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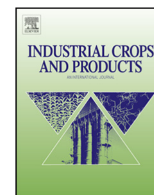

Application of response surface methodology for optimizing the recovery of phenolic compounds from hazelnut skin using different extraction methods

Halil İbrahim Odabaş[a,b,*], Ilkay Koca[b]

[a] Gümüşhane University, Faculty of Engineering and Natural Sciences, Department of Food Engineering, Gümüşhane, Turkey
[b] Ondokuz Mayıs University, Faculty of Engineering, Department of Food Engineering, Samsun, Turkey

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ABSTRACT

Hazelnut skin which is a rich source of phenolic compounds is a by-product of hazelnut roasting process. In present study, ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE) and supercritical carbon dioxide extraction (SCE) methods were applied for recovery of phenolic compounds from hazelnut skin. Response surface methodology was used to estimate optimum extraction conditions. Extraction studies were performed according to Box-Behnken Design. Temperature (UAE)/power (MAE), time and ethanol concentration were independent variables for UAE and MAE. The independent variables selected for SCE were temperature, time and pressure. The optimum extraction conditions were 45 min and 67.2–67.6% ethanol concentration, 600 W, 6 min and 55.03–56.23% ethanol concentration and 42.72–49.10 °C, 59.83–60.00 min and 10.01–11.48 bar, for UAE, MAE and SCE respectively. Total phenolic content, FRAP and $1/EC_{50}$ values at the optimum conditions were 122.99–123.01 mg GAE/g, 612.20–613.25 mmol Fe(II)/g and 4.36 mL/mg for UAE, 111.53–111.55 mg GAE/g, 582.44–582.52 mmol Fe(II)/g and 2.48 mL/mg for MAE and 69.59–72.64 mg GAE/g, 426.25–465.52 mmol Fe(II)/g and 2.18–2.27 mL/mg for SCE, respectively. Maceration was performed for comparison with novel methods. UAE was found to be the best method of the extraction of phenolic compounds from hazelnut skin with the highest total phenolic content and antioxidant activity values.

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1. Introduction

Hazelnut (*Corylus avellana* L.) which belongs the Betulacae family is an important ingredient for processed foods, such as bakery, confectionery and dairy products. Only a small quantity of annual hazelnut production (~10%) is consumed raw (Dervisoglu, 2006; Schmitzer et al., 2011). Hazelnut processing, which includes harvesting, cracking, shelling/hulling, and roasting processes, generates by-products such as hazelnut skin, hazelnut hard shell, hazelnut green leafy cover and hazelnut tree leaf (Shahidi et al., 2007). Hazelnut skin is a by-product of roasting process and represents about 2.5% of the total hazelnut kernel weight (Alasalvar et al., 2009). Currently, hazelnut skin has no commercial value. Therefore, finding a feasible way to evaluate this waste product has great importance for the hazelnut industry.

Previous studies have shown that hazelnut and hazelnut by-products are rich sources of phenolic compounds (Alasalvar et al., 2006; Shahidi et al., 2007; Stevigny et al., 2007; Contini et al., 2008; Alasalvar et al., 2009; Del Rio et al., 2011; Altun et al., 2013). In addition, the majority of hazelnut phenolics are located in the hazelnut skin (Shahidi et al., 2007). The high antioxidant activity of hazelnut skin extracts were measured as 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity (Shahidi et al., 2007; Contini et al., 2008; Contini et al., 2009; Alasalvar et al., 2009; Locatelli et al., 2010; Contini et al., 2012; Montella et al., 2013), hydrogen peroxide and superoxide radical scavenging activity (Shahidi et al., 2007), antiperoxyl radical efficiency (Contini et al., 2008), ferrous chelating capacity and ferric reducing ability (Contini et al., 2009) in different studies. Hazelnut skin phenolic extract has better DPPH radical scavenging activity than most common natural (α-tocopherol) and synthetic (BHA, BHT) antioxidants (Contini et al., 2008; Contini et al., 2012). Furthermore, *in vivo* studies indicate

* Corresponding author at: Department of Food Engineering, Faculty of Engineering and Natural Sciences, Gümüşhane University, 29100, Gümüşhane, Turkey.
E-mail address: hiodabas@gumushane.edu.tr (H.İ. Odabaş).

