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Composition and antioxidative activities of supercritical CO_2-extracted oils from seeds and soft parts of northern berries

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ABSTRACT

The present study investigated the composition and the antioxidative activities of oils from the seeds and the soft parts of a range of northern berries extracted by supercritical CO_2. The seed oils of the species of *Rubus*, *Vaccinium*, *Empetrum*, *Fragaria* and *Hippophaë* were rich in linoleic (18:2n-6, 34–55% of total fatty acids) and α-linolenic (18:3n-3, 29–45% of total) acids with n-6:n-3 ratios of 1:1–1:2. The seed oils of the species *Ribes* contained, in addition to linoleic and α-linolenic acids, γ-linolenic (18:3n-6) and stearidonic (18:3n-4) acids. In seed oils from European rowanberry (*Sorbus aucuparia* L.) and snowball berry (*Viburnum opulus* L.), linoleic and oleic (18:1n-9) acids together exceeded 90% of the total fatty acids. The sea buckthorn (SB) pulp oil had palmitoleic (16:1n-7), palmitic (16:0) and oleic acids as the major fatty acids. The SB pulp oil and snowball berry seed oil were rich in α-tocopherol (120 and 110 mg/100 g oil, respectively), whereas raspberry seed oil contained a high level of γ-tocopherol (320 mg/100 g oil). Seed oils of cranberry (180 mg/100 g oil), Arctic cranberry (190 mg/100 g oil) and lingonberry (120 mg/100 g oil) are rich sources of γ-tocotrienol. The berry seed oils and the SB pulp oil showed varying peroxyl radical scavenging efficacies (300–2300 μmol α-tocopherol equivalent per 100 g oil) and inhibitory effects on perioxidation of microsomal lipids (250–1200 μmol trolox equivalent per 100 g oil) in vitro. The peroxyl radical scavenging activity positively correlated with the total content of tocopherols and tocotrienols of the oils ($r = 0.875$, $P = 0.001$). The SB seed oil and pulp oil were active in scavenging superoxide anions produced by xanthine–xanthine oxidase system and inhibited Cu^{2+}-induced LDL oxidation in vitro. The SB oils also protected purified DNA and rat liver homogenate from UV-induced DNA oxidation in vitro. The current research suggests potential of supercritical CO_2-extracted oils from northern berries as nutraceuticals and ingredients of functional foods.

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1. Introduction

Vegetable oils are important raw materials for food and food ingredients as well as major sources of essential fatty acids and lipid-soluble bioactive components for human diet. During the past century, plant breeding programs aimed for improved oil stability have resulted in decreased proportion of n-3 fatty acids in the vegetable oils most commonly used for industrial food production and homemade aliment preparation (Gunstone & Harwood, 2007). Industrial processing, such as refining and hydrogenation, significantly reduces the content of essential fatty acids and liposoluble vitamins and antioxidants in oil. The imbalance between the intake of fatty acids of n-6 and n-3 families in Western diet has been widely recognized by scientists and nutrition authorities (Simopoulos, 2000, 2001, 2008; de Wilde, Farkas, Gerrits, Kiliaan, & Luiten, 2002; Oddy, de Klerk, Kendall, Mihrshahi, & Peat, 2004). Although increasing the consumption of fish and fish oil has been recommended as an effective way of correcting the deficiency in n-3 fatty acids, it is of great importance that vegetable oils of balanced composition of n-6 and n-3 fatty acids are available for consumers of various cultural backgrounds and dietary habits.

Wild and cultivated berries are widely used as raw materials for food and drinks in Western and Northern Europe and the North America. Wild berries of *Vaccinium* species (e.g. bilberry and lingonberry) and *Rubus* species (e.g. wild raspberry and cloudberry) are commonly harvested and processed in Northern and Western Europe. Strawberry, currants, and raspberry are among the most important cultivated berries in Europe. In addition, cranberry and blue berries are commonly cultivated in the US and Canada. Juice pressing is a common way of industrial berry processing. As by-products of the process, the press residues consist of the pulp/peel fraction and the seeds of the berries. The pulp/peel fractions of different berries are used as valuable raw materials for extraction of phenolic compounds

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with multiple potential health benefits (Cheng et al., 2003; Zheng & Wang, 2003; Seeram et al., 2006; Ogawa et al., 2008; Zhang, Seeram, Lee, Feng, & Heber, 2008).

