentration (mg solute A/g vegetal material)
- k = The constant that describing the released fraction (min⁻¹)

3. RESULTS

3.1. MAE Procedure Behavior

In the present study, different temperatures were tested for the TPC extraction from several semiarid region plants of Mexico using MAE procedure. Two empirical models (two-site kinetic model and saturation simple equation) were assessed to describe the extraction

progress of target compounds during the kinetics performed. R² values of each model were considered for the assessment of model adequacies (**Table 1 and 2**). The results implied that both models used could describe the extraction of total phenolic compounds present in the plants used with the change of temperature in the studied ranges. However, checking model adequacies, *J. dioica* kinetics displayed lower goodness of fit in the two kinetics used with respect to the other plants kinetics. Generally, saturation simple equation was concluded to be the best model describing the extraction of TPC using MAE procedure in the plants used showing high performance to explain the experimental data obtained. Depending on the purpose, both equations could be used to achieve good estimation of extraction system of TPC under the studied range of temperature at least for *F. cernua*, *T. diffusa* and *E. camaldulensis*. Experimental data and theoretical curves fitted of MAE procedure are presented in the **Fig. 1 and 2**.

3.2. Model Fitting for MAE Procedure

Two-site kinetic model is knowledge for present goodness of fit curve extractions, it could be attributed to the model has two term groups separately describing the fast and the slow parts of extraction. **Figure 1A-D** shows the fitted curves for extraction yields of TPC at three different temperatures using the two-site kinetic model. The effect of temperature on the extraction of TPC indicated the maximum recoveries was reached at around 60-70°C for the four plants studied. The plots show that the values calculated from the model adequately match the experimental values ($R^2 > 0.98$). Models parameters used to fit the experimental data with this model are presented in **Table 1**. The equilibrium concentrations calculated for TPC increased with increasing temperature, however no significant difference are presented between 60 and 70°C in *J. dioica*, *F. cernua* and *T. diffusa* extracts. In the other hand, asymptotic values were observed for *E. camaldulensis* extract with change of temperature. The equilibrium concentration of *E. camaldulensis* was found to be increased from 50 to 60°C temperatures and slightly decreased with further increase up to 70°C (**Table 1**). The F values no displayed change for *J. dioica*. In contrast, the values F for *F. cernua* show a slight tendency of increase with increase in extraction temperature and besides these values were very close to 1, which indicated that the higher portion of target solute was removed from material vegetal during the fast rate of process. For the *T. diffusa* and *E. camaldulensis* extracts the influence of increase in extraction temperature on the F values were in asymptotic values, implying that for *T. diffusa* using 60°C temperature

allowed the fraction of extractable solute content removed was faster than the other temperatures, in contrast where 60°C was used in *E. camaldulensis* the fraction of extractable solute decreased.

The effects of extraction temperature on the fast and slow extraction rates constants (k_1 and k_2 , respectively) were already investigated. The fast extraction rate constant (k_1) in *J. dioica* indicated a slight decrease when 70°C were used. In contrast, for *F. cernua* increase in the temperature caused increase in the fast extraction rate constant implying that the extraction at elevated temperatures (60-70°C) provides the fastest extraction of the quickly release solutes in the very early part of the extraction. Similar behavior was observed in the fast extraction rate constant for *T. diffusa* and *E. camaldulensis* where increase in the temperature from 50 to 60°C caused slightly decreased in the k_1 and then increase with increasing temperature in from 60 to 70°C indicated that 60°C is a point where extraction velocity of the faster fraction of extractable solute was slightly slower than 50 and 70°C.

Slow extraction rate constants (k_2) for *J. dioica*, *F. cernua* and *T. diffusa* no significant change were observed with increased in the temperature. Nevertheless, in *E. camaldulensis* shifting the extraction temperature from 50 to 60°C caused an increase in the slow rate constant and negligible shifting was observed from 60 to 70°C.