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that the phenolic extracts obtained from hazelnut skin are biologically active in rats (Contini et al., 2009). Hazelnut skin phenolics are formed mainly monomeric and oligomeric flavan-3-ols (95%). Flavonols, dihydrochalcones (3.5%) and phenolic acids (<1%) are other phenolic subclasses in hazelnut skin (Del Rio et al., 2011). Flavan-3-ols exhibit some beneficial effects to human health with their antioxidant, anticarcinogen, cardiopreventive, anti-microbial, anti-viral and neuro-protective properties (Aron and Kennedy, 2008). Thus, the phenolic extract obtained from hazelnut skin could potentially be used as a natural antioxidant, functional ingredient and dietary supplement in food and pharmaceutical industries (Shahidi et al., 2007; Alasalvar et al., 2009).

The conventional methods for extracting phenolic compounds are commonly solvent extraction, heat reflux and Soxhlet extraction (Bai et al., 2010). Consumption of a large amount of organic solvent (generally ethanol, methanol, acetone, dimethylformamide) and the long extraction time are the main disadvantages of these methods (Li et al., 2005; Joana Gil-Chávez et al., 2013). Longer extraction times and using heat, increase the chance of oxidation, ionization and hydrolysis of phenolic compounds (Li et al., 2005; Naczk and Shahidi, 2004). In this context, various novel methods such as ultrasound-assisted extraction (Muniz-Marquez et al., 2013), microwave-assisted extraction (Wu et al., 2012), supercritical fluid extraction (Castro-Vargas et al., 2010), subcritical fluid extraction (Adil et al., 2007) and pressurized liquid extraction (Santos et al., 2012a) have been used for the extraction of phenolic compounds from plants. Extraction yield enhancement, reduction of solvent consumption, reduction of extraction cost, reduction of pollution to environment and reduction of extraction temperature are main advantages of UAE (Tao and Sun, 2015). MAE has advantages such as shortened extraction time and reduced solvent consumption (Bhuyan et al., 2015). SCE is the effective method with several advantages over conventional methods such as reduction the need for organic solvents, high selectivity, reduction of extraction time and easy separation of CO_2 from the product (Lang and Wai, 2001).

Many authors researched the extraction of phenolic compounds from hazelnut skin by conventional methods (Contini et al., 2008; Alasalvar et al., 2009; Locatelli et al., 2010). However there is limited research has been published about the use of novel extraction methods for the recovery of phenolic compounds from hazelnut skin. In addition to the best of our knowledge, no research has been done on the optimization and comparison of UAE, MAE and SCE process from hazelnut skin. Thus, the objectives of this study are to (1) evaluate the hazelnut skin by extracting phenolic compounds using novel methods (2) optimize UAE, MAE and SCE conditions for the hazelnut skin based on total phenolic content and antioxidant activity by response surface methodology; (3) compare the extracts obtained by novel methods with a conventional method (maceration) in terms of total phenolic content and antioxidant activity.

