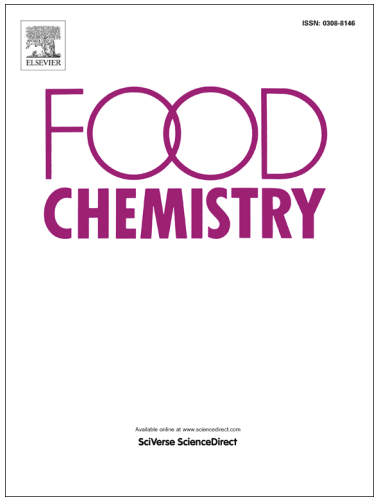

Comparison of Microwave, Ultrasound and Accelerated-Assisted Solvent Extraction for Recovery of Polyphenols from *Citrus Sinensis* Peels

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Full title:

Comparison of Microwave, Ultrasound and Accelerated-Assisted Solvent Extraction for Recovery of Polyphenols from *Citrus Sinensis* Peels

Running title:

Comparison of extraction methods for Polyphenols from *Citrus* Peels

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Abstract

Peel of *Citrus sinensis* contains significant amounts of bioactive polyphenols that could be used as ingredients for a number of value-added products with health benefits. Extraction of polyphenols from the peels was performed using a microwave-assisted extraction (MAE) technique. The effects of aqueous acetone concentration, microwave power, extraction time and solvent-to-solid ratio on the total phenolic content (TPC), total antioxidant activity (TAA) (using DPPH and ORAC-values) and individual phenolic acids (IPA) were investigated using a response surface method. The TPC, TAA and IPA of peel extracts using MAE was compared with conventional, ultrasound-assisted and accelerated solvent extraction. The maximum predicted TPC under the optimal MAE conditions (51% acetone concentration in water (v/v), 500 W microwave power, 122 s extraction time and 25 mL g^{-1} solvent to solid ratio), was 12.20 mg GAE g^{-1} DW. The TPC and TAA in MAE extracts were higher than the other three extracts.

Keywords: *Citrus sinensis*; microwave extraction; ultrasound extraction; accelerated solvent extraction, phenolic compounds; antioxidant activity; response surface method

1. Introduction

Citrus is one of the most important fruit crops in the world. Production of citrus fruits has increased enormously in the last few decades, going from an average of 62 million tons a year in the period 1987–1989 to about 100 million tons in the year 2010 (Food and Agriculture Organization, 2014). Citrus is grown in more than 100 countries all over the world, mainly in tropical and subtropical areas, where favorable soil and climatic conditions prevail for citrus cultivation. Citrus fruits are marketed mainly as fresh fruit or as processed juice. During processing of citrus fruits, huge amount of peels are generated as by-product, which do not add value to the product as these are discarded or dumped. Potential use of citrus peels as value-added products has been widely studied because it contains numerous biologically active compounds including natural antioxidants such as phenolic compounds (Hayat, Hussain, Abbas, Farooq, Ding, Xia, et al., 2009). Citrus phenolics have been the subject of increased interest in the last few years because of their contributions to the quality attributes with color, bitterness, astringency, antioxidant activity and flavor (Legua, Forner, Hernández, & Forner-Giner, 2014). In recent years, the physiological function of foods including fruits, vegetables, legumes and grains, and food components such as phytochemicals has received much attention. Possible correlations between the biologically active compounds and human health have generated interest in *in-vitro* and *in-vivo* studies. Phenolic compounds are a major class of phytochemicals found in plants and consist of a large variety of derivatives including simple phenols, phenylpropanoids, benzoic acid derivatives, flavonoids, tannins, lignans and lignins. These compounds have diverse properties as antioxidants, anti-inflammatory, anti-allergic, and ant-carcinogenic activity, and these properties improve the quality and value of the food (Liu, 2004). The antioxidant property is associated with the ability of the phenolic compounds to scavenge free radicals, break radical chain reactions and chelate metals. It was also found that the phenolic

compounds inhibit human immunodeficiency viral replication (HIV), human simplex virus (HSV), glucosyl transferases of *Streptococcus mutans* (dental carries), ascorbate auto-oxidation (green tea), cytotoxic effects, tumor promotion and xanthine and monoamine oxidases (Proestos, Boziaris, Nychas, & Komaitis, 2006).

Phytochemicals from the citrus peels could add value to the citrus processing industry when the compounds are extracted effectively by applying efficient extraction technologies. Optimization and standardization of extraction parameters for these health benefitting bioactive phytochemicals from citrus peels are important to retain their antioxidative properties. Application of various techniques for sample preparation and processing of bioactive compounds from plant materials have been reviewed by many researchers (Chumnanpaisont, Niamnuy, & Devahastin, 2014; Fernández-Agulló, Freire, Antorrena, Pereira, & González-Álvarez, 2013). The first step of processing is "extraction", which involves separation of phytochemicals from the cellular matrix of citrus peel. There are various methods for extracting phenolic compounds such as leaching-out extraction (Zhang, Bi, & Liu, 2007). Generally, extraction is being carried out using conventional technologies such as solvent extraction (liquid–liquid and solid–liquid extraction) by assistance of external factors (e.g. mechanical agitation, pressing, or heating systems).

The "ideal" extraction method must provide high extraction rates and should be non-destructive and time saving (Rombaut et al., 2014). In addition, as per the environmental requirements and economic impact, the food and nutraceutical industry prefer green extraction and processing to ensure a safe and high quality extract/product (Chemat, Vian, & Cravotto, 2012). Recently, more rapid and automated methods including supercritical fluid extraction (SFE), pressurized liquid extraction (PLE) or microwave-assisted extraction (MAE), ultrasound extraction (UAE) and accelerated solvent extractor (ASE) have been used (Krishnaswamy, Orsat, Gariépy, & Thangavel, 2013; Périno-Issartier, Zill-e, Abert-Vian, &

Chemat, 2011). The above extraction methods are advantageous compared to conventional methods because they can be carried out in the absence of light and oxygen, cope with the demand for a reduction in organic solvent consumption and improve the extraction time due to the possibility of working at elevated temperatures or pressures in inert atmospheres. A thorough literature search did not yield any reference or reports on the optimization of microwave procedure for extraction of phenolic compounds from *C. Sinensis* peels. Response surface methodology (RSM) is a useful tool to evaluate the effects of multiple factors and their interactions on one or more response variables such as phenolic compounds. The central composite design (CCD) is a popular form of RSM and has been applied by a number of researchers for optimization of various food processing methods such as extrusion cooking (Nayak, Berrios, Powers, & Tang, 2011) and extraction (Huang, Ou, Hampsch-Woodill, Flanagan, & Prior, 2002). In this project, we investigated (i) the effects of different extraction parameters on the efficiency and recovery of phenolic compounds from *C. Sinensis* peels; (ii) RSM technique to optimize microwave-assisted extraction parameters such as microwave power, extraction time, solvent-to- solid ratio to maximize the total phenolic content ; (iii) individual phenolic acids of microwave-assisted (MAE), ultrasound-assisted (UAE), accelerated-solvent (ASE) and conventional solvent (CSE) extracted samples. Finally, the total phenolic contents and antioxidant activities (using DPPH radical scavenging assay and ORAC-values) of citrus peels in optimized MAE conditions were compared with UAE, ASE and CSE to understand the most efficient extraction method.

2. Materials and methods

2.1 Plant material

The fruit samples of *C. Sinensis* were collected in the area of Oued Ghir (Bejaia, Algeria). Samples were washed with distilled water and peeled off manually. Peels were dried in a forced-oven at 40 °C to constant weight, and then grounded using an electrical grinder (IKA

model- A11, Staufen, Baden-Württemberg, Germany). The ground powder was passed through a standard 125 µm sieve and only the fraction with particle size < 125 µm was collected and stored at 4 °C in airtight bags until further use. The water activity (a_w) of the sample was measured with a HygroPalm AW1 portable water activity meter, (Rotronic, Bassersdorf, Switzerland) and found to be 0.18 ± 0.02 at 20.6 °C.

2.2 Reagents

Sodium carbonate (Na_2CO_3), Folin-Ciocalteu's phenol reagent and hydrochloric acid (HCl) were purchased from Prolabo (Loire, France). Fluorescein (FL) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), DPPH (1, 1-diphenyl-2-picrylhydrazyl) were purchased from Sigma–Aldrich Co. (St. Louis, MO). HPLC standards such as gallic acid, ferulic acid, caffeic acid, *p*-coumaric acid, chlorogenic acid, rutin, quercetin, catechin and epigallocatechin were purchased from Fisher scientific (Fair Lawn, NJ, USA). 2-2′-azobis (2-amino-propane) dihydrochloride (AAPH) was purchased from Wako chemicals (Richmond, VA, USA). All the solvents used for extraction and HPLC analysis were HPLC grade.