Saturation simple equation described best the experimental data for MAE procedure in the TPC extraction for the present study. Model parameters, equilibrium concentration (C_{eq}) and extraction rate constant (k) were determined by fitting the experimental data and are presented in **Table 2**. High goodness of fit for Model equation indicated that extraction of TPC from plants studied could be adequately described by this model. The experimental and theoretical extraction curves are shown in the **Fig. 2A-D**. The advantage of Monod's equation against Two-site kinetic model is that first one is composed only for two terms whereas second one include four terms making the model more complicated (Karacabey *et al.*, 2011).

In the same way as two-site kinetic model, the equilibrium concentrations (C_{eq}) of TPC indicated the increase in those values with increasing temperature from 60 to 70°C in *J. dioica*, *F. cernua* and *T. diffusa* extracts and asymptotic values were observed for *E. camaldulensis* extract where it was found to be higher value at 60°C temperature (**Table 2**). According to the extraction rate constants, extraction of TPC was accelerated with the increase in temperature from 50 to 70°C when *J. dioica* were used.

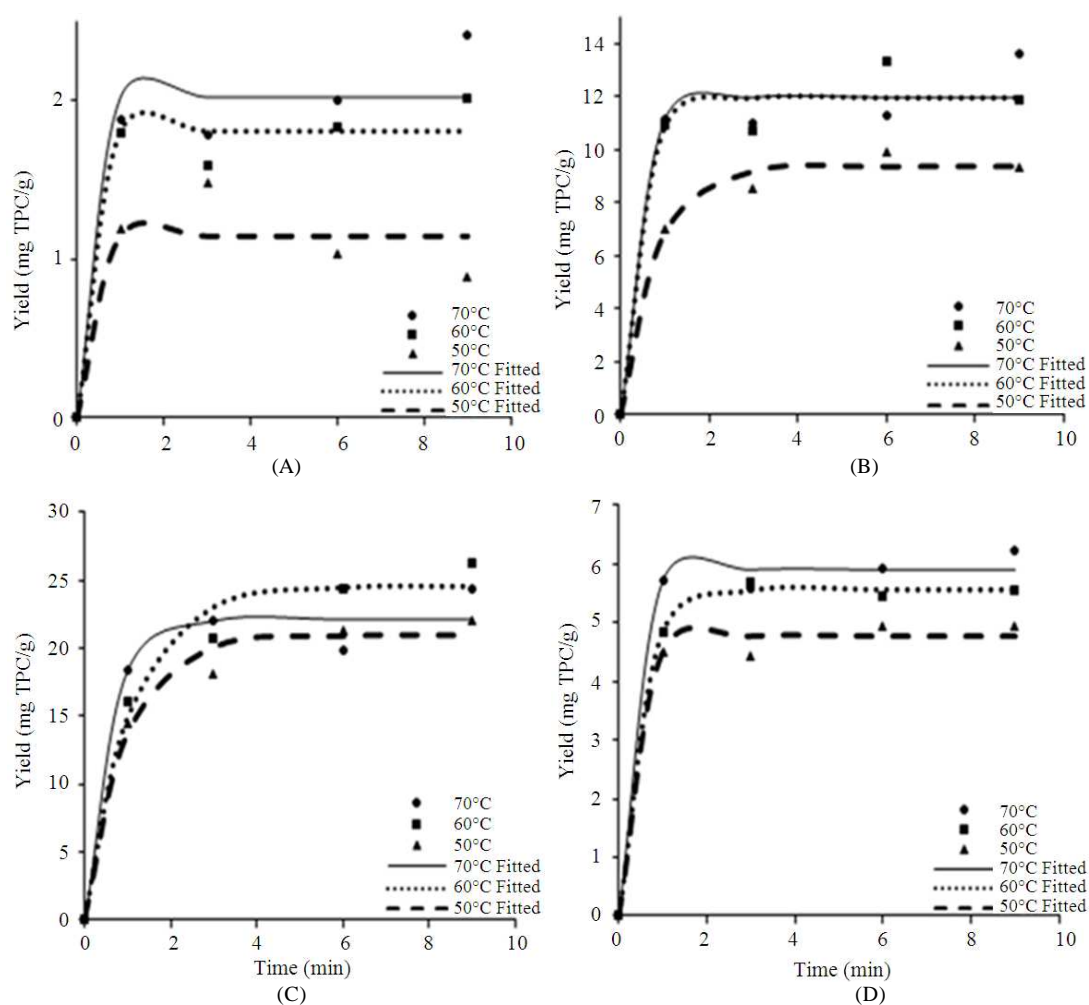
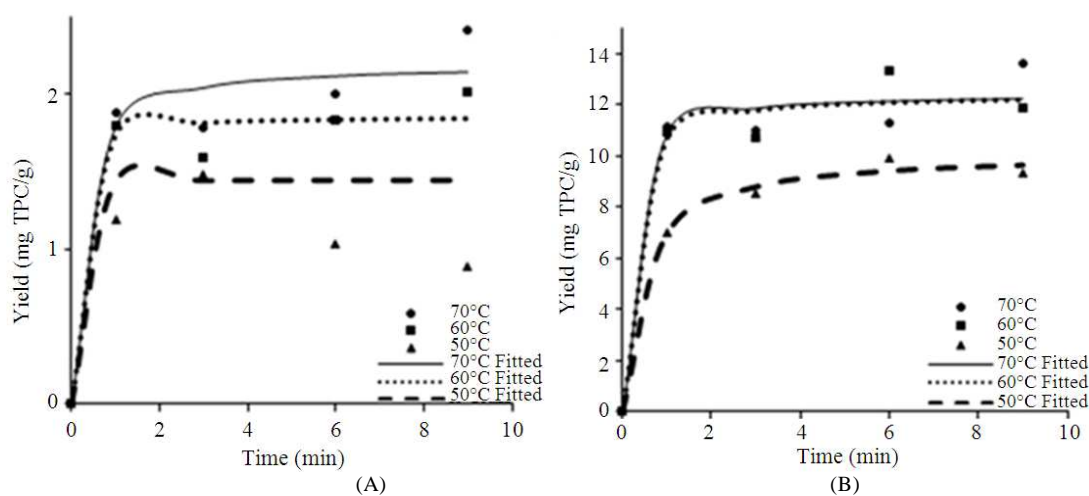


