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Fractionation of sea buckthorn pomace and seeds into valuable components by using high pressure and enzyme-assisted extraction methods

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ABSTRACT

Sea buckthorn pomace and seeds were processed via 3-step fractionation using supercritical carbon dioxide (SFE-CO_2), pressurized ethanol (PLE-EtOH) and enzyme-assisted (EAE) extraction. SFE-CO_2 yielded 146 and 135 g/kg of lipophilic fraction from pomace and seeds, respectively. PLE-EtOH of SFE-CO_2 residues recovered 158 g/kg of polar constituents from the pomace and 5-fold lower amount from seeds. Finally, the treatment of PLE-EtOH residues with cellulolytic and xylanolytic enzyme (Viscozyme, CeluStar XL) preparations increased the amount of soluble constituents by 24–80%, as compared to enzyme-untreated samples. CO_2 extracts of pomace and seeds contained 630 and 2223 mg/kg tocopherols, respectively; while PLE-EtOH extracts contained sugar acids, phenolics and triterpenes. EAE increased glucose, fructose and maltose content. The total phenolic content (2–22 g gallic acid/kg DW prior SFE-CO_2) and radical scavenging capacity (6–166 g Trolox/kg DW) indicate that both soluble and insoluble EAE fractions could be utilized as a low-cost source of functional ingredients for their potential applications in foods.

1. Introduction

Sea buckthorn (*Hippophaë rhamnoides* L.) is cultivated for numerous dietary and pharmaceutical purposes: to produce jams, jellies, sweets, juice, oil, herbal teas, supplements, cosmetics, traditional medicines, etc. (Bal, Meda, & Satya, 2011). Plant seeds, fruits and leaves are good sources of valuable nutrients, e.g. polyunsaturated fatty acids, carotenoids, tocopherols, phytosterols (Bal et al., 2011; Yang & Kallio, 2002), phenolic acids (Michel, Destandau, Le Floch, Lucchesi, & Elfakir, 2012), flavonoids (Chen et al., 2013), and proanthocyanidins (Fan, Ding, & Gu, 2007), demonstrating various medicinal and therapeutic effects: antioxidant, antimicrobial, anti-inflammatory; treatment of stomach ulcers, skin diseases and arsenic poisoning; reducing the risks of cardiovascular disease, diabetes, thrombosis and cancer (Xu, Kaur, Dhillon, Tappia, & Dhalla, 2011).

Pressing of juice produces high amounts of pomace, which currently are discarded as a waste or utilized rather inefficiently; therefore considerable amounts of nutrients are lost annually (Galanakis, 2012). The development of extraction schemes was recently demonstrated for the valorisation of berry pomace from black currant (Kapasakalidis, Rastall, & Gordon, 2009), raspberry (Bobinaitė et al., 2016; Kryževičiūtė, Kraujalis, & Venskutonis, 2016) and black chokeberry (Grunovaitė, Pukalskienė, Pukalskas, & Venskutonis, 2016), as well as for sea buckthorn seeds (Górnaś, Pugajeva, & Segliņa, 2014; Górnaś, Soliven, & Segliņa, 2015; Michel et al., 2012; Yakimishen, Cenkowski, & Muir, 2005). These studies outlined the potential of supercritical carbon dioxide (SFE-CO_2), pressurized liquid (PLE) and enzyme-assisted (EAE) extraction/fractionation to obtain higher added value fractions, rich in bioactive compounds.

Therefore, this study was aimed at consecutive fractionation of sea buckthorn by-products into valuable food-grade ingredients by SFE-CO_2, PLE and EAE, and assessment of phytochemical composition and antioxidant capacity of the products obtained. The results of this research are expected to provide sound evidence for the valorisation of sea buckthorn pomace and seeds for future nutraceutical and pharmaceutical applications.

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2. Materials and methods

2.1. Materials

Sea buckthorn pomace and seeds were obtained from the local juice producer, freeze-dried after juice pressing and ground by ZM 200 mill (Retsch, Haan, Germany) using 0.2 mm sieve. All reagents and solvents were of analytical and HPLC-grade (Supplementary Materials).

2.2. High pressure and enzyme-assisted extraction

SFE-CO_2 (Fig. 1) was performed in a Helix extractor (Applied Separation, Allentown, PA) from 150 g material placed in a 500 mL vessel at the following parameters: temperature 60 °C, pressure 35 MPa, static extraction time 30 min, dynamic extraction time 180 min, CO_2 flow rate 2–3 SL/min at $P_{CO2} = 100$ kPa, $T_{CO2} = 20$ °C, $\rho_{CO2} = 1.8$ g/L (Kraujalis & Venskutonis, 2013).

PLE of SFE-CO_2 residues was performed in an accelerated solvent extractor ASE350 (Dionex Sunnyvale, CA, USA) with ethanol from 25 g material at 70 °C, 10.3 MPa, 5 min pre-heating, 15 min static extraction (3 cycles × 5 min), 100% of cell flush volume, 120 s purge time with nitrogen to collect the extract (Kryževičiūtė et al., 2016).