2.3 Extraction and quantification of total phenolic content (TPC)

2.3.1 Microwave assisted extraction (MAE).

A domestic microwave oven (Samsung model: NN-S674MF, Kuala Lumpur, Malaysia) with cavity dimensions of 22.5 × 37.5 × 38.6 cm and a working frequency of 2.45 GHz was used for extraction of phenolic compounds from *C. Sinensis* peel powders. The apparatus was equipped with a digital control system for measuring extraction time and microwave power (the latter was linearly adjustable from 200 to 1000 W). The oven was modified in order to condense the vapors generated during extraction of the sample. Aqueous acetone in different concentrations was used as a safe and efficient solvent for the extraction of phenolic compounds (Li, Deng, Wu, Liu, Loewen, & Tsao, 2012). The use of acetone allows an

efficient and more reproducible extraction, avoids problems with pectins such as its clotting properties, and permits a much lower temperature for sample concentration Garcia-Viguera, Zafrilla, and Tomas-Barberan (1998).

One gram of *C. Sinensis* peel powder was mixed with aqueous acetone by stirring in preparation for extraction using the MAE system. The MAE extraction parameters were microwave power (300-600 W), extraction time (90–240 s), solvent-to-solid ratio (15-30 mL g^{-1}) and acetone in water concentration (20-80 %, v/v), where the influence of each parameter was investigated in single-factor experiments (*Supplement Table S1*). Each trial was carried out in triplicate. The temperature of the samples were never exceeded 80 °C during 122 s of extraction at 500 W (optimal conditions). After MAE treatment, the extract was filtered through a Whatman No. 1 filter paper lined in a Büchner funnel and the supernatant was collected in a volumetric flask. The extract was stored at 4 °C until further use. Influence of each factor on the yield of total phenolic content was statistically assessed by ANOVA and Tukey's post-hoc test.

2.3.1.1 Experimental Design and Statistical Analyses.

The influence of the process parameters i.e. type of solvent, microwave power, extraction time and solvent-to-solid ratio were investigated using a single-factor-test to determinate the preliminary range of extraction variables (*Supplement Table S1*). Based on the single-factor experimental results, major factors influencing the extraction process were selected for designing experiments using response surface methodology (RSM). Minitab statistical software (Minitab, version 8.0.7.1, State college, PA, USA) package was used to establish a mathematical model and obtain the optimum conditions for maximum recovery of TPC. In the present study, a three-level four-factorial Box–Behnken experimental design (BBD) was applied to investigate and validate extraction process parameters affecting the rate of phenolic compounds from *C. sinensis* peels. The number of experiments (N) required for

159 the development of BBD is defined in Eq.(1) (Dahmoune, Boulekbache, Moussi, Aoun,
160 Spigno, & Madani, 2013).

161 $$N = 2k(k-1) + C_0 \qquad (1)$$

162 where k is number of factors and C_0 is the number of center points. The factor levels were
163 coded as −1 (low), 0 (center point or middle) and 1 (high), respectively. The four factors
164 chosen for this study designated as X_1: aqueous acetone (40-60%), X_2: extraction time (90-
165 150 s), X_3: microwave power (400-600 w) and X_4: solvent-to-solid ratio (20-30 mL g^{-1})
166 (Table 1). The variables were coded according to equation Eq.(2) (Dahmoune, Nayak,
167 Moussi, Remini, & Madani, 2015).

168 $$x_i = \frac{(X_i - X_0)}{\Delta X} \qquad (2)$$

169 Where x_i the (dimensionless) coded value of the variable is X_i and X_0 is the value of X at the
170 center point and ΔX is the step change.

171 The experiments were performed according to the design of experiments shown in
172 Table 1. The output results (TPC yield) were fitted to a second-order polynomial equation
173 (quadratic model), according to the model in Eq.(3).

174 $$Y = B_0 + \sum_{i=1}^{k} B_i X_i + \sum_{i=1}^{k} B_{ii} X^2 + \sum_{i>j}^{k} B_{ij} X_i X_j + E \qquad (3)$$

175 where Y represents the response function (in our case the TPC yield); B_0 is a constant
176 coefficient; B_i, B_{ii} and B_{ij} are the coefficients of the linear, quadratic and interactive terms,
177 respectively, and X_i and X_j represent the actual independent variables. The regression
178 coefficients of individual linear, quadratic and interaction terms were determined using
179 analysis of variance. In order to visualize the relationship between the response and
180 experimental levels of each factor and to deduce the optimum conditions, the regression
181 coefficients were used to generate *3-D* surface plots from the fitted polynomial equation.

Analysis of variance (ANOVA) was performed for response variable using the full models where p-values (partitioned into linear and interaction factors) indicated whether the terms were significant or not. To verify the adequacy of the models, additional extraction trials were carried out at the predicted optimal conditions and the experimental data were compared to the values predicted by the regression model. Microwave extraction method was compared with UAE, ASE and CSE based on the TPC, antioxidant activity (DPPH assay and ORAC-values) and HPLC results.

2.3.2 Ultrasound assisted extraction (UAE)

An ultrasonic system with working frequency fixed at 20 kHz (SONICS Vibra cell, VCX 130 PB, stepped microtips and probes, No. 630-0422, Newtown, Connecticut, USA) was used for extraction of phenolic compounds from the citrus peel under optimal conditions (Dahmoune, Moussi, Remini, Belbahi, Aoun, Spigno, et al., 2014). Briefly, 1 g of peel powder was mixed with 50 mL of 75.79 % acetone concentration in a 250 mL amber glass bottle (Ø × H: 45 × 140 mm and cap size of 28 mm) the obtained suspension was exposed to acoustic waves for 8.33 min extraction time, and 65.94% extraction amplitude. The temperature (27 ± 2 °C) was controlled continuously by circulating cold water using an external cold-water bath and checking the temperature using a T-type thermocouple (Cooking, Thermo-Timer, China). After the UAE treatment, the supernatant was recovered and analyzed as reported in Section 2.3.1 for the optimized MAE extract.

2.3.3 Accelerated solvent extraction (ASE)

An accelerated solvent extractor (ASE, Dionex Corp., Sunnyvale, CA) system was used for extraction of phenolic compounds from *C. Sinensis* following our in-house procedure. Briefly, one gram of peel powder was placed in two layers of diatomaceous earth (about 0.5 g in each layer) in 11 mL Dionex (ASE 200) stainless-steel cell, and phenolic compounds were extracted with 50% acetone. The cells were equipped with a stainless steel

frit and a cellulose filter (Dionex Corp.) at the bottom to avoid the collection of suspended particles in the collection vial. A dispersing agent (diatomaceous earth), was used to reduce the solvent volume. The extraction was performed at 1500 psi and temperature of 120 °C, and then heated for 6 min, followed by three static periods of 5 min (3 static cycles). The sample was flushed with 70% nitrogen for 90 s. Extracts were collected into 50 mL tubes.

2.3.4 Conventional solvent extraction (CSE)

Phenolic compounds in citrus peels were extracted using a conventional solvent extraction method following the procedures recommended by (Giorgia Spigno, Tramelli, and De Faveri, 2007). Briefly, one gram of powder was mixed with 50 mL of 50% aqueous acetone (v/v) in a conical flask (Ø × H: 51 × 150 mm and cap size of 38 mm); the mixture was kept in a thermostatic water bath (model: WNB22, Memmert, Frankfurt, Germany) at 60 °C for 2 hours shaking at a speed of 110 strokes per minute. After the CSE treatment, the supernatant was recovered and analyzed as reported in Section 2.3.1 for the optimized MAE extract.

2.4 Determination of total phenolic content (TPC)

The total phenolic content in the *Citrus sinensis* powder extracts was determined by the Folin–Ciocalteau method (Jaramillo-Flores, González-Cruz, Cornejo-Mazón, Dorantes-Alvarez, Gutiérrez-López, & Hernández-Sánchez, 2003). Briefly, twenty micro- Liter of supernatant was mixed with 150 µL of a 10-fold diluted Folin–Ciocalteau reagent. The solutions were mixed thoroughly and incubated at room temperature (27 °C) for 5 min. After incubation, 150 µL of 6 % sodium carbonate (Na_2CO_3) solution was added and again incubated at 25 °C for 90 min. The absorbance of the reaction mixtures were measured at 725 nm using a Omega FLUOstar plate reader (Model : SpectroScan 50, Nicosia, Cyprus). The absorbance of the extract was compared with a gallic acid standard curve for estimating

concentration of TPC in the sample. The TPC was expressed as mg of gallic acid equivalents (GAE) per gram of powder on dry weight (DW) basis.