Berry seeds are rich in oil (Johansson, Laakso, & Kallio, 1997; Johansson, Laine, Linna, & Kallio, 2000). Some berry seeds contain high levels of polyunsaturated fatty acids with desirable ratios between n-6 and n-3 (Goffman & Galletti, 2001; Johansson et al., 1997, 2000; Johansson, Korte, Yang, Stanley, & Kallio, 2000). The unique fatty acid composition, often in combination with high contents of lipid-soluble antioxidants, makes the seeds of some wild and cultivated berries valuable raw materials for nutraceuticals and functional ingredients of foods (Goffman and Galletti, 2001; Yang, Karlsson, Oksman, & Kallio, 2001; Yang, Koponen, Tahvonen, & Kallio, 2003; Bushman et al., 2004; Parry et al., 2005). In vitro studies have shown antioxidative activities of selected berry seeds and cold pressed berry seed oils (Bushman et al., 2004; Parry et al., 2005).

Cold pressing and solvent extraction with iso-hexane followed by various refining processes are conventional technologies for manufacturing commodity vegetable oils. While cold pressing technology often suffers from low yield, organic solvent extraction imposes the risk of solvent residues in the oil and decreased contents and bioavailability of some important bioactive components due to the refining process. Supercritical fluid extraction technique (SFE) takes advantages of the high penetrating and solvating power of supercritical fluids for extraction of lipids and other bioactive substances from different types of matrices (Stahl, Quirin, & Gerard, 1987; King & List, 1996). Supercritical CO_2 extraction is the most commonly used SFE process for obtaining the lipophilic extracts free of residues of conventional organic solvents (Lenucci et al., 2010). Supercritical CO_2 extractions are often carried out at mild temperature in absence of oxygen; thus, it is possible to avoid thermal and oxidative damages to the bioactive components in the extract. Furthermore, supercritical CO_2 extraction is an environment-friendly process.

The aim of the present study was to investigate the composition and antioxidative activities of a range of oils extracted by supercritical CO_2 from seeds and soft parts of northern berries. The fatty acid composition of the oils was analyzed by gas chromatography (GC-FID), and the tocopherols and tocotrienols by high performance liquid chromatography (HPLC) combined with diode array detection. The antioxidative activities of the oils were studied using different in vitro models.

2. Materials and methods

2.1. Berries and seeds

Wild bilberries (*Vaccinium myrtillus* L.), lingonberries (*Vaccinium vitis-idaea* L.), Arctic cranberries (*Vaccinium oxycoccos* L.), crowberries (*Empetrum nigrum* L.), and cloudberries (*Rubus chamaemorus* L.) were collected in northern Finland. Wild sea buckthorn (*Hippophaë rhamnoides* L. ssp. *rhamnoides*) berries were collected from the Baltic coast in southern Finland. Cultivated raspberries (*Rubus idaeus* L.), blackcurrants (*Ribes nigrum* L.) and redcurrants (*Ribes rubrum* L.) were from the cultivating sites in southern Finland. Juice was pressed from the fresh berries. After juice pressing, the press residues were dried with hot-air in an oven with temperature controlled below 50 °C. The seeds were separated from the dried press residues mechanically by wind-screening. Seeds of snowball berries (European cranberrybush, *Viburnum opulus* L.) were separated from dry berries obtained from Novosibirsk, Russia via a commercial source. Seeds of European rowanberries (*Sorbus aucuparia* L.) and strawberries (*Fragaria X ananassa* Duch.) were separated from press residue from juice processing, supplied by Bayernwald Früchteverwertung GmbH (Hengersberg, Germany). Cranberry seeds (*Vaccinium macrocarpon* L.) were supplied by BRB Seeds, Inc (Prosser, WA).

