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Evaluation of ultrasound assisted and conventional methods for production of olive pomace oil enriched in sterols and squalene

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ABSTRACT

This study highlights the extraction of olive pomace oil enriched in sterols and squalene by using two methods: ultrasound-assisted extraction (UAE) and conventional extraction by various solvent systems. UAE was performed to examine the effect of temperature and solid/liquid ratio on the extraction of these minor compounds using hexane and the optimal conditions were found:1.399 mg/g at 60 °C and 1/12 g/mL for β-sitosterol; 0.220 mg/g at 55 °C and 1/10 g/mL for campesterol; 0.105 mg/g at 50 °C and 1/12 g/mL for stigmasterol and 4.575 mg/g at 60 °C and 1/12 g/mL for squalene recovery. Conventional extraction by various alternative solvent systems (hexane, isopropanol and their mixtures) were used and their effect on the sterols and squalene extraction under Soxhlet (SE) conditions was also investigated. The hexane:isopropanol 3:2 (H:I 3:2) mixture achieved high sterols and squalene recovery (total sterols: 1.706 mg/g; squalene: 5.217 mg/g), which extracted also the highest oil (13.70%) and unsaponifiable matter (4.92%). Comparing the two methods, it was revealed that the application of UAE led to oils with higher sterols and squalene content than the ones obtained by SE using hexane as solvent and with almost similar content with that by SE using H:I 3:2.

1. Introduction

Vegetable oils are mainly composed of triacylglycerols (95–98%) and multiple mixtures of minor bioactive compounds (2–5%), which generate the unsaponifiable matter (USM), mainly including aliphatic and triterpenic alcohols, sterols, hydrocarbons (squalene), volatile compounds and polyphenols. Many studies have reported the health benefits of these minor components present in vegetable oils (Bavisetty & Narayan, 2015; Yuan et al., 2017) and, also their significant role in establishing the quality of the oil, attracting great interest in the past few years. Therefore, the recovery of these minor compounds from food sources or by-products is of great importance.

Sterols are integral natural components of plant cell membranes, which are abundant in vegetable oils, nuts, seeds and grains. The common forms of sterols are β-sitosterol, campesterol and stigmasterol (Ryan, Galvin, O'connor, Maguire, & O'brien, 2007). They have very important beneficial effects, such as hypocholesterolemic, anti-inflammatory, antibacterial, antifungal and antioxidant activity (Sánchez-Machado, López-Hernández, Paseiro-Losada, & López-Cervantes, 2004; Yuan, Ju, Jin, Ren, & Liu, 2015). Moreover, sterols are characteristic compounds of a vegetable oil and can be used for the detection of its adulteration (Gu et al., 2016; Sánchez-Machado, López-Hernández, Paseiro-Losada, & López-Cervantes, 2004).

Squalene is a highly unsaturated aliphatic hydrocarbon ($C_{30}H_{50}$) and an important constituent in numerous edible oils such as olive oil, soya bean oil, rice bran oil and amaranth seed oil. It is proved to be a precursor of cholesterol and Vitamin D biosynthesis; shark liver (*Squaluss* spp.) oil is a rich source of squalene (Lu, Jiang, & Chen, 2004; Ryan et al., 2007). Several studies have indicated that squalene is an important dietary cancer chemo-preventive agent. The protective effect of squalene may be attributed to its ability to serve as an antioxidant; it can protect cells against free radicals, strengthen the immune system of the body and decrease the risk of cancers (Lu et al., 2004).

Olive pomace is the solid by-product resulted from olive oil production process; in general by processing 100 kg olives approximately 35–40 kg of olive pomace are released (Akay, Kazan, Celiktas, & Yesil-Celiktas, 2015). It consists of pulp fragments (21–33% w/w), pit (42–54% w/w), olive fruit skin (10–11% w/w). It contains, also, oil content of 8–12% w/w, that can be recovered and used for consumption after refining and variable moisture content depending on the extraction system, in particular 25–30% w/w, dry basis, for mechanical pressing and 45% and 70% w/w, dry basis, for three-phase and two-phase centrifugal systems, respectively (Akay et al., 2015; Sanchez Moral & Ruiz Mendez, 2006). Olive pomace oil (OPO) displays many