2.5 Determination of antioxidant activity

2.5.1 DPPH radical scavenging assay

The electron donation ability of the obtained acetone extracts was measured by bleaching of the purple-colored solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) following the procedures of Brand-Williams, Cuvelier, and Berset (1995) with modifications. Briefly, 300 µL of $DPPH^{\bullet}$ solution prepared in methanol (70 µM) was mixed with 10 µL of peel extracts and the mixture was incubated at 37 °C. The tests were performed on a micro-plate reader (Omega FLUOstar, BMG Labtech, Cary, NC, USA). Absorbance readings of the mixture were taken at 515 nm over a period of 20 min.

The antioxidant activity was expressed as percentage of inhibition of DPPH radical calculated according to Eq.(4).

$$\%\ inhibition = \frac{A_{blank\ t=20\ \text{min}} - A_{sample\ t=20\ \text{min}}}{A_{DPPH\ t=0\ \text{min}}} \times 100 \qquad (4)$$

where A_{blank} is the absorbance value of the blank (300 µL of DPPH solution plus 10 µL of the solvent in which extract has been dissolved); A_{sample} is the absorbance of the sample extract; t is the time (min) at which absorbance was read and A_{DPPH} is the absorbance of the control at time = 0 min. The effective concentration of sample required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations.

2.5.2 Oxygen radical absorbance capacity (ORAC) assay

The antioxidant activity of peel extracts were also assessed using an ORAC assay following the procedures of Huang, Ou, Hampsch-Woodill, Flanagan, and Prior (2002) with modifications. Briefly, a stock fluorescein solution (Stock #1) was prepared by dissolving

0.0225 g of fluorescein in 50 mL of 0.075 M phosphate buffer (pH 7.0). A second stock solution was prepared by diluting 50 µL of stock solution #1 in 10 mL of phosphate buffer. An aliquot of 800 µL of solution #2 was added to 50 mL of phosphate buffer, mixed on magnetic stir plate and aliquots were dispensed to 10 mL tubes and placed in 37 °C heating block until the start of the assay. AAPH was used as a peroxyl generator and trolox as a standard. Twenty microliters of sample, blank, and trolox calibration solutions were transferred to 96-well microplates in triplicate on the basis of a set layout. The ORAC assays were carried out on Omega FLUOstar plate reader which was equipped with an incubator and two injection pumps. The temperature of the incubator was set to 37 °C. The plate reader was programmed to record the fluorescence of fluorescein during each cycle for 40 min. Four calibration solutions of trolox (6.5, 12, 25, 50 µM final concentration) was also tested to establish a standard curve. All samples were analyzed in triplicate.

The area under the curve (*AUC*) was calculated for each sample by integrating the relative fluorescence curve (Eq.(5)).

$$AUC = \left(0.5 + \frac{f_4}{f_3} + \frac{f_5}{f_3} + \frac{f_6}{f_3} + \cdots + \frac{f_i}{f_3} \right) \times CT \qquad (5)$$

where f_3 initial fluorescence reading at cycle 3, f_i is a fluorescence reading at cycle *i*, and *CT* is cycle time in minutes. The net *AUC* of the sample was calculated by subtracting the *AUC* of the blank. The regression equation between net *AUC* and Trolox concentrations was determined and ORAC values were expressed as µmol trolox equivalents per gram of sample (µmol TE g^{-1}) using the standard curve established in same condition.

2.6 HPLC-DAD analysis

Treated sample and control extracts were fractionated for phenolic acids in four fractions using a SePak C-18 cartridge (Waters, Milford, MA, USA). The columns were activated for neutral phenolics by sequentially passing 50 mL of ethyl acetate, acidified

methanol (0.01% v/v HCl) and acidified water (0.01% v/v HCl). Fraction 1 was obtained by eluting 20 ml of crude extract in the column prior to washing the column with 60 ml of acidified distilled water to remove any organic sugars and acids (fraction 2). The retained fraction of phenolic acids was eluted with 60 ml of ethyl acetate (fraction 3). Anthocyanins plus proanthocyanidins (fraction 4) were eluted with 60 ml of acidified methanol. Finally, after evaporation, all fractions (1, 2, 3 and 4) were re-suspended in methanol (HPLC grade) and stored at 4 °C for further analysis.

Identification of selected individual phenolic acids was performed using a High-Performance Liquid Chromatography (Agilent Technologies, Santa Clara, USA) equipped with a Diode array detector and a column C-18 (5 μm, 4.60 × 250 mm, USA). All samples were centrifuged at 5000 rpm for 10 min before injection into the column with an injection volume of 20 μL and at a flow rate of 0.5 mL min^{-1}. Chromatographic analysis was carried out at 30 °C using simultaneous monitoring of extracts performed at 254, 280, 520, 300 and 700 nm. The mobile phase A was a mixture of 6:94 (v/v) acetic acid in distilled water, whereas mobile phase B consisted of 100% HPLC grade acetonitrile. The solvent gradient in volume ratios was as follows: 0–40 min, 0–25% B; 40–80 min, 25–85% B; 80–90 min, 85–100% B; 90-95 min, 100% B.

Individual phenolic compounds were identified based on their elution time and quantified from peak area at 280 nm. Identified phenolic compounds (phenolic acid and flavonoids) were quantified using external standards. The standard response curve was a linear regression fitted to values obtained at each concentration within the range of 12.5–200 μg mL^{-1} for phenolic acid (Gallic acid, Ferulic acid, Caffeic acid, *p*-Coumaric acid and Chlorogenic acid) and 41.5-333 μg mL^{-1} for flavonoids (Rutin, Quercetin, Catechin and Epigallocatechin).

3 Results and discussion

3.1 Effects of extraction on the visual color of extracts

Extraction is an important step for the recovery and isolation of bioactive phytochemicals from plant materials before analysis. Liquid-liquid and solid-liquid extraction are the most commonly used procedures prior to analysis of phenolic compounds in natural matrix. They are still the most widely used techniques, mainly because of their efficiency, wide-ranging applicability and ease of use to extract of natural antioxidants. In our study, from the color and turbidity of the acetone-water extracts in relation to the extraction methods i.e. CSE, UAE, MAE and ASE, it was observed that the visual color was influenced by the extraction methods. Most importantly, color of the MAE and ASE extracts turned pale brown, differing from the usual pale green after extraction. In MAE, heat is generated by the volumetric heating of samples with the help of electromagnetic waves; in ASE, high temperature extraction of the samples potentially attributed to the degradation of color compounds. A similar trend in the degradation of color compounds was observed in our previous studies on the extraction of phenolic compounds from *C. limon*, *P. lentiscus* and *M. communis* using MAE, ASE, UAE and CSE (Dahmoune, Boulekbache, Moussi, Aoun, Spigno, & Madani, 2013; Dahmoune, et al., 2014).

3.2 Modeling of MAE

3.2.1 Single factor experiments

Selection of extraction solvents is critical for the complex plant materials as it will determine the amount and type of phenolic compounds being extracted. Aqueous solvents particularly acetone, ethanol and methanol are more commonly used in phenolic extraction from botanical materials than the corresponding mono-component solvent system (Spigno, Tramelli, & De Faveri, 2007). In the present study with preliminary single factor experiments, we observed that the type of solvent significantly influenced the TPC yield

during MAE, with 50% aqueous acetone (11.49 ± 0.44 mg GAE g^{-1}) providing higher recovery than water (7.63 ± 1.22 mg GAE g^{-1}), 50% aqueous ethanol (6.29 ± 0.77 mg GAE g^{-1}) and 50% aqueous methanol (9.68 ± 0.99 mg GAE g^{-1}) (*supplement Table S2*). Higher recovery of TPC by aqueous acetone could be attributed to better absorption of microwave energy by the extract during MAE due to volumetric heating. This process increases temperature inside the plant cells, resulting in breaking the cell walls and releasing compounds in to the surrounding solvent. Aqueous acetone was then selected for the RSM trials and for subsequent single-factor trials.

The effect of various percentages of acetone in water (20–80%) as an extraction solvent to recover phenolic compounds was investigated. TPC yield increased with increasing aqueous acetone concentration up to 50% and then decreased slightly at higher concentrations (*supplement Table S2*). Thus, the acquired ratio of TPC increased with decreasing water content following the principles of "like dissolves like" (Zhang, Bi, & Liu, 2007). In the second step, the proportion of acetone in the extraction solvent was varied between 40 and 60% for the optimization design. The solvent with 50% acetone content was then chosen for the determination of optimal microwave power, extraction time and solvent-solid ratio.

Selection of an appropriate microwave power for extraction was the third step in a series of preliminary experiments. Phenolic compounds were extracted from *C. sinensis* peel samples by varying the microwave power using 50% aqueous acetone for 120 s while keeping the solvent-to-solid ratio constant at 20 mL g^{-1}. The results showed that the TPC increased when microwave power increased from 400 to 500 W. Beyond 500 W, TPC decreased sharply and reached a minimum at 800 W (*Supplement Table S2*), possibly due to the degradation of compounds with the higher microwave power during extraction (Proestos & Komaitis, 2008). Based on the preliminary tests, the best microwave power for the

extraction was selected as 500 W. The lower, middle and upper levels of extraction power chosen for RSM were 400, 500 and 600 W, respectively.