Fig. 1. Experimental fitting of two-site kinetic model to TPC recovery data obtained at various temperatures in *J. dioica* (A), *F. cernua* (B), *E. camaldulensis* (C) and *T. diffusa* (D)



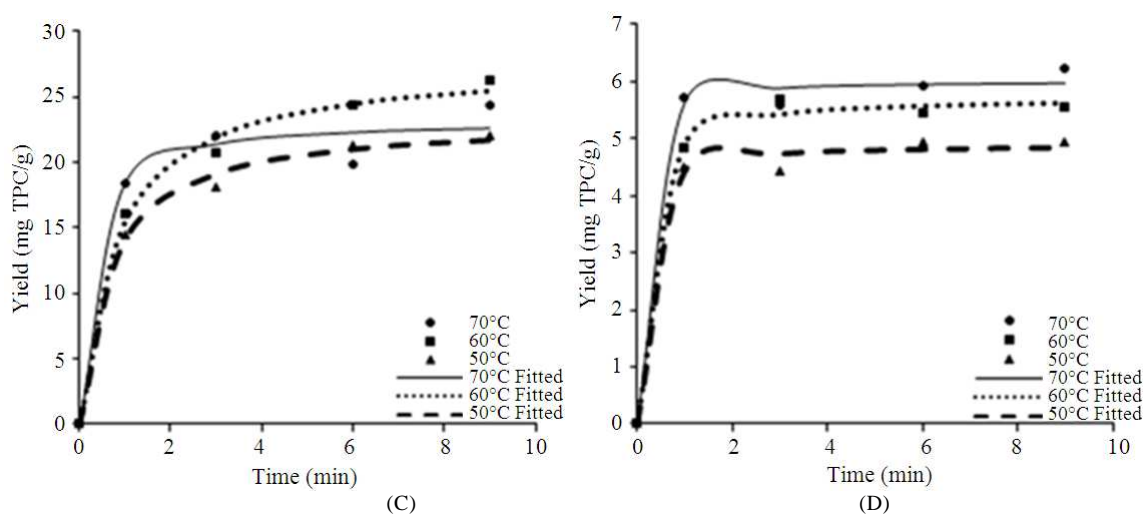


Fig. 2. Experimental fitting of saturation simple equation to TPC recovery data obtained at various temperatures in *J. dioica* (A), *F. cernua* (B), *E. camaldulensis* (C) and *T. diffusa* (D)

