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Supercritical carbon dioxide and pressurized liquid extraction of valuable ingredients from *Viburnum opulus* pomace and berries and evaluation of product characteristics

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ABSTRACT

Supercritical carbon dioxide extraction (SFE-CO_2) of *Viburnum opulus* L. fruits and pomace was optimized using 2 level factorial and central composite design (CCD). The effects and interactions of temperature (T), pressure (P), extraction time (t) and CO_2 flow (v) were estimated for the washed *V. opulus* berry pomace, while 2 most significant factors, P and t were used further to estimate the coefficients of quadratic model and to find optimal extraction parameters by response surface methodology (RSM) for the unwashed pomace. The highest extract yields at optimal parameters (P = 55–57 MPa, t = 120–131 min, T = 50 °C and v = 2.5 L min^{-1}) from washed, unwashed berry pomace and dried whole berries were 19.1, 14.6 and 6.6%, respectively. The oil in lipophilic fractions was composed mainly of oleic (42–51%) and linoleic (42–46%) fatty acids; it contained 963–1157 mg kg^{-1} tocopherols. SFE-CO_2 residue was consecutively re-extracted by pressurized acetone, water and ethanol yielding 11.59 ± 0.95, 27.58 ± 0.79 and 30.71 ± 0.59% of extracts, respectively, which demonstrated strong antioxidant capacity in DPPH and ABTS radical scavenging, oxygen radical absorbance capacity (ORAC) and total phenolics assays. In total, biorefining of unwashed pomace yielded 62.11% of extracts from the initial plant material. The fractions obtained may be considered as valuable functional ingredients for foods, nutraceuticals and other applications.

1. Introduction

The growing interest in natural food ingredients has been an important factor in expanding the studies of less known horticultural plants in recent years. Nevertheless, some berry species remain poorly studied, including the genus of *Viburnum* (>230 species), which is grown for ornamental purposes and edible fruits (common names of *V. opulus*: guelder-rose, water elder, cramp bark, snowball tree and European cranberrybush). Evaluation of antioxidant potential and phytochemicals of several *Viburnum* genotypes demonstrated that berry juices possess strong radical scavenging capacity [1,2], due to high concentrations of phenolic constituents such as chlorogenic and quinic acids [3,4], and antimicrobial activity [1,5]. *V. opulus* fruits have been used as ingredients in sauces, jellies, marmalades and drinks [6,7]. However, the main problem in direct use of *V. opulus* berries for foods is associated with undesirable flavor notes, due to the presence of some unpleasantly smelling constituents [8]. Therefore, there is an interest in developing extraction methods for the isolation of valuable fractions from the whole *V. opulus* berries and their pomace. Biorefining of berry pomace, which are often discarded as a waste or used inefficiently, is of particular importance because large amounts of valuable constituents are lost.

Antioxidant properties of *V. opulus* were evaluated by $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging, total phenolics (TPC), ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assays [3]. Coumaroyl-quinic acid, chlorogenic acid, procyanidin B2, and procyanidin trimer were the strongest antioxidants in juice, while the composition of antioxidants in the extracts of seeds obtained with organic solvents was different [4].

Until now research has been focused mainly on the waste of the major horticultural products; e.g. supercritical fluid extraction with carbon dioxide (SFE-CO_2) was applied for citrus fruits [9] hazelnut, coffee and grape wastes [10,11], olive waste [12], raspberry pomace [13], apple peels [14] and sour cherry [15], while numerous other species remain under explored. It should also be emphasized that green technologies in biorefining agromaterials are highly preferable, particularly when the products are intended for nutrition. Green alternative methods for the isolation of antioxidant bioactive compounds from winery wastes and by-products, which also

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include SFE-CO_2, were recently reviewed [16]. SFE-CO_2 possesses many advantages (nontoxic, nonflammable, inexpensive and yields high purity extracts) and therefore can be successfully explored in food industry. Pure CO_2 is particularly effective in extraction of lipophilic constituents; for instance, oil from sea buckthorn [15,17] and pomegranate [16,18]. However for more effective isolation of polyphenolic fractions polar co-solvents are required as it was demonstrated for guava seeds [17,19] and black chokeberries [20]. The solubility of various substances in CO_2 highly depends on their properties and process parameters; therefore, SFE-CO_2 should be optimized for every individual raw material.

All previously performed studies used conventional extraction techniques for *V. opulus* and only one study applied SFE-CO_2 for *V. opulus* seeds [21]; however the yield of oil was not indicated and extraction parameters were not optimized. A statistical tool, called Response Surface Methodology (RSM) is used for SFE-CO_2 optimization in order to evaluate the effect of multiple factors and their interactions on dependent responses; whereas Central Composite Design (CCD) is the most popular form of RSM.

The main aim of this study was comprehensive evaluation of SFE-CO_2 process for obtaining lipophilic fractions from *V. opulus* berries and their pomaces. The objectives were to determine the optimal conditions of SFE-CO_2 and to evaluate the extracts obtained as possible functional ingredients for food and other applications. In addition, SFE-CO_2 residues were consecutively re-extracted using pressurized liquid extraction (PLE) with increasing polarity solvents, namely acetone, ethanol and water for obtaining higher polarity polyphenolic fractions.

2. Materials and methods

2.1. Materials

The following *V. opulus* samples obtained from the SIA BestBerry (Latvia) were used in this study: (1) whole dried berries (moisture content 8.4%), (2) dried berry pomace (obtained after pressing juice, moisture content 9.3%), and (3) washed berry pomace (obtained after washing berry pomace with water to remove soluble juice residues and drying at 45–50 °C for 6 h; moisture content 9.5%).