Extraction time is another parameter that influences the yield of TPC and should be considered for the optimization procedure. Generally, by increasing the extraction time, the quantity of analytes extracted is increased, although there is a risk that degradation of phenolic compounds may occur (Proestos, Boziaris, Nychas, & Komaitis, 2006). The acquired ratio of TPC extraction gradually increased with increasing extraction time and the maximum TPC was obtained between 30-120 s (*Supplement Table S2*). A significant increase in extraction efficiency was observed as the extraction time increased from 90 to 120 s followed by a significant decrease after 125 s (*Supplement Table S2*). Longer irradiation exposition without temperature control could have induced thermal degradation of phenolic compounds (Yang, Jiang, Li, Chen, Wang, & Zhu, 2009). Since shorter extraction time is also favorable to reduce energy costs, the 90 - 150 s range was selected for the optimization.

The solvent-to-solid ratio can influence the TPC yield. In the present study, solvent-to-solid ratio was set at 15:1, 20:1, 25:1 and 30:1 (mL g^{-1}) respectively. It was observed that the extraction yield increased quickly with the increase of solvent-solid ratio from 15:1 – 25:1 mL g^{-1} (*Supplement Table S2*). Then, extraction yield rapidly decreased with increase in solvent /material ratio from 25:1 – 30:1 mL g^{-1}. The solvent-to-solid ratio significantly influenced the TPC yield, showing similar trend on the extraction of tea polyphenols using microwave as reported by Spigno and De Faveri (2009). However, the decrease in the extraction yield beyond a solvent-to-solid ratio could be due to the non-uniform distribution and exposure to microwave heating (Eskilsson & Björklund, 2000). Furthermore, the optimized solvent volume should be sufficient to ensure that the entire sample is immersed, especially when a sample will swell during the extraction process (Dahmoune, Boulekbache,

376 Moussi, Aoun, Spigno, & Madani, 2013; Eskilsson & Björklund, 2000). Based on statistical
377 analysis, the range 20–30 mL g^{-1} was selected for the RSM optimization.

378 *3.2.2 Optimization of MAE technique*

379 In this study, we evaluated the effects of microwave power, extraction time and
380 solvent-to-solid ratio with the Box-Behnken experimental design. Aqueous acetone was used
381 for all the extraction methods. The data on the yield of TPC obtained from 27-runs of
382 experiments using MAE was analyzed using ANOVA and are shown in Table 1. A quadratic
383 model was fitted to the generated data to test the significance and adequacy of the model.
384 From the significant levels obtained at $p < 0.001$, it was observed that the data adequately fit
385 the developed model (Table 2). In addition, the coefficient of determination (R^2=0.92) and
386 adjusted determination coefficient (Adj. R^2 = 0.89) were reasonably close to 1, indicating a
387 high degree of correlation between the observed and predicted values. Additionally, a low
388 value of coefficient of the variation (CV = 3.96%) indicated a high degree of precision and a
389 good deal of reliability of the experimental values.

390 From the regression equation, it can be observed that the independent variables have a
391 linear effect on the yield of *TPC (Y)* within the experiment range in MAE. The TPC
392 extraction yield was affected more significantly by acetone concentration at $p < 0.001$ (p =
393 0.0001), followed by extraction time at $p < 0.05$ (p =0.0492), while TPC recovery was not
394 affected ($p > 0.05$) by other factors, i.e. extraction power (p = 0.4163) and solvent-solid ratio
395 (p = 0.1257). The quadratic terms X_1^2 , X_2^2, X_3^2 and X_4^2 were highly significant at the level
396 $p < 0.001$ (Table 2).

397 The interaction (cross product) of extraction time and solvent-solid ratio (X_2X_4) was
398 statistically significant at $p < 0.001$ followed by (X_1X_4) at $p < 0.05$ (Table 2). Neglecting the
399 non-significant terms ($p < 0.05$), the final predicted second-order polynomial equation
400 obtained is given in Eq.(6)

401 $$Y = 12.1567 + 0.4967X_1 - 0.4225X_1X_4 - 0.8675X_2X_4 - 1.7291X_1^2 - 1.2404X_3^2 - 0.9091X_4^2 \quad (6)$$

402 The effects of the independent variables and the mutual interaction on the extraction yield of
403 TPC can also be seen on three dimensional response surface curves shown in Figure 1A–F.
404 Each *3D* plot represents the number of combinations of the two-test variable. Figure 1A
405 shows a higher interaction between the solvent-solid ratio and extraction time ($p < 0.001$); the
406 TPC increased roughly from 8.72 to 10.82 mg GAE g^{-1} DW when the ratio was fixed at 20
407 mL g^{-1} and time varies for 90 to 133 s. The decline in TPC was observed after 122 s at a
408 solvent-solid ratio of 25 mL g^{-1}. The TPC yield reach a minimum of 9.25 mg GAE g^{-1} DW
409 at 150 s and at extraction ratio 30 mL g^{-1}.

410 Response surface for TPC yield with varying ratio of solvent to solid samples and
411 aqueous acetone concentration are shown in Figure 1B. Simultaneous increase in the ratio of
412 solvent to solid from 20 to 25 mL g^{-1} and aqueous acetone concentration from 40 to 50%,
413 increased the TPC yields from about 9 to 12.19 mg GAE g^{-1} DW. It was also observed that
414 the extraction yields decreased along with the increase in the ratio of solvent to solid from 25
415 to 30 mL g^{-1} and aqueous acetone concentration of 50 to 60%. The results suggested that the
416 interaction between the ratio of solvent-to-solid and aqueous acetone concentration on the
417 extraction efficiency of TPC was highly significant ($p < 0.01$). Figure 1C shows the effects of
418 aqueous acetone concentration and microwave power on the TPC of the *C. sinensis* peels. By
419 increasing the microwave power from 436 to 496 W with aqueous acetone concentration
420 fixed at 41% (acetone/water, v/v), the TPC increases from 9.45 to 10.04 mg GAE g^{-1} DW.
421 However, the TPC increases from 9.45 to 12.19 mg GAE g^{-1} DW if the aqueous acetone
422 concentration and microwave power were increased simultaneously until 50% and 500 W
423 respectively. The reduction in the TPC up to a value of 9.72 mg GAE g^{-1} DW was noticed
424 when one exceeds the optimal conditions (beyond 500 W and 50% acetone in water). This
425 was due to the increase in the direct effect of microwave energy on the medium of extraction

426 by the dipolar rotation that resulted in a rise in temperature of the medium and caused the
427 degradation of the bioactives substances (Hayat, et al., 2010).

428 The recovery of TPC was affected with response to the interaction between
429 microwave power and solvent-to-solid ratio, when other factors (extraction time and aqueous
430 acetone proportion) were fixed at 120 s and 50% respectively (Figure 1D). The results
431 indicated that TPC yield increased with the increase in microwave power and solvent-solid
432 ratio at the beginning of extraction. The recovery reached its maximum of 12.10±0.15 mg
433 GAE g^{-1} DW at 500 W and a solvent-solid ratio of 25 mL g^{-1} during the MAE process.
434 Increasing the microwave power further to more than 500 W, the extraction recovery of TPC
435 decreased with a solvent-solid ratio of 25 mL g^{-1}. These results are in agreement with those
436 found by Shao, He, Sun, and Zhao (2012), who observed that a strong microwave power
437 leads to an increase in the temperature, which negatively affects the thermo-labile
438 compounds. Figure 1E shows the profiles obtained on the effects of the aqueous acetone
439 concentration and extraction time on the yield of TPC. An increase in the TPC yield was
440 observed with the increase in aqueous acetone concentration and extraction time, but the
441 tendency was reversed when the interaction between solvent-solid ratio and extraction time
442 reached a certain value. A few seconds of peel exposure to microwave showed an excellent
443 yield in TPC, but longer duration enhanced the degradation of the thermo-labile compounds.
444 Figure 1F shows that increase in extraction time and microwave power increased the TPC up
445 to a maximum of 12 mg GAE g^{-1} DW. However, a prolonged extraction time with the
446 microwave power gave reduced TPC yield, which was also noticed during our preliminary
447 study.

448 Under the optimal conditions of microwave power, extraction time, aqueous acetone
449 concentration and solvent-solid ratio, the model predicted a maximum response of 12.20 mg
450 GAE g^{-1} DW. To compare the predicted results with the experimental values, rechecking

was performed using this deduced optimal condition. It led to an experimental yield of TPC equal to 12.09 ± 0.06 mg GAE g^{-1} DW, which is close to the predicted value (12.20 mg GAE g^{-1} DW). The best correlation between these results confirmed that the response model was adequate, and valid enough to reflect the expected optimization results (*Supplement Table S3*).

