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Improvement of supercritical CO_2 and *n*-hexane extraction of wild growing pomegranate seed oil by microwave pretreatment

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ABSTRACT

Microwave radiation was suggested as a pretreatment technique to increase the yield of pomegranate seed oil. Seeds were pretreated at 100, 250 and 600 W during 2 and 6 min and then extracted by supercritical carbon dioxide ($scCO_2$) in high pressure unit as well as by *n*-hexane in Soxhlet apparatus. Even at the lowest microwave pretreatment parameters applied (100 W for 2 min) increased the yield of seed oil obtained by both extraction techniques compared with untreated seeds (from 27.7 to 34.0% and from 21.6 to 25.5% for Soxhlet and $scCO_2$ extractions, respectively). Maximal oil yield in Soxhlet extraction (36.3%) was obtained with microwave radiation of 600 W for 6 min while for $scCO_2$ extraction maximal oil yield (27.2%) was with 250 W for 6 min microwave radiation pretreatment. The qualitative and quantitative composition of fatty acids of the obtained oils was determined by gas chromatography/flame ionization detection and gas chromatography/mass spectrometry. Punicic acid was the most abundant fatty acid in pomegranate seed oil (> 60%). Microwave pretreatment of seeds showed negligible influence on profile and the amount of fatty acids in obtained extracts, compared with its significant influence on extraction yield. Our results recognize microwave pretreatment as a promising technique for intensification of oil extraction from pomegranate seeds.

1. Introduction

Punica granatum L., known as pomegranate, is deep red colored fruit, with leathery skin, grenade-shaped and crowned by the pointed calyx. The fruit can be divided into three parts: the seeds (about 3% of the fruit weight), juice (about 30% of the fruit weight) and the peels, that also include the interior network of membranes (Lansky and Newman, 2007). Pomegranate seeds, usually considered as a waste of juice industry, comprise 12–20% of fatty oil (Lansky and Newman, 2007; Al-Maiman and Ahmad, 2002). According to previously published results, pomegranate seed oil (PSO) consists of 65–80% conjugated linolenic acids (CLnAs), among which the most important is punicic acid (Abbasi et al., 2008). Fatty acids are important components of human cell membranes and are known as precursors to many substances in the body. Preventive role of fatty acids in development of cardiovascular diseases and in alleviation of some other health problem have been reported (Wijendran and Hayes, 2004). Also, they promote the reduction of both, total and HDL cholesterol (Dubois et al., 2007; FAO, 2010). According to their content of phytoestrogens, it is recommended that women in menopause could employ pomegranate seed oil as external and internal phytoestrogen medicaments, as a possible alternative or supplement to conventional hormone replacement therapy (HRT) (Lansky, 1999). Moreover, it has been reported that CLnA can up-regulate the tumor suppressor gene PTPRG, and may have anti-cancer properties (Amarù and Field, 2009). Also, because of low toxicity and confirmed effectiveness in lowering skin irritations, revitalizing dull or mature skin and wrinkles reduction, PSO are usually used in cosmetic industry products. Pomegranate oil inhibits two inflammatory enzymes, cyclooxygenase and lipoxygenase, which may help protect the skin against the age-accelerating threats of ultraviolet light and inflammation, which can help result in younger-looking skin (Ashoori et al., 1994; Schubert et al., 1999).

Abbreviations: ANOVA, analysis of variance; CLnAs, conjugated linolenic acids; FAME, fatty acid methyl esters; FID, flame ionization detection; GC, gas chromatography; HRT, hormone replacement therapy; MS, mass spectrometry; met_ara, methyl arachidate; met_beh, methyl behenate; met_E_11, methyl (E)-11-eicosenoate; met_ela, methyl elaidate; met_lind, methyl linolelaidate; met_lin, methyl linoleate; met_mar, Methyl margarate; met_oct1,2.3,4, Methyl octadecatrienoate isomer 1,2,3,4; met_ole, Methyl oleate; met_pal, methyl palmitate; met_pun, methyl punicate; met_ste, methyl stearate; met_Z_11, methyl (Z)-11-eicosenoate; MUFA, monounsaturated fatty acids; PSO, pomegranate seed oil; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; scCO, $_{22}$supercritical carbon dioxide; SFE, supercritical fluid extraction

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Considering all the beneficial properties of the oil rich in fatty acids, this study focuses on its extraction from pomegranate seed. Different techniques can be used for the extraction of oil from pomegranate seed. Techniques like normal stirring extraction, Soxhlet extraction, ultrasonic baths that employ organic solvents (e.g. *n*-hexane, petroleum, benzene, and acetone) and supercritical fluid extraction (SFE) have different extraction efficiency with the lowest value reported for SFE (Petrovic et al., 2012). Also, it is possible to obtain oil from seed by using cold pressing method and steam distillation. Cold pressing method provide high-quality oil but, in most cases, the process has a low extraction rate and consumes large amounts of energy. Steam distillation is a very simple process, but suffers of many disadvantages, such as thermal degradation, hydrolysis and solubilization of some compounds in water resulting in change of the flavour and fragrance profile of many oils extracted by this technique. Moreover, Goula (2013) obtained higher pomegranate seed oil yields by ultrasound-assisted extraction (302.3–446.3 g oil/kg seeds), compared to those by conventional extraction methods. SFE presents one of the clean and efficient techniques that have been developed in last decades. The most extensively used solvent in SFE process is supercritical carbon dioxide ($scCO_2$) because of its low critical pressure (7.38 MPa) and temperature (31.1 °C), low cost and availability. It can be easily removed from final product without any residues making it suitable for use in medicine, food and pharmaceutical industry (Ivanovic et al., 2014). $scCO_2$ is known for its high diffusion ability in organic matter and it is good solvent for many valuable compounds. It is predominantly used for extraction of non-polar components from plant material matrix such as fatty acids, sterols, terpenes without use of co-solvents (He et al., 2012).

