Food Research International 44 (2011) 2009–2017

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

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

Baoru Yang [a,*], Markku Ahotupa [a], Petri Määttä [b], Heikki Kallio [a]

[a] Department of Biochemistry and Food Chemistry, University of Turku, FI-20014 Turku, Finland
[b] Aromtech Ltd., Veturitallintie 1, 95410 Tornio, Finland

ARTICLE INFO

Article history:
Received 3 December 2010
Accepted 14 February 2011

Keywords:
Antioxidative activity
Berry seed oils
Fatty acids
Supercritical fluids
Tocopherols
Tocotrienols

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.

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

* Corresponding author at: Department of Biochemistry and Food Chemistry, University of Turku, FI-20014 Turku, Finland. Tel.: +358 2 3336844; fax: +358 2 3336860.
E-mail address: baoru.yang@utu.fi (B. Yang).

0963-9969/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2011.02.025

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

295 nm. The tocopherols and tocotrienols were identified by comparing the retention times and the UV absorption spectra of the sample peaks with those of the reference compounds. α-Tocopherol was used as an external standard. A series of methanol solutions of α-tocopherol of varying concentrations (0.01, 0.02, 0.04, 0.08, and 0.16 mg/mL) were analyzed. A calibration curve was constructed by plotting the peak area as the function of the concentration. The correction factors were determined by analysis of reference compounds of tocopherol and tocotrienol isomers of known concentrations and were used in the quantification of isomers of tocopherols and tocotrienols. The analyses were carried out in duplicates, and the average values of the two analyses were presented.

2.6. Determination of peroxyl radical scavenging activity

Peroxyl radical scavenging activity of a range of CO_2-extracted berry seed oils was studied using a method previously described (Ahotupa, Mantyla, & Kangas, 1997) after modifications. 2,2′-Azobis (2,4-dimethylvaleronitrile) (AMVN) was used as a lipophilic source of peroxyl radicals in a chloroform:methanol (6:4, v/v)-based reaction system. The amount of peroxyl radicals was determined by measuring the chemiluminescence resulted from oxidation of luminol. The oil samples were diluted in pure ethanol (dilution factor 1:10–1:100) before addition to the reaction system, whereas pure ethanol was added for the negative control. The chemiluminescence measurements were performed with a Bio-Orbit 1251 Luminometer (Bio-Orbit, Turku, Finland). The lag time between initiation of the reaction and the half peak point of the chemiluminescence (the time required to reach half peak height) was determined to measure the antioxidative activity of berry seed oils. α-Tocopherol was used as a standard compound for quantification of the antioxidant activity, assuming that one mole of α-tocopherol scavenges two moles of peroxyl radical. The stoichiometric factors, i.e. the amount of test compounds required for scavenging one mole of peroxyl radicals, were calculated by comparing the lag time induced by the test oils and that by α-tocopherol at known concentration. The antioxidative activity was also expressed as equivalent of α-tocopherol (micromole) activity in 100 g samples.

2.7. Inhibition of lipid peroxidation by berry seed oils

The antioxidative activity of a range of supercritical CO_2-extracted berry seed oils was evaluated by their potency of inhibiting *tert*-butylhydroperoxide (t-BuOOH)-induced lipid peroxidation in rat liver microsome in vitro (Ahotupa et al., 1997). The microsomes were prepared using a method previously described (Ahotupa et al., 1997). A 20% liver homogenate was prepared in sucrose solution (0 °C) with a Potter-Elvehjem glass-Teflon homogenizer driven by an electric drill at 500 rpm. Microsomes were prepared from the postmitochondrial supernatant fluid (obtained by centrifugation at 12,000×*g* for 10 min at 4 °C) by centrifugation at 10,500×*g* for 10 min at 4 °C. The microsome pellet was collected and resuspended in 0.15 M KCl water solution. The protein content of the microsome preparation was determined by the biuret method (Layne, 1957). An aliquot of 800 μL of buffer (50 mM sodium bicarbonate, pH 10.2 with 0.1 mM EDTA) was transferred into a luminometer cuvette. A volume of 20 μL of dilution of rat liver microsome (1.5 μg protein/mL) was added, followed by 6 μL of luminol solution (0.5 mg/mL) and ethanol solution of berry seed oils (<2% of the incubation volume). For a negative control, pure ethanol was added to a parallel reaction system. The reaction was initiated by adding 50 μL of 0.9 mM t-BuOOH solution at 33 °C. The chemiluminescence was monitored with a Bio-Orbit 1251 Luminometer (as described in previous section) for 45 min with intervals of 1 min between measurements. The integrated area under curve was used to determine the inhibitory effects of the berry seed oils, which were compared with the negative control. Trolox was used as a reference antioxidant in the assay. The results were expressed both as IC_{50} values, i.e. the concentrations needed for the berry seed oils to inhibit the lipid peroxidation by 50% and as trolox equivalent (micromole) activity in 100 g samples.

2.8. Inhibition of LDL oxidation

The effects of supercritical CO_2-extracted sea buckthorn seed oil and pulp oil on the oxidation of human low density lipoprotein (LDL) were investigated in vitro. Oxidation of isolated LDL was induced in vitro by the presence of copper ion (Cu^{2+}) (Kleinveld, Haklemmers, Stalenhoef, & Demacker, 1992). The sea buckthorn oils were dissolved in a small volume of ethanol and added to the oxidation/incubation mixture at different dosage levels. Trolox, a water soluble form of vitamin E as a reference antioxidant (positive control), was added as water solution to the oxidation/incubation system at different dosages. In the negative control, the isolated LDL particles were oxidized without presence of sea buckthorn oils or Trolox. The oxidation process was allowed to proceed at 37 °C for 3 h. After the oxidation, lipids were extracted with chloroform:methanol (2:1, v/v), dried under nitrogen, and redissolved in cyclohexane. Detection of oxidation was performed by spectrophotometric analysis of conjugated dienes in the reaction system. The absorbance intensities at 234 nm were measured with a PerkinElmer Lambda 2 spectrophotometer (PerkinElmer Corp., Norwalk, CT, USA) and used for quantification of conjugated dienes (Ahotupa, Ruutu, & Mantyla, 1996). Results are given as percentage of inhibition caused by the indicated (final) concentration of the oils or trolox in the reaction system and as IC_{50} values, which are the indicated concentration causing a 50% inhibition of the formation of conjugated dienes compared to the negative control (Ahotupa et al., 1996). All the tests were repeated three times on three different days (one repeat per day) and the average values of the repeats were presented.