Table 1. Predicted equilibrium concentrations and kinetic parameters obtained in the two-site kinetic model

Vegetal material	Two-site kinetic model						
	Temperature (°C)	MAE	C_{eq} (mg/g)	F	k_1 (min ⁻¹)	k_2 (min ⁻¹)	R^2
<i>J. dioica</i>	50		1.1475	1.0000	14.8042	19.6244	0.9195
	60		1.8100	1.0000	14.8042	19.6244	0.9827
	70		2.0249	1.0000	14.6585	19.6244	0.9663
<i>F. cernua</i>	50		9.3810	0.9969	1.3176	3.2959	0.9944
	60		11.9661	0.9998	2.3980	3.2993	0.9848
	70		11.9924	0.9999	2.6630	3.2993	0.9824
<i>E. camaldulensis</i>	50		20.9424	0.9918	1.0629	3.2948	0.9912
	60		24.5491	0.9871	0.9261	7.2589	0.9889
	70		22.1160	1.0000	1.7740	7.2592	0.9859
<i>T. diffusa</i>	50		4.7680	0.9999	2.8665	1.8304	0.9952
	60		5.5587	1.0000	2.0413	1.8285	0.9993
	70		5.9004	0.9999	3.4282	1.8304	0.9962

Table 2. Predicted equilibrium concentrations and kinetic parameters obtained in the saturation simple equation

Vegetal material	Saturation simple equation				
	Temperature (°C)	MAE	C_{eq} (mg/g)	k (min ⁻¹)	R^2
<i>J. dioica</i>	50		1.4500	0.0100	0.9184
	60		1.8608	0.0712	0.9840
	70		2.2020	0.2321	0.9770
<i>F. cernua</i>	50		10.1437	0.4555	0.9969
	60		12.4325	0.1648	0.9874
	70		12.4499	0.1469	0.9852
<i>E. camaldulensis</i>	50		23.2701	0.6543	0.9979
	60		27.6869	0.7944	0.9972
	70		23.2560	0.2655	0.9868
<i>T. diffusa</i>	50		4.8942	0.1059	0.9966
	60		5.7213	0.1677	0.9980
	70		6.0116	0.0689	0.9970

Contradictorily, for *F. cernua* adverse influence of increase in the temperature on k values was observed implying a slight decrease on the velocity of extraction. Extraction rate constant in the *T. diffusa* and *E. camaldulensis* newly were observed to have asymptotic values. When 50 and 70°C were used in the extraction of the TPC the k values were lower with respect to 60°C. Accordingly, 60°C accelerated the extraction of TPC in both plants.

3.3. Effect of Temperature and Time in MAE

Generally, for all plants used was observed an increase in the yield of TPC (mg/g) with increase temperature from 50 to 60°C and no significant change was observed from 60 to 70°C. Several studies in MAE had shown that increase in the temperatures could result in the increase of the yield of the target molecules as phenolic compounds (Yan *et al.*, 2010; Li *et al.*, 2012). It could be explained by a phenomenon that has been observed in plant cells after exposure to microwave heating. Which microwave energy is absorbed by solvent causing vibrational effects involves disruption of hydrogen bonds, rotation of molecules and migration of ions that heats the solvent and the free water molecules directly present. The moisture begins to evaporate generates pressure within the cell wall that eventually results in opening of the cell matrix or cell rupture and facility the release of active constituents and allowing the dissolution into the solvent and, therefore improving extraction yield (Ballard *et al.*, 2010; Martins *et al.*, 2010; Chan *et al.*, 2011). Although is knowledge that an increase in the temperature causes a decrease in solvent viscosity and an increase in diffusion, increasing the extraction efficiency. Moreover, increase in temperature causes an increase in the diffusivity, which accelerated the rate of mass transfer and therefore reduced extraction time (Ho *et al.*, 2008) as was described before. However, the effect of temperature on the quality of the phytochemicals of interest depends strongly of the kind of target compounds (Li *et al.*, 2012). For polyphenols, the problem with the use of very high temperatures is that may allow side reactions as thermal degradation of the phenols (Ho *et al.*, 2008; Ballard *et al.*, 2010; Yan *et al.*, 2010; Wu *et al.*, 2012). It could explain the fact that C_{eq} values increase from 50 to 60°C and after decreased from 60 to 70°C. Hence, according to the previously discussed, 60°C was selected as the optimum temperature for extraction in all plants.