3.3 Comparison of extraction methods on recovery of TPC

To evaluate the efficiency of extraction and validate the MAE procedure for polyphenol-rich extract, *C. sinensis* peels were extracted and compared with CSE, UAE and ASE. Recovery of the TPC in extracts were compared using the above selected extraction methods (Table 3). The results of the MAE experiments indicated that microwave assistance enhanced the efficiency of yield of TPC significantly ($p < 0.01$) compared to UAE, CSE and ASE. Higher yield of TPC in MAE could be attributed to the microwaves ability to penetrate cell matrix and interact with polar molecules resulting in volumetric heating of biomaterial, consequently leading to a pressure increase inside the plant cell. This pressure increase leads to breaking of cell walls and release of phenolic analytes. Besides, breakdown of bigger phenolic compounds into smaller ones with their intact properties of the original molecules, as measured by Folin-Ciocalteau assay, could have provided the higher yield TPC (Nayak, Liu & Tang, 2015). However, this observation was in contrary to Pingret (2012, 2013), who reported degradation of bioactive compounds by microwave and ultrasound waves. The lower TPC in the ASE treatments using aqueous acetone at 120 °C may be due to the breakdown of phenolic compounds that were not detected using Folin-Ciocalteau assay. In addition, combined effects of oxidation during the extraction process and of non-phenolic compounds such as sugar, fatty acids interaction with the phenolic compounds might have lowered the TPC in ASE extracts and the recovery might have underestimated since the extract showed the dark color.

3.4 Effects of extraction methods on the antioxidant activity

The antioxidant activities of *C. Sinensis* extracts were evaluated by DPPH radical scavenging assay and ORAC test. The reduction of the DPPH absorbance at 517 nm after 20 min incubation was measured with different concentrations of the extract (Figure 2). Extracts from MAE showed lower IC_{50} (337.16 ± 8.45 ml extract L^{-1}) compared to other extraction methods ($p < 0.05$), indicating that significantly higher antioxidant activities of MAE with higher scavenging of DPPH radicals compared to UAE (IC_{50}: 437.45 ± 1.30 ml extract L^{-1}), CSE (IC_{50}: 357.36 ± 6.02 ml extract L^{-1}) and ASE (IC_{50}: 450.44 ± 4.48 ml extract L^{-1}) methods (Table 3). The higher activity of MAE extract could be explained by microwave treatment that affects the structure of the cell due to the sudden increase in temperature and internal pressure; which can be observed clearly under scanning electron microscope (Dahmoune, Boulekbache, Moussi, Aoun, Spigno, & Madani, 2013; Dahmoune, Nayak, Moussi, Remini, & Madani, 2015). The principle of volumetric heating using microwave energy is based on the direct effect of microwaves on molecules by ionic conduction and dipole rotation. This results in rapid rise of the temperature and fast completion of a reaction. Although ultrasound can break the cell wall with its cavitation power, releasing phenolic compounds into the extraction solvent, the quantity of release depends on the intensity and duration of application. In our study, the ultrasound parameters selected for treatment and extraction duration produced lower recovery of total phenolics and hence less antioxidant activity.

Using the ORAC assay, it was observed that antioxidant activities of extracts were statistically similar for MAE, UAE and CSE, but higher ($p < 0.05$) compared to the ASE (Table 3). The antioxidant activity of peel extracts using ASE was 337.97 ± 23.15 μmol TE g^{-1}. It was observed that ASE-extracts had the lowest fluorescence intensity during the test compared to other extraction methods. As noticed, the fluorescence signal declined

dramatically in the presence of a peroxyl generator (AAPH) beyond 15 min whereas in MAE, UAE and CSE-extracts the tendency was prolonged until 30 min. This mechanism in ASE extracts could explain the lowest antioxidant activity in the sample extracts (Figure S1).

3.5 HPLC-DAD analysis

The identification and quantification of individual phenolic compounds of *C. sinensis* extracts was based on a combination of retention times and calibration curve of external standards using a reverse phase C_{18} column in HPLC. The peaks of the phenolic compounds were detected at a wavelength of 280 nm. The elution times of gallic acid, chlrogenic acid, caffeic acid, ferulic acid, *p*-coumaric acid were 8.7, 23.5, 27.1, 39.5 and 58.6 min, respectively. Similarly, elution times of catechin, rutin and quercitin were 20.5, 38 and 54.5 min, respectively (Dahmoune, Nayak, Moussi, Remini, & Madani, 2015). None of the extraction methods provide a particular trend of quantity of phenolic compounds in the extract. While the quantity of chlorogenic acid (1535 μg g^{-1} DW), catechin (3037 μg g^{-1} DW) and rutin (1253 μg g^{-1} DW) were highest, gallic acid (85 μg g^{-1} DW) was lowest in CSE extracts compared to other extraction methods (Table 4) (Hayat, et al., 2010). Quercitin was not detected in any of the extracts. UAE provided higher recovery of gallic acid (210 μg g^{-1} DW) and *p*-coumaric acid (171 μg g^{-1} DW) than other methods (Table 4). Recovery of caffeic acid (815 μg g^{-1} DW) and ferulic acid (1455 μg g^{-1}DW) was highest in MAE extracts (Figure 3) compared to UAE, CSE and ASE (*figures S2, S3 and S4 in supplemental*). From the recovery of individual phenolic compounds using HPLC, it can be reported that CSE, MAE, UAE and ASE favor particular types of phenolic compounds. For example, MAE and ASE may have produced a harsh extraction condition for gallic acid; ultrasound could have a conducive environment whereas type or longer duration of extraction in CSE could have reduced the recovery of gallic acid. Overall, CSE followed by MAE provided the highest quantity of the

525 total of the selected individual phenolic compounds. In addition, a number of other phenolic
526 acids were also detected in the extracts that were not identified.

527 The results of this study contributed to lighten ways of valorization of the *Citrus*
528 *sinensis* peels based on the "six principles of Green Extraction of Natural Products" as
529 outlined by Chemat, Vian, and Cravotto (2012). Some of the major findings from this
530 investigation support the idea of green extraction. For example, (i) reduction in the
531 processing time (122, 500, 900 and 7200s for MAE, UAE, ASE and CSE, respectively), (ii)
532 reduction in the extraction solvent consumption, (iii) higher extraction recovery of TPC (at
533 the lab-scale batch process, the yield of TPC was 356.75, 305.41, 184.72 and 301.27 Kg
534 ton^{-1} h^{-1} for MAE, UAE, ASE and CSE, respectively), (iv) in the case of MAE, microwaves
535 are selectively absorbed by the residual water present in *Citrus Sinensis* peels (about 68 % of
536 moisture), and (v) possible customer acceptance of the by-products (peels) made through this
537 MAE "cleaner, greener" extraction technology.

538 Industrialization of the proposed techniques can be possible by experimental
539 validation and scaling up of the lab parameters in terms of extraction time, yield, chemical
540 composition and quality of environmentally friendly bioactive compounds to a pilot scale.
541 Small scale-up of extraction techniques have been reported for MAE (Petigny et al., 2014),
542 UAE (Achat et al., 2012) and ASE techniques in the literature. There are few reports
543 available on the use of MAE (Zhang, Yang, & Wang, 2011, Filly et al., 2014), UAE (Virot et
544 al., 2010) and ASE (Rabhi et. al., 2015) in the large-scale industrial processing of plant
545 secondary metabolites.

546 ***4 Conclusion***

547 While almost all of the agricultural and food industries are looking for products processed
548 from pulp or flesh of fruits and vegetables, few have put forth the effort to understand and
549 produce value-added products from downstream by-products. Extraction and standardization

of valuable bioactive phytochemicals are important to obtain most of their value by-products. Innovative technology assisted extractions tremendously reduce the extraction time. They have also been shown to use less extraction solvent for a particular bioactive phytochemical when conditions are optimized. The use of mathematical models can be an option to replace conventional extraction methods, providing optimal and predictable results when coupled with MAE. In our study, it was observed that *Citrus sinensis* peels are rich in phytochemicals with antioxidant activity. We established an improved and optimized procedure for extracting polyphenols from *C. sinensis* peels using MAE method. It was found that MAE not only provided higher recovery of TPC, but also quality phenolic compounds with rich antioxidant activity. In comparison of MAE with CSE, UAE and ASE extracts, it was observed that the mechanism of each extraction i.e. application of microwave or ultrasound or accelerated solvent has its own effects on selected individual phenolic compounds. Further studies concerning benefits of polyphenols from the *C. sinensis* peels are required before large scale utilization is recommended. For the industrial application, this research could be a basis for further pilot-scale trials of MAE as a green extraction technology for the recovery of high-added value compounds from biomass residues.