2.9. LDL incorporation

The test is based on inhibition of LDL oxidation due to LDL incorporation of test substances. The results are indicative of the capacity of selected compounds to incorporate into LDL particles in vitro. Supercritical CO_2-extracted sea buckthorn seed oil and pulp oil were tested in this model in order to investigate the efficiency of the antioxidative components of the oils for incorporating into LDL particles and therefore protecting the LDL particles from oxidative stress in vitro. Incorporation was affected by incubation of the sea buckthorn oils with serum at +37 °C for 3 h. After the incubation, the LDL particles were precipitated with heparin in a precipitation buffer (Ahotupa et al., 1996). The susceptibility of LDL to oxidation was determined by copper-induced formation of conjugated dienes (Kleinveld et al., 1992; Ahotupa et al., 1996). Results are given as percentage of inhibition caused by the indicated concentration of the oil in the incubation system and as IC_{50} values.

2.10. Superoxide anion scavenging capacity

Superoxide anions were produced by a xanthine–xanthine oxidase system and detected by lucigenin-amplified chemiluminescence (Ahotupa et al., 1997). Twenty microliters of xanthine oxidase solution (420 mU/mL), 0.02 mL of lucigenin (5 mM), 0.02 mL of linoleic acid (200 mM in 50 mM KOH), and 0.78 mL of 50 mM potassium phosphate buffer (pH 10.0) were pipetted into a cuvette as the reaction system. Supercritical CO_2 extracted sea buckthorn seed oil, pulp oil or trolox (positive control) was added to the reaction system at different concentrations in order to investigate the efficacies of these materials in scavenging superoxide anion radicals. The reaction system without oil or trolox addition was used as the negative control. The superoxide anion producing reaction was

initiated by automated dispersing 0.11 mL of xanthine (1.45 mM). Chemiluminescence was measured at 35 °C for 6 min at 1-min intervals. The chemiluminescence measurements were performed with a Bio-Orbit 1251 luminometer (Turku, Finland). Duplicate reactions were carried out for each test material and for the negative control. The mean area under curve was automatically calculated using the chemiluminescence as a function of reaction time. The superoxide anion radical scavenging activities of test samples were expressed as concentrations needed for 50% inhibition of chemiluminescence compared to the negative control (IC_{50} values).

2.11. DNA oxidation study

Oxidation of pure DNA and DNA in rat liver homogenates was induced by a 24-h exposure of UV-light at room temperature. α-Tocopherol and trolox were used as positive controls. The oxidation induction was carried out with samples of pure DNA or rat liver homogenates in the presence of test oils, α-tocopherol or trolox at different concentrations. The test oils were added to the test mixture as such, and the antioxidants were dissolved in ethanol before addition to the test mixture. DNA and liver homogenate without addition of test oil or antioxidants were used as negative controls. 8-Oxo-deoxyguanosine (8-oxo-dG) was determined as an indication of oxidative DNA modification (Helbock et al., 1998), and the extent of DNA oxidation was quantified by measuring the 8-oxo-deoxyguanosine/deoxyguanosine ratio. DNA from rat liver homogenate was isolated by a non-enzymatic method (Lahiri & Nurnberger, 1991). Pure DNA was dissolved in HPLC-grade water and deferoxamine mesylate was added to reduce artificial oxidation. DNA was hydrolyzed to nucleotides on incubation with nuclease P1; thereafter, the samples were further hydrolyzed to nucleosides with alkaline phosphatase. The nucleosides were separated with a C18 reverse-phase column. The amount of 8-oxo-dG was determined using HPLC equipped with an electrochemical detector, and deoxyguanosine was determined with a UV detector at 290 nm (Helbock, Thompson, Yeo, & Ames, 1996). HPLC configuration was: system controller SCL-10Avp, solvent delivery module LC-10ADvp, degasser PGU-14A and UV-VIS-detector SPD-10Avp from Shimadzu (Kyoto, Japan) and electrochemical detector Intro from ANTEC (Leyden, the Netherlands). The cell potential of the electrochemical detector was 700 mV and its range value was 0.2 nA/V. The HPLC system was operated and data from both detectors were acquired by Shimadzu CLASS-VP software.

2.12. Statistical analysis

The correlations between the total contents of tocopherols and tocotrienols of the oils and their peroxyl radical scavenging activities and inhibitory effect on peroxidation of microsomal lipids were analyzed with Pearson Correlation using the statistical software SPSS 16.0. Correlations reaching a confidence level of 95% ($P<0.05$) were considered as statistically significant.

3. Results and discussion

3.1. Fatty acid composition

Table 1 presents the yield and fatty acid composition of the oils extracted from different seeds by supercritical CO_2. In most of the berry seed oils analyzed, the two essential fatty acids, linoleic (18:2n-6) and α-linolenic (18:3n-3) acids, were the major fatty acids. Typically the two fatty acids together represented 60–80% of the total fatty acids, the ratio between the two ranging from 1:1 to 2:1. These oils are optimal as food ingredients or food supplements for increasing the intake of n-3 fatty acids. The content of oleic acid (18:1n-9) was 11–46% of total fatty acids in the most seed oils analyzed, lowest level found in raspberry seed oil and the highest in snowball berry seed oil.

Two exceptions were snowball berry seed oil and European rowanberry seed oil, which contained a high proportion of linoleic acid (50% and 62%, respectively) and oleic acid (46% and 27%, respectively) but only a negligible amount of α-linolenic acid (<1%). Sea buckthorn berry oil consisted of mostly oil of the fruit flesh and peel fraction with a high proportion of palmitoleic (16:1n-7, 39%) and palmitic (16:0, 29%) acids. The n-6:n-3 ratio was 3.7:1 in blackcurrant (*R. nigrum*) seed oil and 1.9: 1 in redcurrant (*R. rubrum*) seed oil. In addition to the essential fatty acids, the seed oils of blackcurrant and redcurrant contained γ-linolenic acid (18:3n-6) and stearidonic acid (18:4n-3), the metabolites of linoleic and α-linolenic acids, respectively, by the action of Δ^6-desaturase. The activity of Δ^6-desaturase is generally considered as a rate limiting factor in the metabolic cascade of essential fatty acids. Dietary supplementation with γ-linolenic acid (18:3n-6) and stearidonic acid (18:4n-3) may bypass deficiency in Δ^6-desaturase activity. The results of the current study are in agreement with the report of Goffman & Galletti, 2001 indicating a clearly lower content of γ-linolenic acid in the seed oil of *Ribes rubrum* L. than that of *Ribes nigrum* L.

