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Effect of different operating conditions on the extraction of phenolic compounds in orange peel

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ABSTRACT

The effects of different operating conditions on four extraction techniques were studied and compared. The criteria analyzed were total phenol and flavonoid contents, individual flavonoids and antioxidant activity. The common operating conditions of extraction are ratio m/v: 5 g:50 ml; 80% ethanol with mechanical agitation. It appears that the highest values for the total phenol and flavonoid contents were reached when ultrasound assisted extraction (UAE) was carried out at 125 W during 30 min at 35 °C, microwave assisted extraction (MAE) at 200 W during 180 s, supercritical CO_2 extraction (SCE) at 80 °C, 10 MPa and high pressure extraction at 50 MPa during 30 min at 35 °C. These conditions are not optimum to obtain the highest antioxidant activity for MAE (300 W) and HPE (100 MPa).
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1. Introduction

In order to substitute synthetic additives such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), due to their carcinogenic (Ak and Gülçin, 2008) and toxic effects (Moure et al., 2001), natural compounds have received a great deal of interest over last few years. Among these compounds, phenolic extracts from orange peel seemed to be an interesting alternative. In fact, these extracts exhibit anti-carcinogenic, anti-inflammatory, antioxidant and anti-atherogenic properties (Ghasemi et al., 2009) due to the presence of phenolic acids and flavonoids (Bocco et al., 1998). Depending on orange variety, the total phenol contents varied from 0.67 to 19.62 g/100 g dry basis (Magda et al., 2008; Goulas and Manganaris, 2012). The limiting step to the use of these compounds is their extraction from the raw material. Different techniques have been reported such as solvent extraction, hot water extraction, alkaline extraction, enzyme-assisted extraction, ultrasound assisted extraction, microwave assisted extraction, high hydrostatic pressure and supercritical fluid extraction (M'hiri et al., 2014). However, the majority of the studies on these techniques have been conducted to increase the extraction efficiency without taking the behavior of the biological activities of these molecules into account. The phenolic compounds are sensitive to their environment, so the application of high temperature and/or pressure can lead to their degradation or alter their biological activities. The purpose of this work is to compare various techniques of extraction (conventional solvent extraction (CSE), microwave assisted extraction (MAE), ultrasound assisted extraction (UAE), high hydrostatic pressure extraction (HPE) and supercritical fluid extraction (SCE)) and analyze their effects both on the efficiency of the extraction and on the antioxidant activity of the extract.

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2. Material and Methods

2.1. Plant material and sample preparation

About 20 kg of fresh oranges (*Citrus sinensis*) of Maltese variety were collected in March 2012 from Manzel Bouzalfa (Nabeul, Tunisia) in their commercial maturity. All fruits were of eating quality, and without blemishes, or damage. On arrival at the laboratory, the orange fruits were immediately washed using tap water, and peeled. The remaining orange peel accounted for approximately 40% of the total fruit. The peels were stored at −80 °C before any further treatments. Orange peels were dehydrated using a freeze dryer (CHRIST Alpha 1-2 LD, France) for 72 h (at −50 °C and 0.001 mbar) and then finely ground using a coffee grinder (Moulinex®, France) to achieve a standard size of particles of ∼0.315 mm. The orange peel powder was placed in vacuum packaging bags and stored in a freezer maintained at 4 °C before experiments.

2.2. Chemicals and reagents

All chemicals were of analytical or HPLC grade purity. Standards of eriocitrin, narirutin, naringin, hesperidin, neohesperidin, didymin, sinensetin, nobiletin, tangeretin and 3′,4′,5,5′6,7,-hexamethoxyflavone were purchased from Extrasynthese® (Lyon, France). High-purity water was produced in the laboratory using an Alpha-Q system® (Millipore, MA). Potassium persulfate was purchased from Fluka® (Switzerland). Rutin, sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azinobis (3 ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), Folin-Ciocalteu's phenol reagent, sodium carbonate (Na_2CO_3), and gallic acid monohydrate (GA) (purity ≥ 98.0%) were purchased from Sigma-Aldrich® (Germany). Sodium hydroxide (NaOH) and methanol were obtained from Carlo Erba-SDS® (France). Ethanol (purity ≥ 95.0%) and acetic acid were obtained from VWR® (Belgium).