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691 **Figure captions**

692 **Figure 1.** Response surface analysis for the total phenolic yield from *Citrus sinensis* peels
693 with microwave assisted extraction with respect to solvent-solid ratio and extraction time (A);
694 solvent-solid ratio and acetone concentration (B); microwave power and acetone
695 concentration (C); solvent-solid ratio and microwave power (D); extraction time and acetone
696 concentration (E); microwave power and extraction time (F).

697 **Figure 2.** Antioxidant activity of *Citrus sinensis* peel extracts by microwave-assisted
698 extraction (MAE), conventional solvent extraction (CSE), ultrasound-assisted extraction
699 (UAE) and accelerated solvent extraction (ASE) as assessed using % inhibition of DPPH
700 radicals. The effective concentration of extracts required to scavenge DPPH radical by 50%
701 (IC_{50} value) was obtained by linear regression analysis.

702 **Figure 3.** Chromatograms of phenolic acids in different fractions (A: fraction 1; B: fraction
703 2; C: fraction 3; D: fraction 4) of microwave-assisted extracted (MAE) of *Citrus sinensis* peel
704 extracts. Phenolic compound 1: gallic acid; 2: chlorogenic acid; 3: caffeic acid; 4: ferulic
705 acid; 5: p-coumaric acid and 6: rutin. Elution times and maximum absorbance of individual
706 phenolic acids were determined using a reverse phase C18 column in HPLC. The mobile
707 phase A was a mixture of 6:94 (v/v) acetic acid in distilled water and mobile phase B
708 consisted of 100% HPLC grade acetonitrile. The solvent gradient in volume ratios was as
709 follows: 0–40 min, 0–25% B; 40–80 min, 25–85% B; 80–90 min, 85–100% B; 90-95 min,
710 100% B.

Table 1

Experimental design with the observed responses for the recovery of the TPC from *Citrus sinensis* peels using MAE. The codes (−1, 0, 1) and actual values for X_1 (40, 50, 60), X_2 (90, 120, 150), X_3 (400, 500, 600) and X_4 (20, 25, 30).

Run	X_1 Acetone Concentration (% v/v)	X_2 Extraction time (s)	X_3 Microwave power (W)	X_4 Solvent-solid ratio (mL g^{-1})	Recovery of TPC (mg GAE g^{-1} DW)
1	-1(40)	0 (120)	0 (500)	-1 (20)	9.99 ± 1.60
2	-1 (40)	1 (150)	0 (500)	0 (25)	8.83 ± 1.17
3	0 (50)	1 (150)	1 (600)	0 (25)	10.14 ± 2.07
4	0 (50)	1 (150)	0 (500)	1 (30)	9.22 ± 1.25
5	-1(40)	-1 (90)	0 (500)	0 (25)	8.49 ± 1.43
6	0 (50)	0 (120)	-1 (400)	1 (30)	10.25 ± 1.64
7	0 (50)	0 (120)	0 (500)	0 (25)	11.57 ± 1.28
8	0 (50)	-1(90)	-1 (400)	0 (25)	10.31 ± 1.66
9	-1(40)	0 (120)	1 (600)	0 (25)	9.76 ± 2.30
10	0 (50)	0 (120)	-1 (400)	-1 (20)	9.65 ± 1.13
11	0 (50)	0 (120)	1 (600)	1 (30)	10.41 ± 2.84
12	0 (50)	-1 (90)	0 (500)	1 (30)	10.22 ± 1.79
13	-1(40)	0 (120)	1 (600)	0 (25)	8.94 ± 1.69
14	0 (50)	-1 (90)	1 (600)	0 (25)	8.82 ± 2.07
15	-1(40)	1 (150)	0 (500)	0 (25)	9.92 ± 1.55
16	-1(40)	0 (120)	0 (500)	1 (30)	9.25 ± 1.58
17	-1(40)	0 (120)	-1 (400)	0 (25)	10.29 ± 1.39
18	0 (50)	1 (150)	-1 (400)	0 (25)	10.57 ± 1.26
19	-1(40)	0 (120)	0 (500)	-1 (20)	8.54 ± 2.36
20	0 (50)	-1(90)	0 (500)	-1 (20)	8.33 ± 1.36
21	-1(40)	0 (120)	-1 (400)	0 (25)	8.66 ± 2.93
22	0 (50)	1 (150)	0 (500)	-1 (20)	10.8 ± 2.45
23	0 (50)	0 (120)	0 (500)	0 (25)	11.81 ± 1.28
24	0 (50)	0 (120)	1 (600)	-1 (20)	9.62 ± 2.05
25	-1(40)	0 (120)	0 (500)	1 (30)	9.49 ± 2.24
26	-1(40)	-1(90)	0 (500)	0 (25)	9.70 ± 1.89
27	0 (50)	0 (120)	0 (500)	0 (25)	12.09 ± 1.71

GAE: gallic acid equivalents ; **TPC** : total phenolic contents ; **MAE** : microwave-assisted extraction.

Table 2

Analysis of variance (ANOVA) for the effects of acetone concentration, microwave power, extraction time and solvent-solid ratio on TPC of *Citrus sinensis* peels.

Parameter	Estimated coefficients	Standard error	*F*-value	Prob > *F*
Model			26.49	< 0.0001
Intercept				
B_0	12.1566	0.2062	58.94	<0.0001***
Linear				
X_1	0.4966	0.1031	4.82	0.0001***
X_2	0.2175	0.1031	2.11	0.0492*
Quadratic				
X_1^2	−1.7291	0.1568	−11.18	<0.0001***
X_2^2	−1.1679	0.1568	−7.55	<0.0001***
X_3^2	−0.9091	0.1568	−5.88	0.0001***
Interaction				
X_1X_4	−0.4225	0.1786	−2.36	0.0294*
X_2X_4	−0.8675	0.1786	−4.86	0.0001**
Lack of fit			0.24	0.9629
R^2			0.92	
$Adj\,R^2$			0.89	
C.V. %			3.96	
RMSE			0.35	

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **TPC**: total phenolic content; $\boldsymbol{X_1}$: Acetone concentration (% v/v); $\boldsymbol{X_2}$: Extraction time (s); $\boldsymbol{X_3}$: Microwave power (W); $\boldsymbol{X_4}$: Solvent-solid ratio ($mL.g^{-1}$).

Table 3

Comparison of the TPC and antioxidant activity (using DPPH radical scavenging asssay and ORAC-values) of *Citrus sinensis* peels using extraction methods of MAE, UAE, ASE and CSE. Results are expressed as means ± standard deviation.

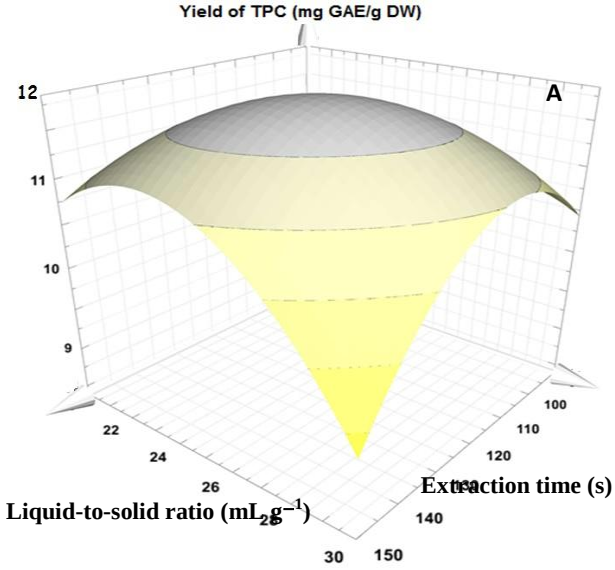
Extraction methods	TPC (mg GAE g^{-1} DW)	DPPH (IC_{50}, mL extract L^{-1})	ORAC of extract (µM TE g^{-1})
MAE	12.09 ± 0.06^{a}	337.162 ± 8.45^{c}	482.27 ± 57.43^{a}
UAE	10.35 ± 0.04^{b}	433.084 ± 7.62^{a}	456.94 ± 35.09^{a}
ASE	6.26 ± 0.23^{c}	450.443 ± 9.49^{a}	337.97 ± 23.15^{b}
CSE	10.21 ± 0.01^{b}	358.456 ± 5.15^{b}	523.04 ± 48.16^{a}

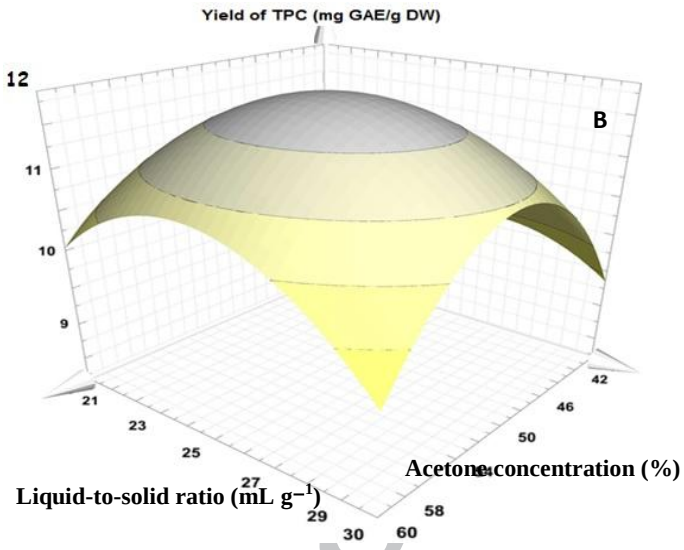
Same letters in the same column refer to means not statistically different according to ANOVA and Tukey's test; **TPC**: total phenolic content, **GAE**: gallic acid equivalents; **DW** : dry weight; **TE** : trolox equivalent ; **MAE** : microwave-assisted extraction ; **UAE** : ultrasound-assisted extraction ; **ASE** :accelerated solvent extraction ; **CSE** : conventional solvent extraction.