3.2. Tocopherols and tocotrienols

The contents of tocopherols and tocotrienols in the seed and berry oils are summarized in Table 2. In most of the oils, α- and γ-tocopherols were the major tocopherol isomers, whereas δ-tocopherol was present in significant amount only in oils from the seeds of currants, raspberry and snowball berry. The raspberry seed oil was an excellent source of γ-tocopherol (320 mg/100 g oil), whereas sea

Table 1
Fatty acid composition of CO_2 extracted oils. Extraction pressure, 350 bars; extraction temperature, 50 °C.

Oils	Fatty acids (% w/w of total fatty acids)[a]											
	16:0	16:1n-7	18:0	18:1n-9	18:1n-7	18:2n-6	18:3n-3	18:3n-6	18:4n-3	20:0	20:1n-9	Total
Sea buckthorn seed oil	8.9		1.9	20.3	2.3	37.4	29.0			0.2		100
Sea buckthorn pulp oil	29.1	38.6	1.2	16.6	8.2	3.6	2.4			0.3		100
Blackcurrant seed oil	5.7		1.5	13.4	0.7	46.1	13.6	15.0	2.8		1.0	100
Redcurrant seed oil	3.9		1.5	17.9	0.4	41.4	22.3	8.8	3.8	0.1		100
Raspberry seed oil	2.6		0.9	11.3	0.7	55.4	28.8			0.4		100
Strawberry seed oil	4.5		1.5	17.8	0.6	41.2	33.3			0.8	0.3	100
Cloudberry seed oil	3.3		1.6	17.8	0.7	43.6	29.8			1.0	1.8	100
Bilberry seed oil	4.5		1.1	21.8	0.6	35.9	36.1					100
Cranberry seed oil	5.2		1.0	23.6	0.5	35.3	34.3			0.1		100
Arctic cranberry seed oil	4.8		1.2	23.4	0.7	35.0	34.6			0.2		100
Crowberry seed oil	3.0		1.3	13.7	1.0	42.5	37.4			0.8		100
European rowanberry seed oil	7.1		1.3	26.6	0.6	62.4	0.8			0.5	0.4	100
Snowball berry seed oil	1.6		0.7	45.7	1.0	50.1	0.7			0.1		100
Lingonberry seed oil	1.0		0.2	19.3	0.6	34.1	44.8					100

[a] Values are averages of two replicates; the coefficient of variation of the method is below 5%.

Table 2
Content of tocopherols (T) and tocotrienols (Tr) in supercritical CO_2-extracted oils. Extraction pressure, 350 bars; extraction temperature, 50 °C.

Oils	Tocopherols (T) and tocotrienols (Tr) (mg/100 g oil)[a]							
	α-T	β-T	γ-T	δ-T	α-Tr	β-Tr	γ-Tr	δ-Tr
Sea buckthorn seed oil	90		80					
Sea buckthorn pulp oil	120		50					
Blackcurrant seed oil	10		90	10				
Redcurrant seed oil	0		60	30				
Raspberry seed oil	70		320	10				
Strawberry seed oil	0		20					
Cloudberry seed oil	110		150					
Bilberry seed oil					10		30	
Cranberry seed oil			4		10		180	1
Arctic cranberry seed oil			5		5		190	
Crowberry seed oil			10				30	10
Snowball berry seed oil	110		40	60				
Lingonberry seed oil					10		120	

[a] Values are averages of two replicates; the coefficient of variation of the method is below 5%.

buckthorn berry oil (120 mg/100 g oil), cloudberry seed oil (110 mg/100 g oil), and snowball berry oil (110 mg/100 g oil) were rich in α-tocopherol. In sea buckthorn seed oil and cloudberry seed oil, α- and γ-tocopherols were almost equally abundant.

α-Tocopherol is the most important lipid-soluble antioxidant in human body. Dietary sources of natural *d*-α-tocopherol are often preferred to the synthetic *dl*-α-tocopherol due to its higher bioavailability in oral application (Traber & Blatt, 2002). The content of α-tocopherol in sea buckthorn berry oil was close to the level found in wheat germ oil and clearly higher than the levels found in the vegetable oils commonly used for food such as soybean oil, sunflower oil and rapeseed oil (Gunstone and Harwood, 2007).

Although γ-tocopherol has much lower vitamin E activity compared with α-tocopherol, the former isomer is likely a more potent scavenger of nitrogen-containing reactive molecules such as nitric oxide (NO), nitrogen dioxide (NO_2), nitrous acid, and peroxynitrite ($ONOO^-$) (Stone & Papas, 2003). Studies have shown anti-inflammatory potential of γ-tocopherol and its metabolites probably via inhibition of cyclo-oxygenase-catalyzed synthesis of prostaglandin E_2 (PGE_2) (Reiter, Jiang, & Christen, 2007; Wagner, Jiang, Harkema, Illek, Patel, Ames et al., 2007; Jiang et al., 2008; Jiang, Moreland, Ames, & Yin, 2009; Wagner, Harkema, Jiang, Illek, Ames and Peden, 2009). The content of γ-tocopherol in raspberry seed oil is several times higher than the contents reported in commonly used vegetable oils (10–60 mg/100 g) (Gunstone & Harwood, 2007). Cloudberry seed oil is also an excellent source of γ-tocopherol (150 mg/100 g), the content being close to the level reported of hemp seed oil (Gunstone & Harwood, 2007).