The effect of the extraction time is a point very important in the obtaining of extracts and is necessary to select a proper extraction time to assure the complete extraction of the target compounds. Results in **Fig. 1 and 2** shows that the solvent saturation using 60°C was achieved

around of 1 and 3 min for *J. dioica*, *F. cernua* and *T. diffusa*, however as no significant deference was observed between 1 and 3 min, 1 min was selected for the next experiment for these plants. Similarly, for *E. camaldulensis* the solvent saturation using 60°C was achieved around of 6 and 9 min (**Fig. 1c and 2c**), hence the extract obtained at 6 min was selected for the next experiment for this plant.

3.4. Comparison Between HRE and MAE

Conventional extraction was performed in order to evaluate the extraction efficiency of the yield, phenolic composition and effect in the activity of the extracts using MAE. As shown in **Table 3**, there are no differences in the extraction yield when *J. dioica* and *F. cernua* were used in HRE and MAE, however taking into consideration the time needed for extraction, MAE was found to be the best feasible approach for the rapid and efficient extraction of bioactive phenolic constituents. In contrast, there are a significant difference between HRE and MAE for *E. camaldulensis* and *T. diffusa*, where MAE improve the extraction efficiency in a 50% *E. camaldulensis* and 31% *T. diffusa* extracts and reduced the extraction time at 1 and 6 min, respectively. This improve in the extraction efficiency in both plants *E. camaldulensis* and *T. diffusa* could be due to these plants have structure less rigid and contained more free water possibly increasing the degree of cell rupture. Similar results were observed when phenolic compounds were extracted of tomato cultivar using MAE extraction (Li *et al.*, 2012). These authors observed that increasing the temperature allowed increase in the yield and reduced in the extraction time.

The effect of the MAE procedure and HRE on the TPC yield is shown in the **Table 3**. In the same way, a significant increase (72%) in TPC was obtained for the MAE as compared to that of the same sample extracted using the HRE method. On the other hand, for *J. dioica*, *F. cernua* and *T. diffusa* was observed that TPC was obtained in the same levels between MAE and HRE. Nevertheless, interestingly the HPLC profiles of MAE and HRE extracts were not similar at least for *J. dioica* and *F. cernua* (**Table 4**), which suggests that MAE caused modifications in the polyphenolic extracts composition but no in the TPC. In *J. dioica* using MAE procedure allowed the extraction of the quercetin compound which it was detected in the HPLC analysis for MAE and not in HRE extracts. In contrast, when *F. cernua* was used in MAE procedure two from three compounds obtained in HRE were not detected in HPLC analysis, chlorogenic and coumaric acids. These compounds are slightly similar in its structure, which

indicated that MAE irradiation affected directly at these compounds and could originate its degradation. Notwithstanding, MAE procedure allowed to the extraction of four important different phenolic compounds in *F. cernua* extracts using MAE procedure as namely pyrogallol, resorcinol, methyl gallate and catechin. Only, quercetin compound was present in the both method extraction MAE and HRE in *F. cernua*. In *E. camaldulensis* and *T. diffusa* similar profiles were observed under the MAE conditions as compared to the conventional extraction method indicated that no phenolic compounds were degraded (Table 4). Actually, there are no reports for the extraction of TPC in these plants using MAE. However, our results are in accordance with findings by other researchers for the extraction of phenolic compounds from different plant materials by MAE, where a few minutes are need to extract the total phenolic content using MAE (Yan *et al.*, 2010; Aspé and Fernández, 2011; Li *et al.*, 2012).