Many reports describe pretreatment of plant material with different techniques (microwave, rapid gas decompression, milling, etc.) in order to intensify extraction of valuable plant constitutes (Meyer et al., 2012; Sayyar et al., 2011). Microwave pretreatment has been reported as an effective method for increasing the oil yield from seeds (Ramesh et al., 1995). The use of microwave radiation offers reduced processing times and energy savings because the energy is delivered directly to materials through molecular interaction with the electromagnetic field resulting to heat generation throughout the material. Microwave radiation also allows rapid and uniform heating of relatively thick materials (Uquiche et al., 2008). Singh and Heldman (2001) reported that microwaves use radio waves to convey energy and convert it to heat at a frequency between 300 MHz and 300 GHz. In this frequency range, waves are mostly absorbed by water with a sufficiently polar oxygen group. Although, seeds are dried, they still contain traces of moisture that serves as the target for microwave heating. The moisture when heated up inside the materials due to microwave effect, evaporates and generates tremendous pressure on the seed cell membrane. The pressure pushes the cell membrane from inside, stretching and ultimately rupturing it, which facilitates leaching out of the active constituents from the pores (Wang and Weller, 2006). As a result, penetration of solvent into the seed cells as well as release of oil from inside of the seeds gets facilitated (Uquiche et al., 2008).

To the best of our knowledge, there is no information in the available literature on the application of microwave radiation in a treatment of pomegranate seed prior to the oil extraction process. The objective of this work was to evaluate the effects of microwave pretreatment on the Soxhlet as well as supercritical fluid extraction efficiency of PSO from wild growing pomegranate and to compare qualitative and quantitative composition of fatty acids in obtained oil bearing in mind the potential use of the oil in the treatment of cardiovascular disease, certain types of cancers, and type II diabetes mellitus.

2. Materials and methods

2.1. Plant material

Wild growing pomegranate fruit was collected in Bosnia and Herzegovina in the village Do during November 2014 from a natural locality (GPS coordinates: 43.086°N 18.140°E, altitude 498 m).

Pomegranate fruit skin and other impurities were previously separated from the seeds. Seeds were then washed with distilled water and air-dried at ambient temperature (4–6 days). Cleaned and dried seeds were ground with a high-speed mill (MMB 1000/05, Bosch). Ground seeds were fractionated by a set of sieves with mesh widths of 0.2, 0.5, and 1 mm to obtain the uniform particle size distribution. Fine powder ensure larger surface area, which provides better contact between the seed and the solvent, and which can enhance the extraction. Also, finer particles will allow improved or much deeper penetration of the microwave. Moisture content was determined by drying of the seed samples (8 g) at 105 ± 0.5 °C for 5 h. Determined moisture values were 2.58 and 5.01 wt.% for ground and whole (non-ground) pomegranate seeds, respectively.

2.2. Microwave pretreatment

Ground pomegranate seeds were placed in a single layer on a Pyrex petri dish (9 cm diameter), in the middle of the turntable plate of a microwave oven (NN-GD 469 M, Panasonic). Throughout microwave radiation, the sample rotates inside of the oven. This configuration allowed the samples to move through the equable electromagnetic field pattern formed inside the oven, allowing uniform energy absorption in the seeds. Samples were treated at a frequency of 2450 MHz for two times of radiation (2 and 6 min) and three levels of power (100, 250 and 600 W). The temperature of microwave heating varied from 63 to 136 °C. Microwave pretreated samples were subsequently placed in an apparatus for the extraction and extracted. Ground pomegranate seed sample without microwave radiation was used as a control.

2.3. Extraction procedures

2.3.1. Soxhlet extraction

Approximately 20 g of ground pomegranate seeds with and without microwave radiation treatment were extracted with 250 mL *n*-hexane for 8 h by Soxhlet extraction. After extraction was completed, *n*-hexane was evaporated at 30 °C under reduced pressure using a rotary evaporator (Laboxact SEM842, KNF, UK). The quantity of obtained extracted oil was expressed in percentage, which is defined as mass of oil extracted over mass of the sample (pomegranate seeds) used for extraction. The obtained extracts were collected in vials and stored at −20 °C until further analysis.

2.3.2. Supercritical fluid extraction

Supercritical fluid extraction (SFE) was performed in the high pressure unit (HPEA 500, Eurotechnica, Germany) previously described elsewhere (Ivanović et al., 2014) which was modified according to requirements of the process (Fig. 1).

Sample of 10 g of ground pomegranate seed with and without microwave radiation treatment was filled into the stainless steel basket with a perforated bottom and top and then placed in stainless steel extraction vessel. Liquid CO_2 supplied from CO_2 cylinder (commercial CO_2, 99% purity, Messer Tehnogas, Belgrade, Serbia) with a siphon tube was cooled in cryostat between the cylinder outlet and the pump to prevent CO_2 vaporization. After the system was heated, the CO_2 was pumped by a liquid metering pump (Milton Roy, France) until the required pressure is obtained. Than valve V-3 is opened and the extraction started. Depending on the specific flow mode, system pressure was maintained using a back-pressure valve (BPR). Applied operating pressure and temperature (47 °C and 37.9 MPa) were pre-