Oils from seeds of cranberry and lingonberry, two species of *Vaccinium*, contained exceptionally high levels of γ-tocotrienol (180 mg and 120 mg per 100 g oil, respectively). Tocotrienols differ from the corresponding tocopherols by the presence of three double bonds in the phytyl side chain, which gives the molecules better mobility within the cells. Some evidence suggests that tocotrienols are more potent in their antioxidative and anticancer effects than tocopherols due to the differences in chemical structures (Goh, Hew, Norhanom, & Yadav, 1994; Nesaretnam, Stephen, Dils, & Darbre, 1998; Stone & Papas, 2003; Nesaretnam, 2008). Palm oil and rice bran oil are known as rich sources of tocotrienols (30–60 mg/100 g oil), γ-tocotrienol being the major isomer accompanied by small quantities of α-, β-, and δ-isomers. Cranberry seed oil is the richest known source of natural γ-tocotrienol (Stone & Papas, 2003).

3.3. Peroxyl radical scavenging activities of oils from berries

In this assay peroxyl radicals were generated by thermal degradation of AMVN [2,2′-azobis(2,4-dimethylvaleronitrile)]. The capacity of the berry seed oils for scavenging peroxyl radicals was measured indirectly as inhibition of chemiluminescence generated by oxidation product of luminol. Among the oils tested, raspberry seed oil showed the highest capacity in quenching the peroxyl radicals (2315 μmol trolox equivalent in 100 g oil), followed by Arctic cranberry seed oil (1543 μmol trolox equivalent in 100 g oil), cloudberry seed oil (1157 μmol trolox equivalent in 100 g oil) and blackcurrant seed oil (1068 μmol trolox equivalent in 100 g oil) (Table 3). The study was carried out in chloroform:methanol-based reaction system, where lipid-soluble antioxidants such as tocopherols and tocotrienols were well dissolved and had good access to peroxyl radicals generated in the system. The peroxyl radical scavenging activity of different berry seed oils in this study, corresponding to lipophilic oxygen radical absorbance capacity (lipophilic ORAC), correlated positively with the total content of tocopherols and tocotrienols in the oils (Fig. 1A, $r = 0.875$, $P = 0.001$).

Bushman et al. (2004) analyzed the fatty acids and tocopherols of cold pressed oil from seeds of five *Rubus* species commonly grown in Northwestern United States and Canada: red raspberry, black raspberry, boysenberry, Marion blackberry, and evergreen blackberry. The oils contained 53–63% linoleic acid, 15–31% α-linolenic acid, and 3–8% saturated fatty acids. The raspberry species had higher percentages of oil and the highest amounts of α-linolenic acid, but the lowest amounts of saturated fatty acid. The oxygen radical absorbance capacity (ORAC, micromole trolox equivalent per 100 g oil) of the cold pressed seed oils was measured both as hydrophilic ORAC and as lipophilic ORAC in vitro. The ORAC values did not

Table 3
Antioxidative activities of supercritical CO_2-extracted berry seed oils.[a]

	Peroxyl radical scavenging activity		Inhibition of microsomal lipid peroxidation	
	L/mol[b]	μmol α-tocopherol equivalent in 100 g oil	IC_{50} (μL/L)[c]	μmol trolox equivalent in 100 g oil
Cranberry seed oil	57	975	144	338
Strawberry seed oil	188	296	48	1015
Cloudberry seed oil	48	1157	75	650
Blackcurrant seed oil	52	1068	102	478
Lingonberry seed oil	186	299	148	329
Raspberry seed oil	24	2315	115	424
Bilberry seed oil	107	519	41	1188
Arctic cranberry seed oil	36	1543	122	399
Sea buckthorn seed oil	49	1323	199	245
Sea buckthorn pulp oil	42	1134	177	275

[a] Values are averages of three replicates; the coefficients of variation of the methods are 2.1%/3.3% for peroxyl radical scavenging, and 4.0%/5.2% for inhibition of microsomal lipid peroxidation (within-assay/between-assay precision, respectively).
[b] Amount of berry seed oil required for scavenging one mole of peroxyl radicals.
[c] Concentration needed to inhibit lipid peroxidation by 50%.

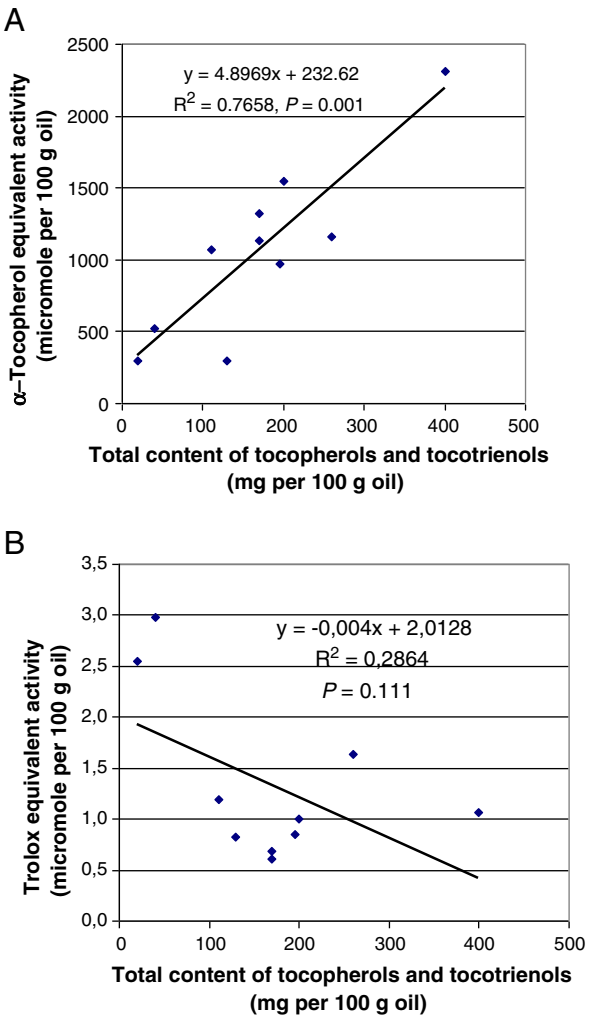

Fig. 1. Correlation between the total contents of tocopherols and tocotrienols in supercritical CO_2-extracted oils and their peroxyl radical scavenging activities (A) and inhibitory effects on peroxidation of microsomal lipids (B) in vitro. The values shown are averages of three replicates carried out on three different days. The coefficients of variation of the methods are 2.1%/3.3% for peroxyl radical scavenging and 4.0%/5.2% for inhibition of microsomal lipid peroxidation (within-assay/between-assay precision, respectively).

correlate with the total content of phenolics or tocopherols (Bushman et al., 2004).