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similarities to olive oil (OO); both oils are derived from olives, especially the OO by mechanical extraction process (pressing or centrifugation, without solvent usage) while the OPO by solvent extraction. Although these oils may not significantly differ from their fatty acid profile, there are many studies reported that OPO has higher unsaponifiable matter content than OO (Antonopoulos, Valet, Spiratos, & Siragakis, 2006; Rodríguez-Gutierrez, Lama-Muñoz, Ruiz-Méndez, Rubio-Senent, & Fernández-Bolaños, 2012; Sánchez-Gutiérrez, Ruiz-Méndez, Jiménez-Castellanos, & Lucero, 2017); particularly, it is richer in sterols and polyunsaturated fatty acids such as linoleic acid (Antonopoulos et al., 2006; Sánchez-Gutiérrez et al., 2017). Total sterols content is found to be more or equal to 1600 mg/kg in OPO and more or equal to 2500 mg/kg in crude OPO. β-sitosterol (apparent β-sitosterol: delta-5,23-stigmastadienol + chlerosterol + β-sitosterol + sitostanol + delta-5-avenasterol + delta-5,24-stigmastadienol) and campesterol make up 93% and 5% of the total sterol fraction, respectively. Other sterols are present in OPO in traces or in very small amounts, including cholesterol, campestanol, stigmasterol, sitostanol, 5,24-stigmastadienol, Δ7-stigmasterol and Δ7-avenasterol (Boskou, 2009). Furthermore, OPO contains higher amounts of erythrodiol and uvaol (> 4.5 of total sterol fraction) than OO (< 4.5 of total sterol fraction); as a consequence, the total amount of erythrodiol and uvaol is a quality parameter to detect adulteration in OOs with OPO (Paiva-Martins & Kiritsakis, 2017). Squalene is a compound, which is found in both OO and OPO at concentrations ranging up to 7 mg/g (with a range variation comprised between 0.09 and 8.70 mg/g) and up to 3.4 mg/g respectively, accounting for more than 50% of the unsaponifiable fraction of the oil (Beltrán, Bucheli, Aguilera, Belaj, & Jimenez, 2016; Boskou, 2011; Firestone, 2001; Ghanbari, Anwar, Alkharfy, Gilani, & Saari, 2012; Rodríguez-Gutiérrez et al., 2012; Yuan et al., 2017). Nowadays, the recovery of sterols and squalene as well as their addition for enrichment of products intended for food, nutraceuticals and pharmaceuticals industrial sectors are more than important. Therefore, according to the modern trend for searching new ingredients from natural sources, the valorization of olive pomace appears to be particularly attractive.

The production of OPO is commonly carried out by solvent extraction using hexane. Hexane has been selected as an excellent extraction solvent for many years due to its nonpolar properties, efficient recovery and reuse, high selectivity to oils, efficient extraction and low energy cost (Li, Pordesimo, & Weiss, 2004). A potential new technology that may improve extraction of lipophilic compounds from plants sources is ultrasound. Ultrasound has been suggested to disrupt plant cell walls facilitating the release of extractable compounds and enhance mass transport of solvent from the continuous phase into plant cells (Li et al., 2004). The method offers many advantages, such as improved efficiency, reduced extraction time, low solvent consumption and high level of automation compared to conventional extraction method (Chanioti & Tzia, 2018a; Jerman, Trebeb, & Mozetiz Vodopivec, 2010). The application of ultrasound has been widely studied for the extraction of oil from plant materials including those from olive paste (Clodoveo, Durante, La Notte, Punzi, & Gambacorta, 2013; Leone et al., 2018), soybeans (Li et al., 2004), *Isatis indigotica* Fort (Li, Qu, Zhang, & Wang, 2012) and almond powder (Zhang et al., 2009). In our previous work, the UAE of OPO and its USM has been studied and optimized in order to determine the optimal process parameters achieving high oil yield (Chanioti & Tzia, 2017).