Fatty acid methyl esters (FAME) and tocopherols, DL-α-T (99.9%), rac-β-T (90 +%), γ-T (99%) and δ-T (95.5%), were from Supelco Analytical (Bellefonte, PA, USA); 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetra-methyl-chroman-2-carboxylic acid (Trolox), microcrystalline cellulose (20 μm), fluorescein (FL) sodium salt, [2,2-azobis(2-methylpropionamidine) dihydro-chloride] (AAPH), Folin–Ciocalteau's reagent and HPLC grade solvents were from Sigma-Aldrich Chemie (Steinheim, Germany). Ascorbic acid was from Across Organics (Geel, Belgium), KOH from Lachema (Neratovice, Czech Republic), *n*-hexane from Reachem Slovakia (Bratislava, Slovakia). Randomly methylated β-cyclodextrin (RMCD, Trappsol) was from CTD Holdings (High Springs, FL, USA), CO_2 (99.9%) and helium from AGA (Vilnius, Lithuania).

2.2. Milling and particle size determination

The samples were ground in an ultra-centrifugal mill ZM 200 (Retsch, Haan, Germany) using 500 μm hole size sieve. Dried berries before grinding were frozen in liquid nitrogen. Particle size distribution of ground berries was measured on a Mastersizer, Hydro 2000S (A) analyzer (UK) operating on a laser diffraction method [22]. Water was used as a dispersant for wet analysis, dispersant refractive index was 1.33, and particle refractive index was 1.53. Volumetric mass of ground materials was as follows (in g cm^{-3}): berries 0.479 ± 0.003, unwashed pomace 0.442 ± 0.003, washed pomace 0.431 ± 0.004.

2.3. Supercritical carbon dioxide extraction (SFE-CO_2)

SFE-CO_2 was performed in a Helix extractor (Applied Separation, Allentown, PA) from 20 g of ground sample placed in a 50 mL cylindrical vessel (320 × 14 mm) between two layers of cotton wool (Fig. 1). The temperature of the extraction vessel was controlled by a surrounding heating jacket. The flow rate of CO_2 in the system (v) was controlled manually by the micro-metering valve (back-pressure regulator). The volume of CO_2 consumed was measured by a ball float rotameter and a digital mass flow meter in liters per min (L min^{-1}) at standard state: pressure (P) = 100 kPa, temperature (T) = 20 °C, density (ρ) = 0.0018 g mL^{-1}. The conditions for extraction were set as shown in Table 1. Optimized T and P conditions were further used for the up-scaling SFE-CO_2 using 10 L extraction vessel in a pilot extractor (Applied Separation, Allentown, PA).

2.4. Pressurized liquid extraction (PLE)

PLE was performed on an accelerated solvent extraction apparatus ASE 350 (Dionex, Sunnyvale, CA, USA) in 34 mL extraction cells applying two cellulose and metal filters at the top and at the bottom. Unwashed *V. opulus* berry pomace residues (15 g) obtained after SFE-CO_2 at optimal parameters was consecutively re-extracted by PLE with acetone, ethanol and water at the following parameters: 70 °C, 10.3 MPa, 5 min pre-heating, 15 min static extraction (3 cycles × 5 min), 100% of cell flush volume and 100 s purge time with N_2 for acetone and ethanol, and 120 °C with 7 min pre-heating for water extraction. Dried material before extraction with water was mixed with diatomaceous earth (1:1). These extraction parameters were selected from our previous extraction experiments with raspberry pomace [13]. The extracts obtained by PLE were collected in the separate vials, acetone and ethanol were evaporated in a Büchi V–850 Rotavapor R–210 (Büchi Labortechnik AG, Flawil, Switzerland), while water residues were freeze-dried. Dry extracts were stored at −18 °C.

Soxhlet extraction with hexane was used as a reference method [23] using 20 g of material. The solvent was removed in a rotary vacuum evaporator at 40 °C.

2.5. Determination of tocopherols

Oily extracts were saponified by placing 0.1 g extract and 0.05 g antioxidant ascorbic acid in a screw-capped tube with 5 mL ethanol (95%) and 0.5 mL KOH (60%). After vortexing 60 s the tubes were flushed with nitrogen 60 s, capped and mixed for 2 h at room temperature. Afterwards 3 mL deionized water and 5 mL hexane were added and again vortexed 60 s. The suspension was extracted four times with 5 mL hexane. The organic layer was collected and evaporated to dryness with nitrogen; the residue was diluted with HPLC mobile phase to a final concentration of 1.6%.

Tocopherols were determined by HPLC [24] using Shimadzu HPLC system with solvent delivery unit (LC-20A), system controller (CBM-20A), auto-sampler (SIL-20A), column oven (CTO-20A) and fluorescence detector. A reverse-phase C_{30} column (particle size 5 μm, 250 × 4.6 mm) was termostated at 30 °C applying isocratic elution with acetonitrile:methanol:dichlormethane (72/22/6, v/v/v). Injection volume was 20 μL, flow rate 1 mL min^{-1}. Tocopherols were detected using fluorescence detector at 290 nm excitation and 330 nm emission and identified by comparing their retention times with reference solutions prepared at different concentrations (0–10 μg mL^{-1}) using mobile phase. The calibration