2. Materials and methods

2.1. Materials

Hazelnut skins were obtained from the Fiskobirlik Integrated Hazelnut Processing Plant (Giresun, Turkey). The skins were filled in the polyethylene bags and delivered to the laboratory in a cooler. Hazelnut skins were ground using a blender (Waring Laboratory, Torrington, USA) and passed through a 1 mm sieve. Ground skins were defatted for 6 h using diethyl ether by Soxhlet apparatus. Defatted skins were dried in a vacuum oven (Nüve, Ankara, Turkey) to remove diethyl ether at 40 °C for 1 h and stored in polyethylene bags at −18 °C during the experiments. All chemicals and reagents were analytical grade. Diethyl ether, Folin–Ciocalteau reagent, hydrochloric acid, glacial acetic acid, gallic acid, sodium carbonate, sodium acetate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Iron(II) sulfate heptahydrate and Iron(III) chloride hexahydrate (Sigma Aldrich Chemie GmbH, Steinheim, Germany); 2,4,6-tripyridyl-s-triazine (TPTZ) (Acros Organics, New Jersey, USA) and ethanol (Carlo Erba, Milan, Italy) were used in the experiments.

2.2. Methods

2.2.1. Ultrasound assisted extraction (UAE)

An ultrasonic processor (VC 1500, Sonics and Materials Inc., Newtown, USA) with a 13 mm diameter probe was used for ultrasound assisted extraction. The probe was immersed to a depth of 25 mm in the sample. Samples were processed at a fixed power (1500 W), amplitude level (60%) and frequency (20 kHz). 1 g of defatted hazelnut skin sample was placed in a glass beaker and 50 mL of solvent was added. After extraction, the beaker was cooled to room temperature. The extract was filtered through Whatman paper No 1 and the solution was collected in a dark flask.

2.2.2. Microwave assisted extraction (MAE)

Defatted hazelnut skin sample (3 g) was taken into a 500 mL round bottom flask and mixed with 150 mL solvent. A household microwave oven (MW71B, Samsung Electronics Ltd., Seoul, South Korea) with some modifications was used for microwave assisted extraction (Fig. 1). Frequency of microwave oven was constant and at 2450 MHz. After extraction, the flask was cooled to room temperature. The extract was filtered through Whatman paper No 1 and the solution was collected in a dark flask.

2.2.3. Supercritical carbon dioxide extraction (SCE)

A supercritical fluid extractor (Spe-ed SFE-2, Applied Seperations Inc., Pennsylvania, USA) was used in this study. Extraction system has two pumps, main pump fitted with a chiller on the pump head for addition of liquid CO_2 and a second pump (LabAlliance Model 1500, Scientific Systems Inc., Pennsylvania, USA) for the addition of co-solvent. 80% aqueous ethanol was used as co-solvent in this study. SC-CO_2 and co-solvent was pumped at a fixed flow rate (2 L/min and 0.5 mL/min respectively) at the all extraction points. 2 g of defatted hazelnut skin sample was placed in the 10 mL of stainless steel extraction vessel. Extraction was started after reaching the desired pressure and temperature. After each extraction, the extract was collected in a glass vial.

2.2.4. Maceration (CSE)

Defatted hazelnut skin (1 g) was extracted in a glass beaker with 50 mL of ethanol (50%, 70%, 90%) at a refrigerator (+4 °C) for 24 h. The extract was filtered through Whatman paper No 1 and the solution was collected in a dark flask.

2.2.5. Experimental design and statistical analysis

The three level Box-Behnken design with three factors was carried out to optimal levels of X_1 (Temperature for UAE and SCE, Power for MAE), X_2 (Extraction time for UAE, MAE and SCE) and X_3 (Ethanol concentration for UAE and MAE, Pressure for SCE). Actual and coded values of the independent variables are shown in Table 1. Combinations of temperature (20, 40,60), extraction time (15, 30, 45 min), ethanol concentration (50, 70, 90%); power (300, 450, 600 W), extraction time (2, 4, 6 min), ethanol concentration (50, 70, 90%); temperature (40, 50, 60 °C), pressure (10, 25, 40 bar), extraction time (20, 40, 60 min) were selected as independent variables for UAE, MAE and SCE, respectively. Total phenolic content, FRAP and DPPH radical scavenging activity (as $1/EC_{50}$) values of the extracts taken as the responses (Y) for the design experiment. The 15 experimental points including three replicates at the central

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