In the plant cells, phospholipids are located at interfaces and during the extraction process they block the access of hexane to the oil, delaying the extraction (Tir, Dutta, & Badjah-Hadj-Ahmed, 2012). Therefore, a co-solvent is sometimes used in order to increase the polarity of the liquid phase and to allow extraction of more components with different polarities in the same process. Many studies have shown that the addition of polar alcohols, such as isopropyl alcohol as a co-solvent to a non-polar solvent improves its extraction efficiency (Balasubramanian, Yen, & Obbard, 2013; Ryckebosch et al., 2014). Isopropyl alcohol is an efficient and advantageous extraction solvent for oilseeds having high availability, bio-renewability and low toxicity, and producing high-quality oil enriched in valuable bioactive compounds such as certain compounds of unsaponifiable matter, thus increasing the nutritional and functional value of the extracted oils (Capellini, Giacomini, Cuevas, & Rodrigues, 2017; Seth, Agrawal, Ghosh, Jayas, & Singh, 2007; Tir, Dutta, & Badjah-Hadj-Ahmed, 2012). Although isopropyl alcohol has a higher vaporization temperature (boiling point 82 °C) than the n-hexane, only a small portion of the total isopropyl alcohol in the system requires vaporization resulting thus in energy savings (Seth et al., 2007). Navarro, Capellini, Aracava, and Rodrigues (2016) mentioned that the use of alcohols as co-solvents for oil extraction enables higher concentration of compounds in the oil, such as sterols, to be obtained. To the best of our knowledge, the extraction of the OPO enriched in individual minor bioactive compounds such as sterols and squalene by various solvent systems and in particular by using isopropyl alchohol as a co-solvent was not reported yet. Moreover, no studies have looked so far at extraction of these interesting compounds using ultrasound and there is also a great interest to study the effect of the processing parameters, such as the temperature and the solid/liquid ratio, on the UAE of sterols and squalene and to optimize their recovery.

According to the aforementioned, the present work aims to evaluate the UAE of olive pomace oil enriched in sterols and squalene and to optimize their recovery as well as to investigate the conventional extraction of OPO enriched in these individual compounds by various solvent systems (hexane-isopropanol mixtures).

2. Materials and methods

2.1. Raw materials and chemicals

Olive pomace was obtained as a by-product by mechanical extraction of olives by a local olive oil mill equipped with a continuous three - phase centrifugation system. The solid residue (initial moisture 45.0% (w/w)) was air dried by an airstream oven (35 °C for 24 h) until the major moisture content removed (final moisture 4.5% w/w) and was ground by a cutting mill (Pulverisette 15, FRITSCH, Idar-Oberstein, Germany) to an average particle size of 1 mm. Hexane, isopropanol, methanol (HPLC grade), acetonitrile (HPLC grade) were purchased from Sigma Aldrich Chemical Co. (St Louis, MO). Sterol standards: β-sitosterol, campesterol, stigmasterol, erythrodiol, cholesterol, ergosterol as well as squalene were procured from Sigma Aldrich Chemical Co. (St Louis, MO).

2.2. Extraction of OPO experiments

2.2.1. Ultrasound-assisted extraction (UAE) experiments

The ultrasound-assisted extraction experiments were conducted as described by Chanioti and Tzia (2017). The ground olive pomace with a predetermined volume of n-hexane (solid: liquid ratio: 1:4–1:12 g/mL) were sonicated in an ultrasonic cleaning bath (Elmasonic. S30(H), 60.0 kHz, 280 W, Elma Schmidbauer GmbH, Germany) at required temperatures (40–60 °C) for 60 min.

2.2.2. Conventional extraction experiments by various solvent systems using soxhlet (SE) apparatus

The ground olive pomace was extracted in a Soxhlet apparatus using hexane, isopropanol and their binary mixture (hexane:isopropanol 9:1, 4:1, 3:1 and 3:2) for 8 h. The extraction temperature was kept constant in all assays in the range of the boiling point of each solvent system.

At the end of each experiment for both methods, the mixture was separated by filtration through Whatman No. 42 filter paper. The solvent was removed with a rotary vacuum evaporator (BUCHI 461, Buchi Laboratotiums Technik AG, Flawil, Switzerland) and its traces were removed by a nitrogen stream. Each experiment was performed in