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Seeds recovered from industry by-products of nine fruit species with a high potential utility as a source of unconventional oil for biodiesel and cosmetic and pharmaceutical sectors

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ABSTRACT

The composition of fatty acids and phytosterols of oils recovered from the seeds of nine industrial fruit by-products: watermelon (*Citrullus lanatus*), honeydew melon (*Cucumis melo*), sea buckthorn (*Hippophae rhamnoides*), red currant (*Ribes rubrum*), pomegranate (*Punica granatum*), Japanese quince (*Chaenomeles japonica*), grape (*Vitis vinifera*), gooseberry (*Ribes uva-crispa*) and apple (*Malus domestica*) were studied. The oil yield in the investigated fruit seeds ranged from 11.8% (sea buckthorn) to 28.5% (watermelon). The main phytosterol identified in all fruit seed oils was β-sitosterol with the concentration ranging between 0.5 and 3.1 mg/g of oil, in watermelon and Japanese quince, respectively. The fatty acid composition was unique for each fruit seed oil. The majority of samples had high linoleic acid content (38.0–70.7%), whereas the pomegranate seed oil was extremely rich in punicic acid (86.2%). Japanese quince seed oil had the highest potential value for biodiesel production; while the unique profile of bioactive compounds recorded in pomegranate seed oil indicated great potential for utilization in cosmetic and/or pharmaceutical industries. The ΣPUFA/(ΣSFA + ΣMUFA) ratio of nine fruit seed oils highly correlated ($r > 0.9$, $p < 0.0001$) with all biodiesel properties, with the exception of the cold filter plugging point.

1. Introduction

Demand for plant oils in pharmaceutical, cosmetic and biodiesel industries is increasing, since they are valuable natural sources of lipophilic compounds. Natural origin of ingredients of products used in daily life has greater acceptability by the international community over their synthetic counterparts. Simultaneously, an increasing amount of the by-products generated by the fruit processing industry, partly in the form of seeds, are usually discarded and may otherwise be utilized as an unconventional source of oil. Based on the data of global fruit production in 2013, and the content of seeds in fruits of various crops (FAOSTAT, 2015; Fromm et al., 2012; Górnaś et al., 2014b; Hernández et al., 2012; Melgarejo et al., 1995) the amount of seeds with potential utilization has been estimated on 114–441 thousands of tons for pomegranate, 81–566 thousands of tons for apple, 32–57 thousands of tons for quince, 738 thousands of tons for melons and 2–5 millions of tons for grape. Despite the fact that not all fruits are processed by industry, the amount of seeds that can be recovered from the by-products and utilized as a natural source of lipophilic compounds is significant. Seeds, kernels and their oils recovered from fruit by-products are rich in bioactive compounds such as, tocochromanols (Górnaś, 2015; Górnaś et al., 2015a,b,d,f), essential fatty acids (Fromm et al., 2012; Goffman and Galletti, 2001; Van Hoed et al., 2009), phytosterols (Caligiani et al., 2010; Piironen et al., 2003; Van Hoed et al., 2009), carotenoids (Górnaś et al., 2014b; Yang and Kallio, 2002) and squalene (Caligiani et al., 2010; Górnaś et al., 2013). The chemical composition of plant oils has a significant impact on their suitability for specific branches of industry. For example, the composition of fatty acids and the content of steryl glycosides have a significant impact on the quality of the biodiesel (Aguirre et al., 2014; Ramos et al., 2009). Globally better biodiesel properties have been reported for the plant oils characterized by the higher content of monounsaturated fatty acids (Ramos et al., 2009). While, the most valuable plant oils for the pharmaceutical and cosmetic industry are those rich in polyunsaturated fatty acids, phytosterols and squalene (de Jesus Raposo et al., 2013; Fatima et al., 2013; Huang et al., 2009; Vermaak et al., 2011). Since there is a limited knowledge about various fruit seed oils, the aim of this study was to explore their composition (fatty acids, phytosterols and squalene) and potential

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utilization in different industry sectors. The seeds were recovered from nine fruit crops grown in different parts of the world: watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), honeydew melon (*Cucumis melo* L.), sea buckthorn (*Hippophae rhamnoides* L.), red currant (*Ribes rubrum* L.), pomegranate (*Punica granatum* L.), Japanese quince (*Chaenomeles japonica* (Thunb.) Lindl. ex Spach), grape (*Vitis vinifera* L.), gooseberry (*Ribes uva-crispa* L.) and apple (*Malus domestica* Borkh.) to demonstrate the global challenge for utilization of agro-industrial by-products.

2. Materials and methods

2.1. Reagents

n-Hexane, *tert*-butyl methyl ether (HPLC grade), 5α-cholestane (≥97%, GC) and brassicasterol, campesterol, stigmasterol, β-sitosterol, cholesterol, squalene (≥95%, GC) were purchased from Sigma–Aldrich (Steinheim, Germany). The Sylon BTZ and fatty acid methyl ester mix were received from Supelco (Bellefonte, PA, USA) and (Steinheim, Germany), respectively. Other reagents were of analytical grade, purchased from Sigma–Aldrich (Steinheim, Germany).

