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Evaluation of physicochemical properties and antioxidant activities of Persian walnut oil obtained by several extraction methods

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ABSTRACT

An analytical study on the fatty acids, physicochemical characteristics, phenolic compounds, tocopherol content and antioxidant activities of walnut oils extracted by different extraction methods (maceration, Mac; modified Bligh–Dyer, MBD; and cold-press, CP) was conducted. Antioxidant activity of oil was measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azinobis (3-ethylbenzthiazoline sulfonate) (ABTS) radical scavenging capacity and β-carotene bleaching assays. Results showed that fatty acid content was not influenced by extraction method. MBD method was found to be the best process for extracting oil with a favorable quality characteristics when compared to Mac and CP. Higher total phenolics content (TPC), ortho-diphenol content (ODC) and total tocopherol content (TTC) and DPPH and ABTS scavenging capacities were also obtained with MBD. According to both principal component and correlation analyses, TPC and ODC were correlated with the β-carotene bleaching activity and TTC was correlated with the ABTS of the oils extracted by the different methods in the current study.

1. Introduction

There are three major walnut species: *Juglans regia* Linn, *Juglans cinerea* Linn and *Juglans nigra* Linn. Among these species, *J. regia* Linn is larger, sweeter and easier to crack owing to its thin shell (Sze-Tao and Sathe, 2000). *J. regia* Linn was originated from Persia and therefore, these walnuts are generally referred to as Persian walnuts. Persian walnuts are also known as English walnuts because English marine merchants traded them around the world (Sze-Tao and Sathe, 2000; Crowe et al., 2002). Iran is ranked third in the world with an annual production of 150,000 tones of Persian walnut, equivalent to 11% of the world's walnut production (Sze-Tao and Sathe, 2000).

Polyunsaturated fatty acids (PUFAs) and phenolic and non-phenolic antioxidant compounds have recently gained enormous attention for cosmetic products having high functional properties (Maranz et al., 2003). Generally, antioxidants can reduce the rate of initiation in the free radical chain reactions and they are considered functional at their low concentrations (0.01% or less) (Zhang et al., 2009). Walnut is a good source of essential fatty acids and tocopherols that contribute to the reduced risk of coronary heart disease and cancer types (Sze-Tao and Sathe, 2000; Miraliakbari and Shahidi, 2008a). Walnut oil (WO) is composed of PUFAs such as linoleic and linolenic acids, which are susceptible to oxidation. Although the amount of α-tocopherol as an antioxidant, in walnut is lower than that in other nuts including almonds, hazelnuts, peanuts, etc., walnut is readily preserved. This implies that the walnut contains antioxidants inhibiting lipid auto-oxidation (Fukuda et al., 2003). Moreover, existance of non-tocopherol phenolics in the oil influence the sensory and its nutritional characteristics and possibly improve its stability (Alasalvar et al., 2003; Miraliakbari and Shahidi, 2008a). Extraction of oil and other valuable components is strongly dependent on the method used for the extraction. A proper extraction method is necessary to extract compounds of interest (Turkmen et al., 2006). Chloroform/methanol and hexane are generally considered as the best extraction solvents for most of the edible oils in laboratory-scale. The solvent extraction method could be driven by the oil characteristics and may induce partial alteration of the majority of minor ingredients that have many antioxidative, functional and pro-oxidative effects (Koski et al., 2002). However, cold-press (CP) is a commercial method applicable to the extraction of oil from oilseeds. In the cold-pressed products,

Abbreviations: Mac, maceration; MBD, modified Bligh–Dyer method; CP, cold-press; FAME, fatty acid methyl ester; FID, flame ionization detector; SFAs, saturated fatty acids; PUFAs, polyunsaturated fatty acids; TPC, total phenolic content; ODC, ortho-diphenols content; TTC, total tocopherol content; GAE, gallic acid equivalent; DPPH, 1,1-diphenyl-2-picrylhydrazyl; IC_{50}, concentration corresponding to 50% inhibition; TEAC, Trolox equivalent antioxidant capacity; ABTS, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; AnV, anisidine value; PV, peroxide value; IV, iodine value; SV, saponification value; PCA, principal component analysis; PC-1, first principal component; PC-2, second principal component; HPLC, high-performance liquid chromatography.

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minor components are affected, thus color, flavor and other quality players of the oil are preserved (Koski et al., 2002; Parry and Yu, 2004).

Although many studies concerning the physicochemical and antioxidant properties of nut oils have been reported (Savage et al., 1999; Crowe et al., 2002; Maguire et al., 2004; Arranz et al., 2008; Martínez and Maestri, 2008; Martínez et al., 2008; Miraliakbari and Shahidi, 2008a), only a few investigations are found on the effect of the extraction methods on such attributes of oils. Therefore, the aim of this study was to compare different extraction methods with respect to the oil content, fatty acid profile, physicochemical characteristics, phenolic and tocopherol compounds, and also antioxidant activities of the extracted oils from Persian walnut kernel.

2. Materials and methods

2.1. Chemicals

Chloroform, methanol, hexane, acetone, anhydrous sodium sulfate, sodium carbonate, potassium hydroxide, cyclohexane, linoleic acid and β-carotene were supplied from Merck Chemical Company (Darmstadt, Germany). Tween 40, toluene, gallic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$), hexane and 2-propanol (HPLC grade), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), tocopherol standards, Folin–Ciocalteu's reagent, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS$^{\bullet+}$) and manganese dioxide were provided from Sigma–Aldrich (Oakville, ON, Canada). Dimethyl sulfoxide (DMSO), pyrogallol, β-cyclodextrin and cathechol, by Fisher Scientific (Ottawa, Ontario, Canada).