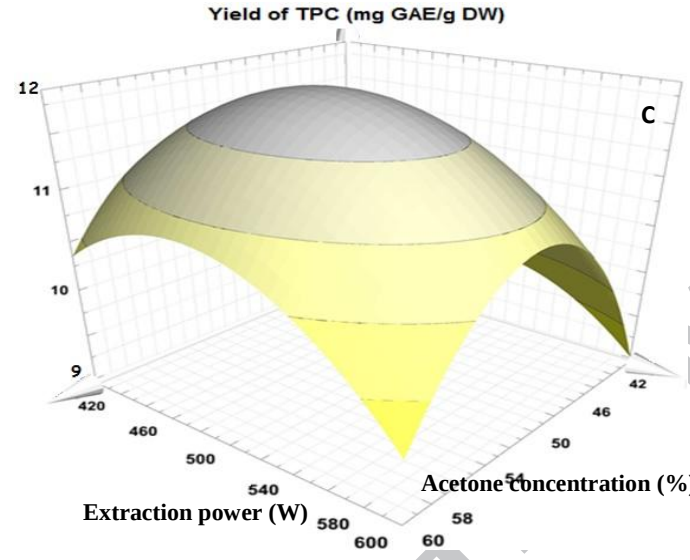
Table 4 Quantity of selected individual phenolic compounds (μg g^{-1} DW) in CSE, MAE, UAE and ASE *Citrus sinensis* peel extracts.

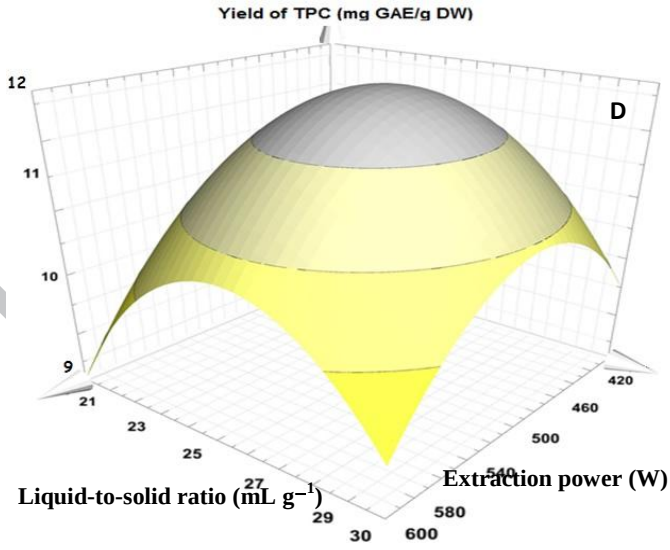
	Extraction methods	Gallic acid	Chlorogenic acid	Caffeic acid	*p*-coumaric acid	Ferulic acid	rutin	Quercetin	Catechin	SPC
Fraction 1*	**CSE**	ND	216.34	59.29	ND	ND	ND	ND	ND	275.64
	MAE	ND	210.12	62.52	ND	ND	ND	ND	ND	272.64
	UAE	64.85	455.46	127.82	ND	17.12	ND	ND	ND	648.14
	ASE	44.20	187.55	59.30	ND	ND	ND	ND	ND	291.06
Fraction 2**	**CSE**	85.28	796.41	192.07	ND	ND	ND	ND	201.45	1275.21
	MAE	142.69	679.12	166.38	ND	ND	ND	ND	ND	988.20
	UAE	145.80	837.06	47.62	ND	ND	ND	ND	521.47	1551.95
	ASE	70.81	315.41	80.01	ND	ND	ND	ND	ND	466.24
Fraction 3**	**CSE**	ND	109.42	284.69	5.08	1227	161.09	ND	ND	1787.27
	MAE	ND	273.92	417.11	23.66	1356	199.57	ND	533.91	2270.6
	UAE	ND	74.83	197.85	5.54	623	74.54	ND	ND	976.51
	ASE	ND	253.46	174.98	ND	ND	ND	ND	ND	428.45
Fraction 4**	**CSE**	ND	413.61	235.50	41.05	ND	1092.66	ND	2836.06	4618.89
	MAE	ND	224.97	169.93	101.28	99.22	389.56	ND	1969.69	2954.65
	UAE	ND	74.84	130.07	165.93	128.27	908.24	ND	463.06	1870.43
	ASE	ND	366.47	221.07	30.71	327.91	1155.51	ND	ND	2101.68
Total	**CSE**	85.28	1535.78	771.55	46.14	1227	1253.75	ND	3037.51	7957.02
	MAE	142.69	1388.13	815.95	124.95	1455	589.13	ND	2503.60	7020.01
	UAE	210.65	1442.19	503.36	171.47	769	982.79	ND	984.54	5064.15
	ASE	115.01	1122.91	535.38	30.71	327.91	1155.51	ND	ND	3287.44

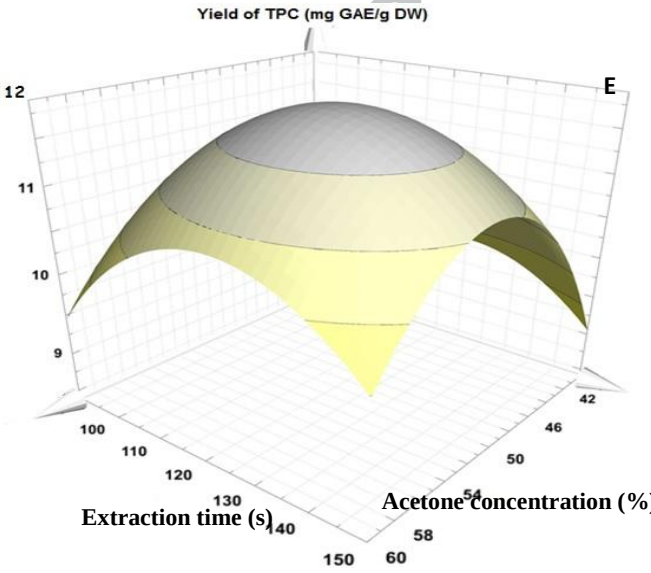
SPC: sum of individual phenolic content; **ND**: Not Detected; *****: the fraction eluted from the crude extract; ******: Fractions 2, 3 and 4 obtained from elution with acidified distilled water, ethyl acetate and acidified methanol, respectively.