3.5. Antioxidant Capacity

Figure 3 shows the potential antioxidant (DPPH and lipid oxidation inhibition) of the extract obtained by MAE and HRE procedure for all plants used. At the concentration used (1 mg mL^{-1}) the extracts of *F. cernua*, *T. diffusa* and *E. camaldulensis* shows the higher antioxidant capacity in DPPH radical scavenging around of 80%, in both extraction methods, MAE and HRE (Fig. 3A). Lower DPPH radical scavenging was obtained in *J. dioica* extracts, with 26% approximately.

In contrast, interestingly differences between MAE and HRE were observed in antioxidant activity when lipid oxidation inhibition was tested (Fig. 3B). The extracts obtained by MAE procedure in *J. dioica* were higher than extracts obtained by HRE with an increase of 50% in the inhibition of lipid oxidation, approximately. While in *F. cernua* extracts the activity was higher using HRE procedure than MAE in an 30%. The HPLC analysis (Table 4), could explain these results, as discussed before, using MAE affected positively the composition in *J. dioica*, while in *F. cernua* some polyphenolic compounds were degraded causing decreased in the activity antioxidant. Despite that in *F. cernua* appears other compounds, it could be in low concentration and the compounds degraded could be the mainly responsible in the contribution of lipid oxidation inhibition. Similarly, in the antioxidant activity of *E. camaldulensis* extracts obtained by MAE procedure was observed higher lipid oxidation inhibition than HRE extracts (Fig. 3B). According to the results obtained in TPC yield (Table 3) and HPLC assays (Table 4), the yield was increased in MAE and only one predominant phenolic compound was observed in the both extracts (MAE and HRE) suggesting that the increase in its activity observed in MAE could be relation whit an increase in the presence and concentration of this compound. Zuurro and Lavecchia (2013) reported that phenolic compounds extraction was correlated to antioxidant acitivity from other plant material, however generally in our case no correlations were observed.

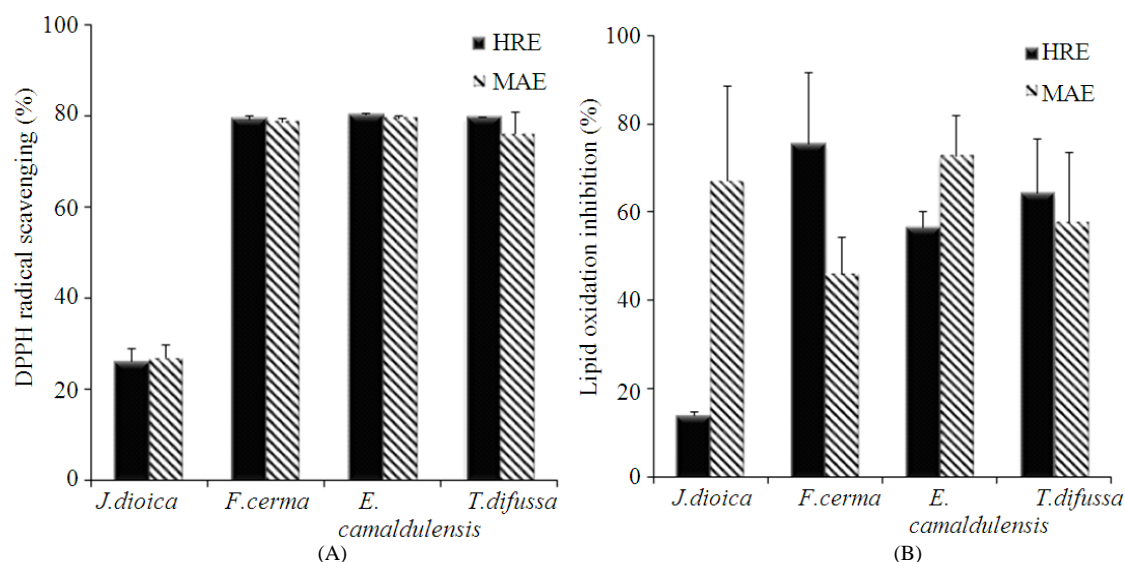


Fig. 3. Percentages of antioxidant activity in DPPH free radical scavenging (A) and lipid oxidation inhibition (B) tests of the extracts obtained in MAE and HRE