2.2. Sample preparation

The Persian walnuts were harvested from Toyserkan city located in Hamedan Province (Iran). They were immediately transported to the laboratory and held in an oven for 3 days at 30 °C. They were stored in the shell in closed plastic bags at −20 °C until the analysis time. Before every chemical analysis, the walnuts were manually cracked and shelled, then chopped in a 643 MX mill (Moulinex, Spain).

2.3. Extraction procedures

2.3.1. Cold-press extraction

A screw press (Model NA 21 T, Zeith, Kerman, Iran) with a 5-mm restriction die and a screw speed of 20 rpm was used for the CP oil expression from walnut kernels. The screw press was first run for 20 min without seed material but with heating via an electrical resistance-heating ring attached around the press barrel to raise the screw-press barrel temperature to the desired temperature (50 °C) monitored by a digital thermometer inserted into the restriction die. Before the extraction, the moisture content of the kernels were adjusted to 7.5% using a desiccator (Martínez et al., 2008).

2.3.2. Modified Bligh and Dyer extraction method

A part of sample was extracted based on an MBD method as described previously by Crowe et al. (2002). 50 g of walnut kernel was first ground into a fine powder and suspended in a mixture of 180 ml water, 200 ml methanol and 100 ml chloroform and homogenized using a laboratory polytron (model PT-1200C, Switzerland) at 5000 rpm for 2 min. An additional 100 ml of chloroform was added to the mixture and blended for another 2 min. The mixture was centrifuged at 500 × *g* for 10 min and the upper layer was removed by aspiration and the bottom layer was vacuum-filtered through a Whatman No. 1 filter paper. The residue was re-extracted with another 200 ml of chloroform and filtered again. The chloroform layer containing the extracted lipids was passed through anhydrous sodium sulfate, which was rinsed again with 100 ml chloroform. Finally, the solvent was removed by using a vacuum-rotary evaporator at 30 °C.

2.3.3. Maceration extraction

Mac method consisted of a cold maceration of the walnut powder in hexane as solvent to avoid thermal degradation. The procedure described by Miraliakbari and Shahidi (2008a,b) was used for the extraction of WO with slight modifications. In brief, 4.0 g of walnut powder was mixed with 400 ml of hexane and homogenized by a laboratory scale Polytron homogenizer (model PT-1200C, Switzerland) at 7000 rpm for 5 min. The obtained mixture was filtered through a Whatman No. 4 filter paper and using an aspirated Büchner funnel.

The residue was re-extracted two more times and the filtrates from the three extraction stages were combined and the solvent was removed under vacuum at 40 °C by a rotary evaporator (model VV2000, Heidolph, Schwabach, Germany). The obtained oil was passed through a layer of anhydrous sodium sulfate placed over a filter paper in a funnel. In the next step, the oil was weighed and transferred into 15-ml sample vials, gently flushed with nitrogen gas, capped and stored at −20 °C until analysis.

2.4. Analytical methods

2.4.1. Physicochemical properties of walnut oils

The acid values of the extracted oils were measured using the AOCS method Cd 3a-63 (AOCS, 1998). K_{232} and K_{270} extinction coefficients were measured based on the absorptions of oil at 232 and 270 nm, respectively, with a UV–visible spectrophotometer (DR/4000U-HACH, USA) using a 1% solution of oil in cyclohexane and a path length of 1 cm. Saponification values (SVs) and unsaponifiable matters of the WOs were measured according to the AOCS methods Cd 3-25 and Ca 6b-53, respectively (AOCS, 1998). Peroxide value (PV) was determined using the International Dairy Federation method described in detail by Savage et al. (1999). Anisidine value (AnV) was determined at 350 nm (cell width = 1 cm) using a solution containing 1.0 g of oil in 100 ml of *iso*-octane according to the method Cd 18-90 of the AOCS (AOCS, 1998). The total oxidation (totox) value of oils extracted by different methods was calculated by Eq. (1):

$$\text{totax value} = 2 * \text{PV} + \text{AnV} \quad (1)$$

Iodine value (IV) was determined from fatty acid percentages by the recommended formula by Martínez and Maestri (2008):

$$\text{IV} = (\%\ \text{oleic acid} \times 0.899) + (\%\ \text{linoleic acid} \times 1.814) + (\%\ \text{linolenic acid} \times 2.737).$$

The oxidative stability was determined by Rancimat analysis. Air flow rate was set at 20 l h^{-1} and temperature of the heating block was maintained at 110 °C (Martínez and Maestri, 2008). Color value was determined using a Lovibond tintometer (Model F, Greenwich, England). The oil viscosity was determined using a Brookfield digital viscometer (model DV-II+Pro, USA). The oil density was determined by a digital densitometer (AP-PAAR DMA 46, Germany) with an accuracy of 10^{-4} g cm^{-3}. The refractive index of oil samples was analyzed using an Abbe refractometer (model G manufactured by Carl-Zeiss, Germany).

2.4.2. Fatty acid profile

In order to evaluate the fatty acid compositions of the extracted WOs, a 30 m × 0.22 mm, 0.25 μm film thickness