3.4. Inhibitory effects of berry oils on lipid peroxidation

In the lipid peroxidation assay, the effects of various oils in inhibiting the peroxidation of membrane lipids of microsomes were studied using t-butylhydroperoxide-induced oxidation model. The lower the IC_{50} value is, the higher is the antioxidative efficacy of the oil. The strongest inhibitory effects on lipid peroxidation were seen in bilberry seed oil (IC_{50} value, 41 μL/L) and strawberry seed oil (IC_{50} value, 48 μL/L), which showed relatively low activities in the peroxyl radical assay. On the contrary, raspberry seed oil (IC_{50} value, 115 μL/L) and cranberry seed oil and Arctic cranberry seed oil (IC_{50} value, 144 and 122 μL/L, respectively) were among the oils showing lowest inhibitory activities in this assay, although they showed relatively high peroxyl radical scavenging activities than the other oils (Table 3). There was no significant correlation between the antioxidative efficacies and the total contents of tocopherols and tocotrienols of the oils (Fig. 1B, $r = -0.535$, $P = 0.111$).

The lipid peroxidation assay differed from the peroxyl radical scavenging assay in way of initiation of the oxidation process, the substrate and the reaction media. The organic solvent-based medium used in the peroxyl radical assay ensured a good distribution and mobility for the relatively nonpolar molecules (tocopherols and tocotrienols) and thus efficient quenching of radicals, whereas the radicals were probably better accessed on the lipid-water interface by molecules of high polarities in the aqueous system used in the lipid peroxidation assay. It is generally known that the antioxidative activities of antioxidants often vary considerably among different assays. The mechanism that is operative or dominant in a particular test is dependent on the experimental conditions and will affect the reaction kinetics and hence the antioxidative activity (Antolovich, Prenzler, Patsalides, McDonald, & Robards, 2002; Murakami, Yamaguchi, Takamura, & Matoba, 2002). Therefore it is often recommended to use more than one single assay when studying the antioxidative activities of specific antioxidants especially of natural extracts consisting of different types of antioxidative compounds.

3.5. LDL oxidation, LDL incorporation and superoxide anion scavenging capacity

Oxidation of LDL is commonly considered as an initiator of atherosclerosis. Increased circulating levels of oxidized LDL are associated with coronary heart disease (CHD) (Imazu et al., 2008). Antioxidants protecting LDL from oxidation may have potentials in maintaining cardiovascular health (Willcox, Curb, & Rodriguez, 2008).

Both the sea buckthorn seed oil and the pulp oil showed clear protective effects on LDL against copper ion-induced oxidation in vitro (Table 4). The amount of oil needed for a 50% inhibition of LDL oxidation was lower for the pulp oil (1.1μL/mg LDL) than for the seed oil (2.0 μL/mg LDL) (Table 4), corresponding to about 1 mg pulp oil and 1.8 mg seed oil for protection of each mg of LDL particles based on calculation using a density of 0.9 g/mL of the oils. The corresponding value for trolox was 1.6 μg/mg LDL (Table 4).

In the LDL incorporation study, sea buckthorn seed and pulp oils were first incubated with serum. After incubation, the LDL particles were precipitated and subjected to copper ion-induced oxidation. Addition of the seed oil at the dosage level close to 14 μL per mg of LDL

Table 4
Effects on LDL oxidation and superoxide anion scavenging activity of supercritical CO_2-extracted sea buckthorn seed and pulp oils.

	Inhibition of LDL oxidation	LDL incorporation	Superoxide anion scavenging activity
	IC_{50} (μL/mg LDL)[a]	IC_{50} (μL/mg LDL)[b]	IC_{50} (mL/L)[c]
Sea buckthorn seed oil	2.0	13.9	2.3
Sea buckthorn pulp oil	1.1	1.2	0.6
Trolox	1.6 μg/mg LDL		8.2 mg/L
a-Tocopherol		9.5 μg/mg LDL	

[a] Amount of oil or antioxidant needed to inhibit LDL oxidation by 50% compared to the negative control. The values are averages of three replicates; the coefficients of variation of the method are 3.6% (within-assay precision) and 4.9% (between-assay precision).

[b] Amount of oil or antioxidant needed for sufficient incorporation of antioxidants into LDL particles to produce a 50% inhibition of LDL oxidation compared to the negative control. The values are averages of three replicates; the coefficients of variation of the method are 6.0% (within-assay precision) and 8.2% (between-assay precision).

[c] Concentration of oil or antioxidant required for a 50% inhibition of production of chemiluminescence compared to the negative control The values are averages of three replicates; the coefficients of variation of the method are 5.1% (within-assay precision) and 6.7% (between-assay precision).

resulted in incorporation of antioxidative components of the oil, causing a 50% inhibition of LDL oxidation in the Cu^{2+}-induced model (Table 4). The corresponding dosage required for the pulp oil was one-tenth of the level of the seed oil (Table 4). The IC_{50} value for α-tocopherol was 9.5 μg/mg LDL, six times as high as the IC_{50} value (1.6 μg/mg LDL) dosage in the LDL oxidation model (Table 4).

The sea buckthorn seed oil and pulp oil showed scavenging activities for superoxide anion radicals created in vitro, the pulp oil (IC_{50} value, 0.6 mL/L corresponding to about 0.5 g oil/L) being more efficient compared with the seed oil (IC_{50} value, 2.3 mL/L corresponding to 2 g oil/L) (Table 4).