Table 3. Comparison between extraction and TPC yields obtained in MAE and HRE extracts under each conditions selected

Method	<i>J. dioica</i>		<i>F. cernua</i>		<i>E. camaldulensis</i>		<i>T. diffusa</i>	
	Extraction yield (%)	TPC yield (mg/g)	Extraction yield (%)	TPC yield (mg/g)	Extraction yield (%)	TPC yield (mg/g)	Extraction yield (%)	TPC yield (mg/g)
HRE	10.34±1.58	2.34±0.93	14.07±0.75	10.24±1.19	13.25±1.70	14.12±3.22	9.95±0.60	4.70±0.38
MAE	10.50±0.57	1.80±0.48	15.54±1.22	10.91±0.52	20.06±1.35	24.29±4.61	13.09±0.77	5.44±0.54

Table 4. Comparison of the phenolic compounds profile detected by HPLC assay in the MAE and HRE extracts

Phenolic compound	Retention Time (min)	Plants extracts							
		<i>J. dioica</i>		<i>F. cernua</i>		<i>E. camaldulensis</i>		<i>T. diffusa</i>	
		HRE	MAE	HRE	MAE	HRE	MAE	HRE	MAE
Pyrogallol	6.50	-	-	-	+	-	-	-	-
Gallic acid	7.46	-	-	-	-	-	-	-	-
Resorcinol	9.51	-	-	-	+	-	-	-	-
Chlorogenic acid	11.05	-	-	+	-	-	-	-	-
Methyl gallate	12.99	-	-	-	+	-	-	-	-
Coumaric acid	15.09	-	-	+	-	-	-	-	-
Catechin	15.65	-	-	-	+	+	+	-	-
2-hydroxycinnamic acid	17.76	-	-	-	-	-	-	-	-
Ellagic acid	18.83	-	-	-	-	-	-	-	-
Quercetin	20.11	-	+	+	+	-	-	+	+
Cinnamic acid	21.05	-	-	-	-	-	-	-	-

Presence (+) and absence (-)

4. DISCUSSION

Several studies have shown that the phenolics with multiple hydroxyl groups are generally the most efficient for preventing lipid oxidation (Moure *et al.*, 2001). For *T. diffusa* extracts no significant differences between MAE and HRE procedures were observed in lipid oxidation inhibition with 60% of activity (Fig. 3b). Quality and quantity of the extracts dependent of the target compounds structures, natural sources and type of processes (Karacabey *et al.*, 2011), this explain the fact that with MAE procedure obtained different phenolic composition in the extracts. These findings suggest that MAE procedure is an alternative method to obtain extracts with high potential antioxidants of these plants in a few minutes, compared with the conventional method. MAE has a promising future, due to the great advantages and the development of equipment for large-scale commercial operation (Taamalli *et al.*, 2012). Besides, the extracts obtained are a source of natural antioxidants which could be used in the food industry. Is important mention that naturally in antioxidants also need safety test (Moure *et al.*, 2001). In the cases of eucalyptus leaf extract, it is included as an antioxidant natural food additive in the list of Existing Food Additives in Japan (Amakura *et al.*, 2002).

5. CONCLUSION

The results obtained of solid-liquid extraction of TPC using MAE procedure were accurately described with the models used in the present study. The best temperature of extraction was 60°C, resulting in an increase on TPC yield and reduced the time from hours to minutes. The MAE procedure is a good alternative for the extraction of natural antioxidants in the semiarid plants. *E. camaldulensis* extract was the higher in TPC and antioxidant activity. In contrast, *J. dioica* was the extract that showed the lower TPC and antioxidant activity. MAE procedure is a promising technique in recovery of bioactive compounds of plants in an environmentally sustainable way, but further studies about optimization in MAE procedure in the plants investigated is necessary in order to evaluate all parameter that affect the extraction efficiency. Also, it is important to mention that is necessary to determine the bioavailability the phenolic compounds of the extracts.

6. ACKNOWLEDGEMENT

Researchers Wong-Paz and Muñoz-Marquez are grateful to Mexican Council for Science and Technology (CONACYT) for the financial support as graduate scholarship.

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