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Valorization of *Citrus limon* residues for the recovery of antioxidants: Evaluation and optimization of microwave and ultrasound application to solvent extraction

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ABSTRACT

Ultrasound assisted extraction (UAE) and microwave-assisted extraction (MAE) were optimized (by response surface methodology, RSM) and compared for the recovery of total phenolic compounds (TPC expressed as gallic acid equivalents (GAE)) from *Citrus limon* peels. The optimized result for MAE was 48% ethanol as extraction solvent, 28:1 mL/g of solvent: solid ratio, 123 s and 400 W for irradiation time and power. The optimized result for UAE was 63.93% ethanol as extraction solvent, 40 mL/g of liquid/solid ratio, 15.05 min of holding time and 77.79% for amplitude. Maximum predicted TPC recoveries under the optimized conditions for MAE and UAE were 15.74 and 15.08 mg GAE/g respectively, which were close to the experimental values of 15.78 ± 0.8 and 15.22 ± 0.88 mg GAE/g, indicating suitability of the employed model and the success of RSM in optimizing the extraction conditions. The antioxidant activity determined by the DPPH and reducing power tests confirmed the suitability of MAE for the preparation of antioxidant-rich plant extracts.

1. Introduction

Citrus is the most important fruit crop in the world. The production of citrus fruits has been increasing enormously in the last few decades, going from an average of 48 million tons a year in the period 1970–1979, to more than 100 million tons in 2004–2005 (Dugo and Di Giacomo, 2002; González-Molina et al., 2010). Citrus fruits are cultivated in more than 100 countries all over the world, mainly in tropical and subtropical areas, where favorable soil and climatic conditions prevail (Dugo and Di Giacomo, 2002).

Among fruit and vegetables, citrus fruits are reported to be a very rich source of health promoting substances (Artés-Hernández et al., 2007). Although their health-related properties have always been associated with their content of vitamin C, it has recently been shown that flavonoids also play a role in this respect. In ancient medicine *Citrus limon* (*C. limon* Burm) and melissa (*Melissa officinalis* L.) have long been used as natural insect repellents (Oshaghi et al., 2003).

Citrus is an important crop mainly used in food industries for fresh juice production. Peels, the main waste fraction of citrus fruits, represent roughly half of the fruit mass and have been widely studied because they contain numerous biologically active compounds including natural antioxidants such as phenolic acids and flavonoids (Hayat et al., 2009, 2010).

The first step for both analysis and exploitation of medicinal plant bioactive constituents is their extraction from the cellular matrix. The "ideal" extraction method should be quantitative, non-destructive, and time saving. Besides conventional solvent extraction processes commonly used for the recovery of phenolic compounds (Proestos et al., 2006), non-conventional, more rapid and automated methods have been recently used, e.g. supercritical fluid extraction (SFE), pressurized liquid extraction (PLE), microwave-assisted extraction (MAE) and ultrasound assisted extraction (UAE) (Aybaster et al., 2013; Liazid et al., 2007). Actually, the state of the art in these fields has shown that ultrasound and microwave radiation could accelerate the extracting process improving bioactive compounds extraction, particularly phenolic compounds (Garofulić et al., 2013; Muñiz-Márquez et al., 2013; Morelli and Prado, 2012; Li et al., 2011).

MAE is attractive because it allows for rapid heating of aqueous samples and presents advantages over conventional extraction techniques, such as improved efficiency, reduced extraction time, lower solvent consumption, higher selectivity toward target

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molecules and higher level of automation (Han et al., 2011; Singh et al., 2011; Zhang et al., 2008). In addition of these advantages, a wider range of solvents can be used in MAE, as the technique is less dependent on solvent affinity (Yang and Zhai, 2010).

UAE is particularly attractive for its simplicity, low cost of equipment (Carrera et al., 2012), efficiency in extracting analytes from different matrices (Herrera and de Castro, 2005), low energy required and reduced solvent- and time-consuming method (Xia et al., 2011). The enhancement of the extraction process by ultrasounds is attributed to the disruption of the cell walls, reduction of the particle size and the increase of the mass transfer of the cell content to the solvent caused by the collapse of the bubbles produced by acoustic cavitation (Xia et al., 2011; Rodrigues et al., 2008).

To our best knowledge, no literature report exists on the optimization and comparison of MAE and UAE procedure for the extraction of total phenolic compounds (TPC) from *C. limon* peels. Therefore, the objectives of the current study were to:

- investigate the effects of different parameters on the extraction efficiency (in terms of recovery and antioxidant activity of total phenolic compounds) by MAE and UAE processes;
- optimize the MAE and UAE conditions by response surface methodology (RSM);
- compare the optimized MAE and UAE results with a reference Conventional Solvent Extraction procedure (CSE).