2.2. Reagents and reference compounds

α-, β-, γ- and δ-Tocopherols were purchased from Sigma-Aldrich Co. (St. Louis, MO). α-, β-, γ- and δ-Tocotrienols were purchased from Davos Life Science Pte Ltd. (Singapore). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was from Aldrich Chem. Co. (Milwaukee, WI). Methanol (HPLC grade) was from J. T. Baker (Deventer, Holland), acetonitrile (HPLC grade) was from VWR International Oy (Espoo, Finland), and hexane (HPLC grade) was from Rathburn Chemicals Ltd. (Walkerburn, Scotland). *tert*-Butyl hydroperoxide (t-BuOOH) (70% aqueous solution) and sodium linoleate (sodium salt of *cis*-9,12-octadecadienoic acid) were from Sigma Chemical Co. (St. Louis, MO). Lucigenin (*bis*-N-methylacridinium nitrate) and luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) were from Bio-Orbit Ltd. (Turku, Finland), 2,2′-Azobis(2,4-dimethylvaleronitrile) (AMVN) was purchased from Cayman Chemicals Co. (Ann Arbor, MI). Ethanol of analytical grade was from Primalco Oy (Rajamäki, Finland).

2.3. Supercritical CO_2 extraction of berry seeds

The supercritical CO_2 extractions were carried out at Aromtech Ltd. (Tornio, Finland). Seeds and dried fruit pulp/peel were milled with a Murska 220 SM roller mill (Aimo Kortteen Konepaja Oy, Finland). Milled seeds (500 g for each sample) were extracted with a pilot CO_2 extraction facility using a 2 L extraction vessel (Chematur Ecoplanning, Tampere, Finland). The extraction temperature was 50 °C, and the extraction pressure was 350 bars. The flow rate of CO_2 was 0.4 L/min. The extraction time was 120 min. The pressure and temperature of the separator were 100 bars and 50 °C, respectively.

2.4. Analysis of fatty acid composition of berry seed oils

Methyl esters of fatty acids (FAMEs) were prepared by transesterification of the berry seed oil using a sodium methoxide catalysis method (Yang et al., 2000). The FAMEs were analyzed with a PerkinElmer AutoSystem gas chromatograph combined with a flame ionization detector, controlled by TotalChrom Workstation version 6.3.1 (PerkinElmer, Waltham, MA). A silica capillary column PE-FFAP (30 m, i.d. 0.32 mm, d_f, 0.25 μm, PerkinElmer, Waltham, MA) was used with a temperature program started at 110 °C, increased to 210 °C at a rate of 5 °C/min and held at 210 °C for 5 min. The injector temperature was 240 °C, that of the detector 280 °C. FAMEs were identified by comparing their retention times with those of the fatty acid standard mixture 68D of known composition (NuChek Prep, Elysian, MN). The fatty acid composition was expressed as weight percentages of individual fatty acids of the total. The analyses were carried out in duplicates, and the average values of the two analyses were presented.

2.5. Analysis of tocopherols and tocotrienols in berry seed oils

An aliquot of 500–700 mg seed oil was weighed accurately and transferred quantitatively to a 25 mL volumetric bottle using 20 mL of methanol as solvent. The sample was put into an ultrasonic bath for 10 min to speed up the dissolution of the oil in methanol. After this, the sample was left to stand for 10 min, followed by addition of methanol to make a final volume of 25 mL. After a thorough mixing, the sample was left to stand for 20 min followed by filtration through a PTFE syringe filter (0.45 μm). The samples were analyzed by a Series 200 high performance liquid chromatograph equipped with a diode array detector (PerkinElmer, Waltham, MA). A reverse-phase Brownlee C-18 column (5 μm, 100×4.6 mm, PerkinElmer, Shelton, CT) was used for the analysis of tocopherols and tocotrienols. The mobile phase was methanol:acetonitrile:water (50:44:6, v/v/v). The flow rate of the mobile phase was 1.0 mL/min. The peaks were detected at

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