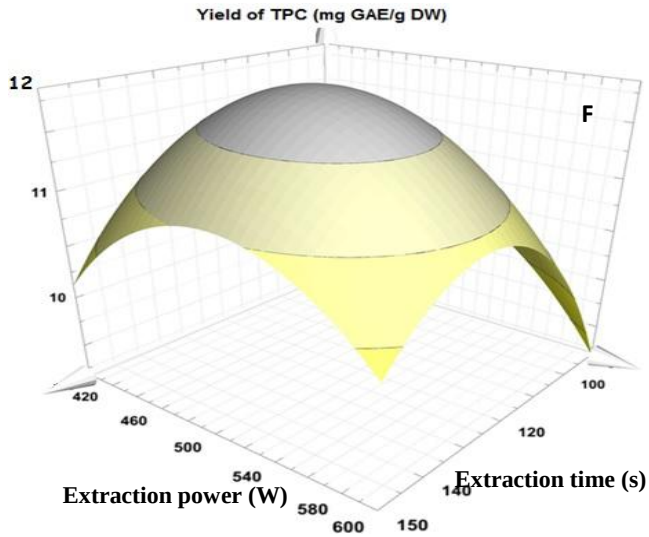


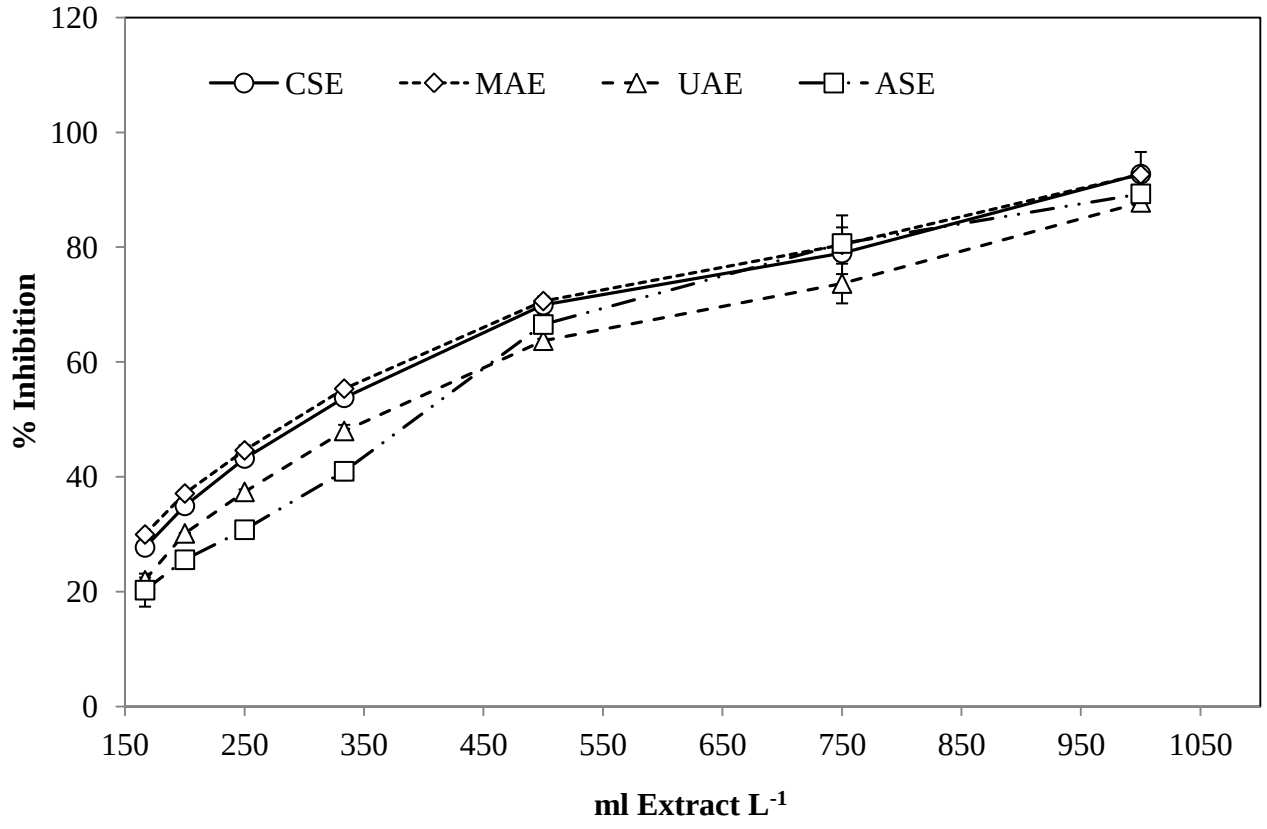


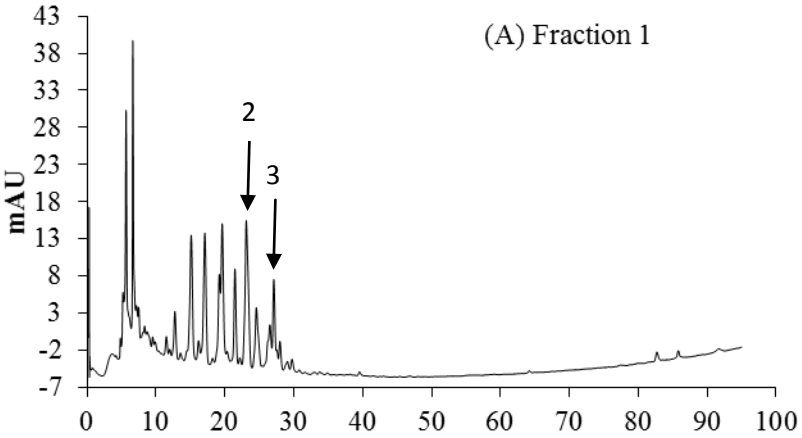


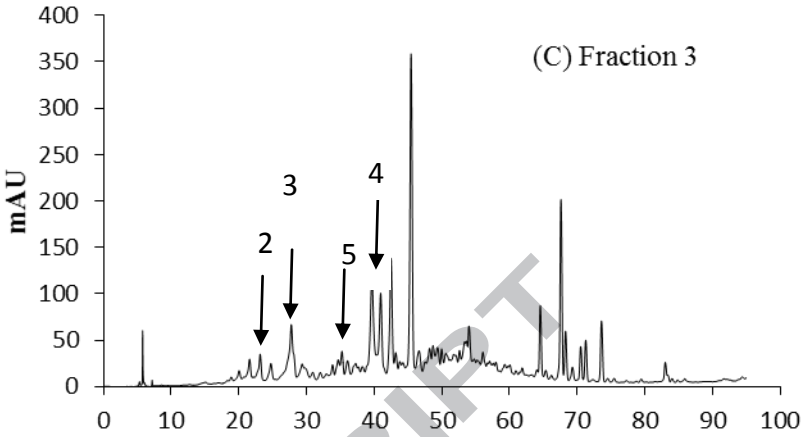


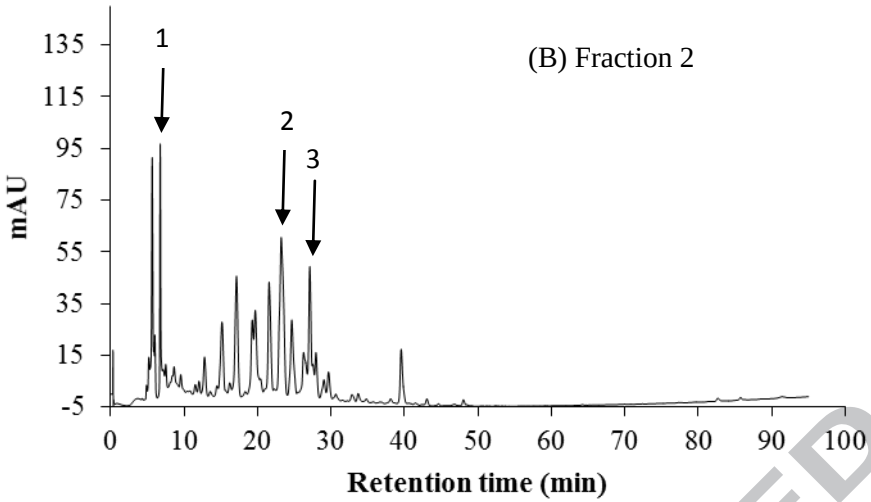


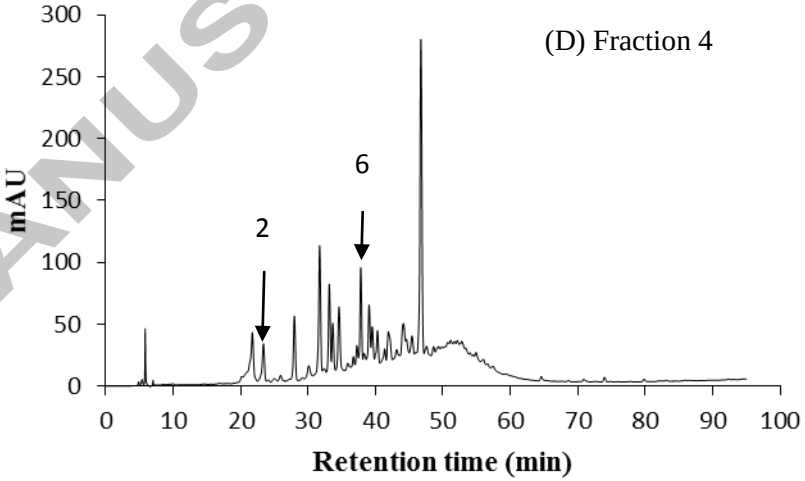












Highlights

- RSM was applied to optimize TPC extraction from *C. sinensis* peels using MAE.
- MAE method was optimized and compared to UAE, CSE and ASE in term of TPC.
- Antioxidant activity of peels using DPPH and ORAC methods retained in MAE.
- Individual phenolic compounds identified in four fractions of peel extracts.