2. Materials and methods

2.1. Plant material

The fruit samples of *C. limon* were collected in the area of Sidi Aich (Bejaia, Algeria), washed with distilled water and peeled off with hands. Peels were dried for about 15 days at room temperature in a ventilated darkroom to protect the active compounds content from light oxidation. Dried peels were ground with an electrical grinder (IKA model A_{11} Basic, Germany), the powder was passed through standard 125 μm sieve and only the fraction with particle size <125 μm was used. The powder was stored in airtight bags until use. The water activity (a_w) was determined by HygroPalm AW and was 0.25 ± 0.02 at 23 °C.

2.2. Reagents

Sodium carbonate (Na_2Co_3), Folin–Ciocalteau's phenol reagent and disodium hydrogen phosphate (Na_2HPO_4) were obtained from Prolabo (made in CE) and 1,1-diphenyl-2-picryl-hydrazil (DPPH) from Sigma Aldrich (Germany). Gallic acid, ferric chloride ($FeCl_3{\cdot}6H_2O$), potassium ferricyanide ($C_6N_6FeK_3$), trichloroacetic acid and sodium dihydrogen phosphate (NaH_2PO_4) were purchased from Biochem-chemopharma (UK). All solvents used were of analytical grade and purchased from Prolabo (CE).

2.3. Experimental work

For optimization of the MAE and UAE procedure, the influences of the process parameters were firstly separately investigated in single-factor experiments to limit the total experimental work (Tables 1 and 2). When one variable was not studied, it was kept constant. In the MAE trials, the constant values for irradiation time, solvent-to-solid ratio and ethanol concentration were 120 s, 20 mL/g and 50%, respectively. Microwave power was set at 500 W in the trials to investigate the influence of solvent type and ethanol concentration, and at 400 W in the trials to investigate the influence of solvent-to-solid ratio. In the UAE trials, the constant values of sonication time, solvent-to-solid ratio and ethanol concentration were 10 min, 40 mL/g and 50%. Radiation amplitude was set at 50% in the trials to investigate the influence of ethanol concentration and sonication time, and at 60% in the trials to investigate the influence of solvent-to-solid ratio.

On the basis of the single-factor experimental results, major influence factors were selected. Then, an RSM based on a Box–Behnken Design (BBD) for MAE and Central Composite Rotatable Design (CCRD) for UAE was conducted to optimize both processes (Tables 3 and 4) (Vázquez et al., 2012; Wu et al., 2013). Regression analysis of the data to fit a second-order polynomial equation (quadratic model) was carried out according to the following general equation (Eq. (1)) which was, then, used to predict the optimum conditions of extraction process.

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

where Y represents the response function (in our case the TPC yield); B_0 is a constant coefficient; B_i, B_{ii} and B_{ij} are the coefficients of the linear, quadratic and interactive terms, respectively, and X_i and X_j represent the coded independent variables. According to the analysis of variance, the regression coefficients of individual linear, quadratic and interaction terms were determined. In order to visualize the relationship between the response and experimental levels of each factor and to deduce the optimum conditions, the regression coefficients were used to generate 3-D surface plots from the fitted polynomial equation. The factor levels were coded as −1 (low), 0 (central point or middle) and 1 (high), respectively. The variables were coded according to the following equation (Eq. (2)):

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

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

Analysis of variance was performed for the response variable using the full model 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 optimal conditions predicted with the RSM and the obtained experimental data were compared to the values predicted by the regression model.

Efficiency of the two non-conventional methods (MAE and UAE) was compared based on the TPC recovery and the quality of the recovered phenolic compounds in terms of antioxidant activity (according to DPPH assay and Fe reducing ability) which was measured only on the extracts obtained under the optimum conditions selected by RSM.

Optimized MAE and UAE conditions were then compared to a reference CSE procedure.

Finally, in order to investigate the influence of ultrasound, microwave and maceration on the microstructure of the samples powder, the peel powder samples (before and after the different extraction processes) were observed by scanning electron microscopy (SEM).

2.3.1. Microwave assisted extraction

A domestic microwave oven (NN-S674MF, Samsung, Malaysia) with cavity dimensions of 22.5 cm × 37.5 cm × 38.6 cm and 2450 kHz working frequency was used. The apparatus was equipped with a digital control system for irradiation time and microwave power (the latter linearly adjustable from 200 to 1000 W). The oven was modified in order to condensate into the sample the vapors generated during extraction giving a constant sample volume.

For the extraction, one gram of the peel powder was placed in a 250 mL volumetric flask containing the extraction solvent. The suspension was irradiated at regular intervals according to