EAE of PLE-EtOH residues was performed by suspending 10 g sample in 100 mL of 50 mmol/L sodium acetate buffer (pH 3.5), adding 0.6 mL of cellulolytic (Viscozyme L) or xylanolytic (CeluStar XL) enzyme preparation; incubating in a thermostatically controlled shaker (800 rpm, 40 °C, 7 h); boiling 10 min in a water bath (EAE termination); rapid cooling and centrifugation at 9000 rpm, 10 min (Kapasakalidis et al., 2009). Resulting water-soluble supernatants and water-non soluble solid residues were collected, freeze-dried and kept at −20 °C. Control (pomace + buffer, seeds + buffer), Blank A (enzyme + buffer) and Blank B (buffer) samples were prepared simultaneously.

2.3. Determination of tocopherols, phytochemicals and sugars

Concentrations of α, β, γ and δ tocopherols (α-T, β-T, γ-T, δ-T) in SFE-CO_2 extract solutions (5 g/L) were determined on a Perkin Elmer Series 200 HPLC system (Norwalk, CT, USA) under isocratic elution conditions using $CH_3CN/MeOH/CH_2Cl_2$ (720/220/60 mL/L) as mobile phase (Kraujalis & Venskutonis, 2013).

The content of glucose, fructose and maltose in EAE supernatant solutions (1 g/L) was determined on an Acquity UPLC H-class system (Waters, Milford, MA, USA) under isocratic elution conditions using 750/250 mL/L CH_3CN/ultra-pure H_2O with 1 mL/L NH_4OH as mobile phase (Grunovaitė et al., 2016).

Phytochemical composition of extract solutions (1 g/L) was screened by modified procedure of Grunovaitė et al. (2016) on an Acquity UPLC (Waters, Milford, MA, USA) system (Supplementary Material).

2.4. In vitro antioxidant activity

Total phenolic content (TPC), Trolox Equivalent Antioxidant Capacity (TEAC in $ABTS^{\cdot+}$ scavenging) and oxygen radical absorbance capacity (ORAC) of EAE supernatants (0.1–1 g/L) was evaluated by the modified procedures of Singleton, Orthofer, and Lamuela-Raventós (1999), Re et al. (1999) and Prior et al. (2003)

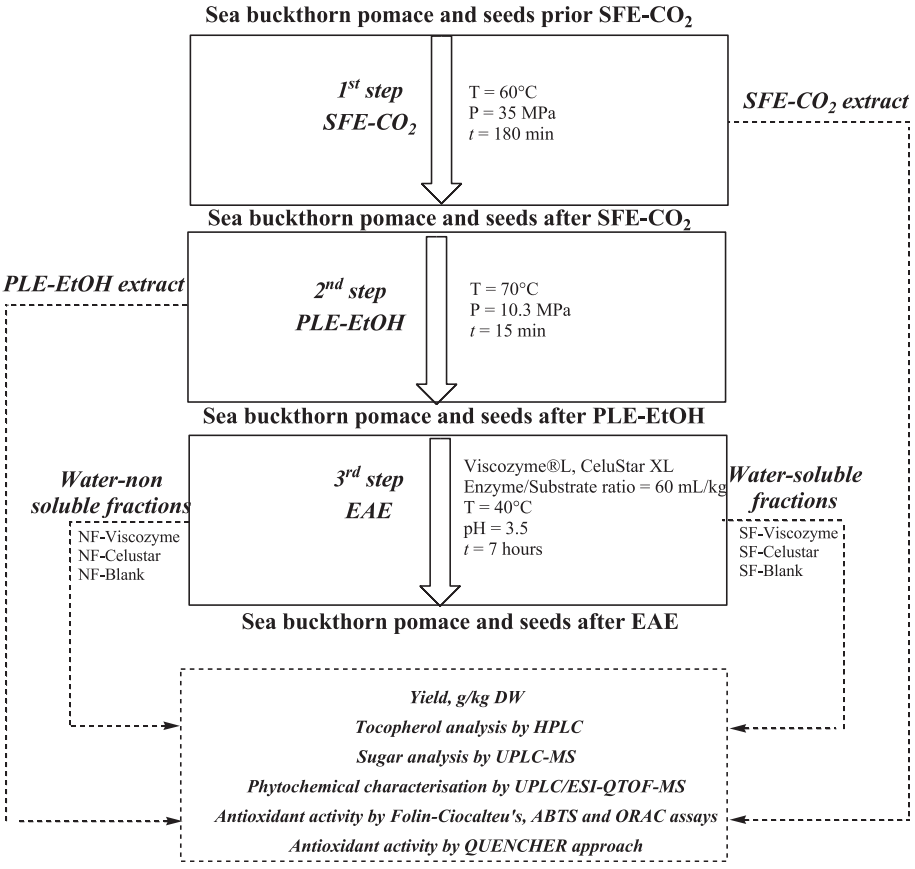

Fig. 1. Schematic representation of fractionation of sea buckthorn pomace and seeds and product analysis.