2.2. Plant material

Ripe fruits of nine crops, free from the diseases and injuries, were obtained: apple (*M. domestica* Borkh.)—cv. 'Iedzēnu', red currant (*R. rubrum* L.)—cv. 'Niva', gooseberry (*R. uva-crispa* L.)—cv. 'Koknese' and Japanese quince (*C. japonica* (Thunb.) Lindl. ex Spach)—mixed sample of hybrid material were collected at the Latvia State Institute of Fruit-Growing (hereinafter—the LSIFG); sea buckthorn (*H. rhamnoides* L.)—cv. 'Prozračnaja' was obtained from Baltplant Ltd. Latvia; grape (*V. vinifera* L.) from Poland; pomegranate (*P. granatum* L.) from India; watermelon (*C. lanatus* (Thunb.) Matsum. & Nakai) from Bulgaria and honeydew melon (*C. melo* L.) from Spain were provided by a local supplier (cultivars could not be verified). Before sampling fruits were stored according to standard commercial procedure suitable for particular fruit crop. Fruits of each crop were randomly separated in three batches before processing. The seeds were recovered from by-products obtained after preparation of juice or fresh cut-salads in the LSIFG processing facility according to the scheme shown in Fig. 1. The seeds were separated from fruit pulp or residues, then oven-dried (3 h) in Orakas 5600 (Marlemi, Lemi, Finland) with forced hot air circulation at 55 ± 1 °C. The undamaged seeds were selected (~10 g for each batch) and milled in a Knifetec™ 1095 (Foss, Höganäs, Sweden) universal laboratory mill to mesh size of 0.75 mm to finally obtain a powder.

2.3. Extraction of oil

Oil was extracted according to the method introduced earlier method (Górnaś et al., 2014a). Briefly, ground fruit seeds (5 g) were supplemented with 25 mL of *n*-hexane (Sigma–Aldrich, Steinheim, Germany) in a centrifuge tube and mixed on a Vortex REAX top (Heidolph, Schwabach, Germany) at 2500 rpm (1 min). Samples were subjected to ultrasound treatment in the Sonorex RK 510 H ultrasonic bath (Bandelin electronic, Berlin, Germany) (5 min, 35 °C) and centrifuged on a Centrifuge 5804 R (Eppendorf, Hamburg, Germany) (10000 × *g*, 5 min, 21 °C). The supernatant was collected in a round bottom flask and the remaining solid residue was re-extracted (twice) as described above. The combined supernatants were evaporated in a Laborota 4000 vacuum rotary evaporator (Heidolph, Schwabach, Germany) at 40 °C until constant weight. The oil content was expressed in% (w/w) dw (dry weight basis—measured gravimetrically) of seeds.

2.4. Fatty acid composition

The fatty acid composition of the extracted fruit seed oil was estimated using gas chromatography according to AOCS (2005). Fatty acid methyl esters were separated using a Hewlett-Packard 5890 II gas chromatograph (GC) (Hewlett Packard, Wilmington, DE, USA) equipped with a Supelcowax 10 capillary column (30 m × 0.20 mm × 0.20 μm) and FID detector. Oven temperature was programmed as follows: initially 60 °C, increased at a rate of 12 °C/min to 200 °C and was held at 200 °C for 25 min. Temperature of the injection port and the detector was held at 240 °C. Hydrogen was used as a carrier gas at a flow rate of 1.0 mL/min. Fatty acids were identified by retention times of fatty acid methyl ester standards and expressed as a percentage of the total peak area of all the fatty acids in the oil sample. The limit of detection was 0.01 mg/g.

2.5. Contents of phytosterols and squalene

Contents of plant sterols and squalene were determined according to AOCS (1997). Detailed description of identification procedure of the sterols using the GC–FID and GC–MS has been published previously (Górnaś et al., 2016a). In brief, fruit seed oil (50 mg) was saponified with 1 M KOH in methanol for 18 h at room temperature, and then unsaponifiables were extracted thrice with hexane/methyl *tert* butyl ether (1:1, v/v). After silylation using a Sylon BTZ phytosterols were separated on a HP 6890 GC (Hewlett Packard, Wilmington, DE, USA) equipped with a DB-35MS capillary column (25 m × 0.20 mm × 0.33 μm; J&W Scientific, Folsom, CA, USA). Samples of 0.5 μL were injected in splitless mode. Column temperature was initially held at 100 °C for 5 min, increased to 250 °C at a rate of 25 °C/min, held for 1 min at 250 °C, then increased to 290 °C at a rate of 3 °C/min and held at 290 °C for 20 min. FID detector temperature was set at 300 °C with the limit of detection 0.01 μg/g. Hydrogen was used as a carrier gas at a flow rate of 1.5 mL/min. An internal standard, 5α-cholestane, was used for sterol quantifications. Phytosterols and squalene were identified by comparing retention data of standards previously verified by mass spectrometry.

2.6. Physicochemical properties of biodiesel

Physicochemical properties of biodiesel were calculated empirically based on the mean values of fatty acid methyl esters (FAME) determined for each oil sample. The proposed equations (Park et al., 2008; Ramírez-Verduzco et al., 2012; Ramos et al., 2009; Wang et al., 2012) are rapid low cost methods for preliminary selection of potential biodiesel feedstock.

2.6.1. Cetane number

$$\phi_i = -7.8 + 0.302 \times M_i - 20 \times N \quad (1)$$

where φ_i is the cetane number of the *i*th FAME, M_i is the molecular weight of the *i*th FAME and *N* is the number of double bounds (Ramírez-Verduzco et al., 2012).

2.6.2. Kinematic viscosity

$$\ln(\eta_i) = -12.503 + 2.496 \times \ln(M_i) - 0.178 \times N \quad (2)$$

where η_i is the kinematic viscosity at 40 °C of the *i*th FAME in mm^2/s (Ramírez-Verduzco et al., 2012).

2.6.3. Density

$$\rho_i = 0.8463 + \frac{4.9}{M_i} + 0.0118 \times N \quad (3)$$

where ρ_i is the density at 20 °C of the *i*th FAME in g/cm^3 (Ramírez-Verduzco et al., 2012).