Tocopherols and carotenoids were the major lipid-soluble antioxidants in the sea buckthorn oils. The total content of α- and γ-tocopherols was 170 mg/100 g in both oils (Table 2). The seed oil contained roughly equal amounts of α- and γ-tocopherols, whereas the pulp oil contained α-tocopherol as the dominating isomer (Table 2). The antioxidative activities differ among the isomers of tocopherols (Stone & Papas, 2003). α-Tocopherol is the major antioxidant in lipoproteins in vivo. Carotenoids, especially lycopene and β-carotene, have been shown to effect synergistically with α-tocopherol to inhibit LDL oxidation (Fuhrman, Ben-Yaish, Attias, Hayek, & Aviram, 1997; Fuhrman, Volkova, Rosenblat, & Aviram, 2000). Carotenoids were more abundant in the pulp oil (300 mg/100 g oil) than in the seed oil (30 mg/100 g oil) (unpublished results). The difference in the composition of tocopherol isomers and the content of carotenoids together may have contributed to the difference in the antioxidative efficacies observed of the two oils.

3.6. Protective effects of sea buckthorn seed and pulp oils on DNA oxidation

Under the experimental condition, both seed and pulp oils of sea buckthorn effectively protected DNA from oxidation. The two oils did not differ significantly in the protection efficacy. In the oxidation study of pure DNA, within the dosage range of 0.5–5 μL/mg DNA sea buckthorn seed and pulp oils showed protective effects in a dose-dependent manner. When the dosage of the oils increased from 0.5 to 5 μL/mg DNA, the DNA oxidation, measured as percentage of the negative control, was decreased from 90% to 42% by the seed oil and from 74% to 38% by the pulp oil. It is worth to notice that for the pulp oil treatment at the dosage level of 2.5 μL/mg DNA, the DNA oxidation was higher than the corresponding level at the dosage of 0.5 μL/mg DNA. When the dosage was further increased from 5 to 100 μL/mg DNA, no further increase in the protective effects was observed. When the dosage reached 500 μL/mg DNA, the protective effects of the both oils were clearly lower than those seen in the lower dosage range (Fig. 2A). The results indicate that under the experimental conditions used, the concentration/dosage range of sea buckthorn oils for maximal protection of DNA from oxidation was 5.0–100.0 μL/mg DNA. α-Tocopherol and trolox showed dose-dependent protective effects within the dosage range from 5.5×10^{-10} to 5.5×10^{-6} mg/mg DNA in the same model (Fig. 2B). At the dosage level of 5.5×10^{-6} mg/mg DNA, α-tocopherol and trolox showed protective effects that are comparable to those of sea buckthorn oils at dosage level of 5.0–100.0 μL/mg DNA (Fig. 2B).

Within the dosage range of 2.3×10^{-2} to 4.0 μL/mg rat liver homogenate, again, the sea buckthorn seed oil and pulp oil showed dose-dependent effects against oxidation of DNA in liver homogenates (Fig. 3A). At the dosage of 4.0 μL/mg liver homogenate, the DNA oxidation was reduced to 48% and 24% of the negative control by the seed oil and the pulp oils, respectively. The protective effect of the pulp oil was consistently higher than the seed oil at most of the tested dosage levels (Fig. 3A).

Again, the protective effects of α-tocopherol and trolox were tested at different doses as positive controls. Within the dose range of 2×10^{-8} to 2×10^{-6} mg/mg liver homogenate, α-tocopherol and trolox inhibited DNA oxidation in a dose-dependent manner, the DNA oxidation being

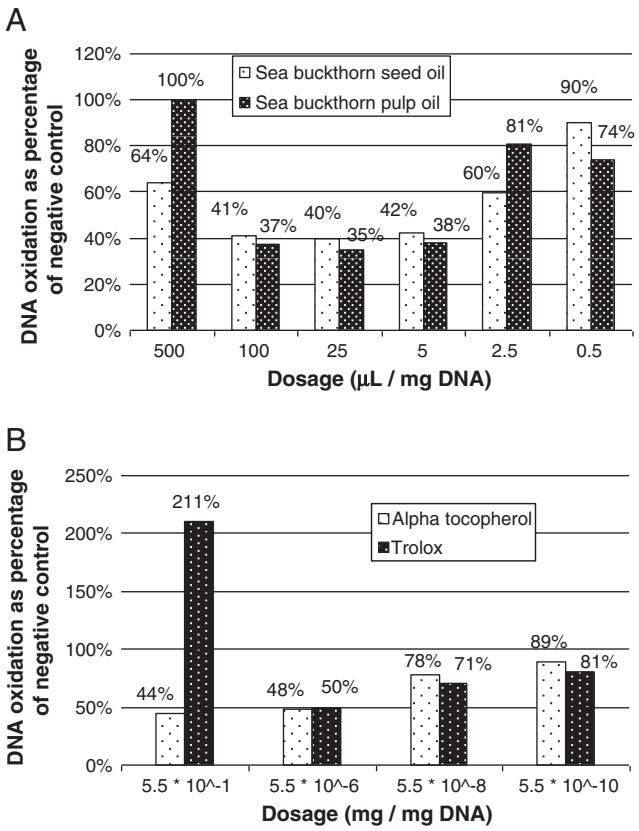

Fig. 2. Protective effects of supercritical CO_2-extracted sea buckthorn seed and pulp oils (A) as well as tocopherol and trolox (B) on oxidation of purified DNA in vitro. The values shown are averages of three replicates carried out on three different days. The coefficient of variation of the method is 9% and 12% for within-assay and between-assay precision, respectively.

reduced to 96–63% of the negative control by alpha tocopherol and 99–85% of the negative control by trolox treatment (Fig. 3B). The activity of α-tocopherol was slightly higher than that of trolox within this range of dosages. At the dose level as high as 2×10^{-5} mg/mg liver homogenate, slight pro-oxidative effects were seen of both α-tocopherol and trolox shown as increased DNA oxidation compared with the negative control (Fig. 3B).

Accumulating evidence indicates that oxidative damages of biomolecules are widely involved in aging and diseases of humans such as neuro-degenerative diseases, atherosclerosis and cancer.