2.3. Extraction methods

2.3.1. Operating conditions of each technique

For each technique except for HPE, the extraction step was repeated three times. For UAE, MAE, SCE and HPE, the experimental domain was defined taking into account the operative limits of the instrument and the preliminary experiments.

2.3.1.1. *Conventional extraction by solvent (CSE)*. Five grams of *C. sinensis* peel powder were extracted with 50 ml of 80% ethanol. The mixture was shaken at 200 rpm in darkness using a mechanical stirrer for 30 min at 35 °C.

2.3.1.2. *Ultrasound assisted extraction (UAE)*. Five grams of *C. sinensis* peel powder were extracted with 50 ml of 80% ethanol in the ultrasound sonicator (VibraCell 75115, Bioblock-Fisher, Illkirch, France) with maximum input power of 200 W. Extraction was carried out for 30 min at 35 °C. The ultrasonic power levels used were 100, 125, 150 and 200 W. The mixture was shaken in darkness and was stirred at the same time using a magnetic stirrer.

2.3.1.3. *Microwave assisted extraction (MAE)*. A laboratory scale microwave extraction apparatus (Multiwave 3000, Microwave Reaction System, Graz, Austria) operated under high pressure and temperature rates was used for extraction. The apparatus was equipped with a digital controlled system for temperature, time and power. Five grams of *C. sinensis* peel powder were extracted with 50 ml of 80% ethanol. Samples were heated for 180 s at 100, 200, 300 or 400 W. The sample temperatures were measured by using a thin thermocouple for each power: 100 W corresponds to 67 °C, 200 W to 76 °C, 300 W to 92 °C and 400 W to 108 °C.

2.3.1.4. *Supercritical CO_2 extraction (SCE)*. Extraction was carried out using a pilot scale extractor (ENSIC, LRGP, Nancy, France) with supercritical CO_2 as a solvent and a maximal pressure of 250 bar. Five grams of *C. sinensis* peel powder were placed in a 50 ml extraction vessel. 80% of aqueous ethanol was chosen as a modifier. Extraction was carried out for 30 min at 35–80 °C and a pressure of 10–22 MPa. The CO_2 flow rate was kept at approximately 15 g/min by adjusting the outlet valve manually.

2.3.1.5. *High pressure extraction (HPE)*. Five grams of *C. sinensis* peel powder were extracted with 50 ml of 80% ethanol in a 3 L reactor unit (ACB Pressure Systems, Nantes, France). Extraction was carried out for 30 min at 35 °C and a pressure of 0.1, 50, 100 MPa.

2.3.2. Post extraction processing

The crude extract provided by each technique was cooled at room temperature, centrifuged at 8000 × *g* for 10 min and the supernatant was filtered through a Millipore paper (0.22 μm). The obtained samples were stored at 4 °C.

2.4. Analytical methods

2.4.1. Determination of total phenol contents (TPC)

Total phenolic compounds were determined colorimetrically at 765 nm and expressed as gallic acid equivalent (GAE), according to the method described by Singleton et al. (1999). The samples were added to Folin-Ciocalteu reagent and Na_2CO_3 solution and placed in a water bath at 40 °C for 30 min before spectrophotometric analysis (spectrophotometer Genesys 10uv screening, Thermo Electron Corporation, France). Total phenol content was expressed as mg of gallic acid equivalent (GAE) per kg of dry matter (DW).

2.4.2. Determination of total flavonoid contents (TFC)

Total flavonoid contents were determined following the modified procedure of Zhishen et al. (1999). 0.5 ml of aqueous extract was placed in a 5 ml volumetric flask, then 2.5 ml of distilled water were added, followed by 0.15 ml of 5% $NaNO_2$. After 5 min, 0.15 ml of 10% $AlCl_3$ were added. 5 min later, 1 ml of 1 M NaOH were added and the volume made up with distilled water. The solution was mixed and absorbance was measured at 510 nm using a spectrophotometer (Genesys 10uv screening, Thermo Electron Corporation, France). Total flavonoid contents were expressed as rutin equivalent g/kg of dry matter.

2.4.3. Determination of antioxidant activity by ABTS assay

The free radical scavenging activities of orange peel extracts were determined by ABTS radical cation decolorization assay (Re et al., 1999) with minor modifications. A stable stock solution of ($ABTS^{\bullet+}$) was produced by reacting a 7 mmol/L aqueous solution of ABTS with 2.45 mmol/L potassium

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