An imbalance between reactive oxygen species and defense and repair mechanisms results in oxidative stress in humans. Excessive DNA damages caused by oxidative stress may overload the repairing system of the body and lead to increased risk of cancer (Kunisada et al., 2005; D'Errico, Parlanti, & Dogliotti, 2008). For example several lines of evidence indicate that oxidative DNA damage plays an important role in the development of skin cancers (Agar et al., 2004; Kunisada et al., 2005). Among the defense mechanisms of the human body are vitamin E, uric acid and serum albumins as well as enzymes such as superoxide dismutase, catalase and glutathione peroxidase. In addition, dietary antioxidants are important for the body in fighting against reactive oxygen species. The current research demonstrated antioxidative potential of sea buckthorn seed oil and pulp oil in various models. In our previous research, oral administration of supercritical CO_2-extracted sea buckthorn seed and pulp oils (Aromtech Ltd., Finland) protected stress-, reserpine-, and pylorus ligation-induced gastric ulcer (Xing et al., 2002). Supplementation with a combination of the two oils inhibited platelet aggregation in healthy men (Johansson et al., 2000). Oral sea buckthorn seed oil protected liver from CCl_4-induced damage and significantly increased

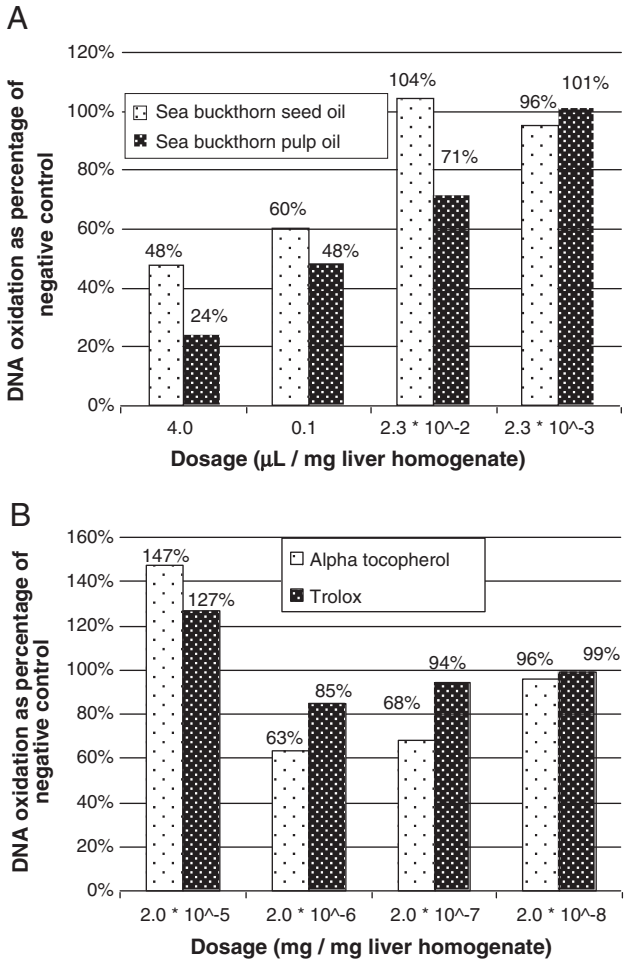

Fig. 3. Protective effects of supercritical CO_2-extracted sea buckthorn seed and pulp oils (A) as well as tocopherol and trolox (B) on oxidation of DNA in rat liver homogenate in vitro. The values shown are averages of three replicates carried out on three different days. The coefficient of variation of the method is 11% and 14% for within-assay and between-assay precision, respectively.

the activities of superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rd), and GSH content in rat liver (Hsu, Tsai, Chen, & Lu, 2009). Sea buckthorn oils have been shown to regulate immune function, reduce inflammation and lower plasma lipid levels (Yang & Kallio, 2002). The results of the current research combined with those of previous investigations suggest that of sea buckthorn oils have great potential in supporting human health.

4. Conclusions

The high content of α-linolenic acid and the low n-6/n-3 ratio (close to 1:1) in northern berry seed oils are optimal for correcting the present imbalance in essential fatty acids in the Western diet. The results of the present study suggested antioxidative potentials of supercritical CO_2-extracted oils from seeds and soft parts of selected berry species. Sea buckthorn seed and pulp oils showed clear antioxidative efficacies in multiple in vitro models, which indicated great potential of the oils as ingredients of functional foods, food supplements and nutraceuticals for supporting human health. It is important to note that antioxidative activities demonstrated by in vitro studies shall not be directly extrapolated to health effects in vivo. Intervention studies are essential in order to evaluate the health effects of oral supplementations of oils from seeds and soft parts of berries.

Acknowledgements

The authors sincerely thank Mrs. Tuija Vaattovaara (Aromtech Ltd., Tornio, Finland) for her skillful help in analytical work. Ms. Leena Söderholm (MCA Research Laboratory, Turku, Finland) is thanked for her contribution in the antioxidation assays.

References

Agar, N. S., Halliday, G. M., Barnetson, R. S., Ananthaswamy, H. N., Wheeler, M., & Jones, A. M. (2004). The basal layer in human squamous tumors harbors more UVA than UVB fingerprint mutations: A role for UVA in human skin carcinogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(14), 4954—4959.

Ahotupa, M., Mantyla, E., & Kangas, L. (1997). Antioxidant properties of the triphenylethylene antiestrogen drug toremifene. *Naunyn-Schmiedeberg's archives of pharmacology*, *356*(3), 297—302.

Ahotupa, M., Ruutu, M., & Mantyla, E. (1996). Simple methods of quantifying oxidation products and antioxidant potential of low density lipoproteins. *Clinical biochemistry*, *29*(2), 139—144.

Antolovich, M., Prenzler, P. D., Patsalides, E., McDonald, S., & Robards, K. (2002). Methods for testing antioxidant activity. *Analyst*, *127*(3), 430.

Bushman, B. S., Phillips, B., Isbell, T., Ou, B., Crane, J. M., & Knapp, S. J. (2004). Chemical composition of caneberry (*Rubus* spp.) seeds and oils and their antioxidant potential. *Journal of Agricultural and Food Chemistry*, *52*(26), 7982—7987.

Cheng, J. Y., Kondo, K., Suzuki, Y., Ikeda, Y., Meng, X. S., & Umemura, K. (2003). Inhibitory effects of total flavones of *Hippophae Rhamnoides* L on thrombosis in mouse femoral artery and in vitro platelet aggregation. *Life Sciences*, *72*(20), 2263—2271.

D'Errico, M., Parlanti, E., & Dogliotti, E. (2008). Mechanism of oxidative DNA damage repair and relevance to human pathology. *Mutation Research-Reviews in Mutation Research*, *659*(1–2), 4—14.

de Wilde, M. C., Farkas, E., Gerrits, M., Kiliaan, A. J., & Luiten, P. G. M. (2002). The effect of n-3 polyunsaturated fatty acid-rich diets on cognitive and cerebrovascular parameters in chronic cerebral hypoperfusion. *Brain research*, *947*(2), 166—173.

Fuhrman, B., Ben-Yaish, L., Attias, J., Hayek, T., & Aviram, M. (1997). Tomato lycopene and beta-carotene inhibit low density lipoprotein oxidation and this effect depends on the lipoprotein vitamin E content. *Nutrition Metabolism and Cardiovascular Diseases*, *7*(6), 433—443.

Fuhrman, B., Volkova, N., Rosenblat, M., & Aviram, M. (2000). Lycopene synergistically inhibits LDL oxidation in combination with vitamin E, glabridin, rosmarinic acid, carnosic acid, or garlic. *Antioxidants & Redox Signaling*, *2*(3), 491—506.

Goffman, F. D., & Galletti, S. (2001). Gamma-linolenic acid and tocopherol contents in the seed oil of 47 accessions from several *Ribes* species. *Journal of Agricultural and Food Chemistry*, *49*(1), 349—354.

Goh, S. H., Hew, N. F., Norhanom, A. W., & Yadav, M. (1994). Inhibition of tumor promotion by various palm-oil tocotrienols. *International Journal of Cancer*, *57*(4), 529—531.

Gunstone, F. D., & Harwood, J. L. (2007). Occurrence and characterisation of oils and fats. In F. D. Gunstone, J. L. Harwood, & A. J. Dijkstra (Eds.), *The Lipid Handbook* (pp. 37—141). Boca Raton, Florida: CRC Press Taylor & Francis Group.

Helbock, H. J., Beckman, K. B., Shigenaga, M. K., Walter, P. B., Woodall, A. A., Yeo, H. C., et al. (1998). DNA oxidation matters: The HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(1), 288—293.

Helbock, H. J., Thompson, J., Yeo, H., & Ames, B. N. (1996). N-2-methyl-8-oxoguanine: A tRNA urinary metabolite—role of xanthine oxidase. *Free Radical Biology and Medicine*, *20*(3), 475—481.

Hsu, Y., Tsai, C., Chen, W., & Lu, F. (2009). Protective effects of seabuckthorn (*Hippophae rhamnoides* L.) seed oil against carbon tetrachloride-induced hepatotoxicity in mice. *Food and Chemical Toxicology*, *47*(9), 2281—2288.

Imazu, M., Ono, K., Tadehara, F., Kajiwara, K., Yamamoto, H., Sumii, K., et al. (2008). Plasma levels of oxidized low density lipoprotein are associated with stable angina pectoris and modalities of acute coronary syndrome. *International Heart Journal*, *49*(5), 515—524.

Jiang, Q., Moreland, M., Ames, B. N., & Yin, X. (2009). A combination of aspirin and gamma-tocopherol is superior to that of aspirin and alpha-tocopherol in anti-inflammatory action and attenuation of aspirin-induced adverse effects. *Journal of Nutritional Biochemistry*, *20*(11), 894—900.

Jiang, Q., Yin, X., Lill, M. A., Danielson, M. L., Freiser, H., & Huang, J. (2008). Long-chain carboxychromanols, metabolites of vitamin E, are potent inhibitors of cyclooxygenases. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(51), 20464—20469.

Johansson, A., Korte, H., Yang, B., Stanley, J., & Kallio, H. (2000). Sea buckthorn berry oil inhibits platelet aggregation. *Journal of Nutritional Biochemistry*, *11*, 491—495.

Johansson, A., Laakso, P., & Kallio, H. (1997). Characterization of seed oils of wild, edible Finnish berries. *Zeitschrift Fur Lebensmittel-Untersuchung Und-Forschung A-Food Research and Technology*, *204*(4), 300—307.

Johansson, A., Laine, T., Linna, M. M., & Kallio, H. (2000). Variability in oil content and fatty acid composition in wild northern currants. *European Food Research and Technology*, *211*(4), 277—283.

King, J. W., & List, C. R. (1996). *Supercritical fluid technology in oil and lipid chemistry*. Champaign, Illinois: AOCS Press.

Kleinveld, H. A., Haklemmers, H. L. M., Stalenhoef, A. F. H., & Demacker, P. N. M. (1992). Improved measurement of low-density-lipoprotein susceptibility to copper-

induced oxidation—application of a short procedure for isolating low-density-lipoprotein. *Clinical chemistry*, *38*(10), 2066−2072.
Kunisada, M., Sakumi, K., Tominaga, Y., Budiyanto, A., Ueda, M., Ichihashi, M., et al. (2005). 8-Oxoguanine formation induced by chronic UVB exposure makes Ogg1 knockout mice susceptible to skin carcinogenesis. *Cancer research*, *65*(14), 6006−6010.
Lahiri, D. K., & Nurnberger, J. I. (1991). A rapid nonenzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic acids research*, *19*(19), 5444.
Layne, E. K. (1957). Spectrophotometric and turbidimetric methods for measuring protein. *Methods in Enzymology*, *3*, 447−454.
Lenucci, M. S., Caccioppola, A., Durante, M., Serrone, L., Leonardo, R., Piro, G., et al. (2010). Optimisation of biological and physical parameters for lycopene super-critical CO2 extraction from ordinary and high-pigment tomato cultivars. *Journal of the Science of Food and Agriculture*, *90*(10), 1709−1718.
Murakami, M., Yamaguchi, T., Takamura, H., & Matoba, T. (2002). A comparative study on the various in vitro assays of active oxygen scavenging activity in foods. *Journal of Food Science*, *67*(2), 539−541.
Nesaretnam, K. (2008). Multitargeted therapy of cancer by tocotrienols. *Cancer letters*, *269*(2), 388−395.
Nesaretnam, K., Stephen, R., Dils, R., & Darbre, P. (1998). Tocotrienols inhibit the growth of human breast cancer cells irrespective of estrogen receptor status. *Lipids*, *33*(5), 461−469.
Oddy, W. H., de Klerk, N. H., Kendall, G. E., Mihrshahi, S., & Peat, J. K. (2004). Ratio of omega-6 to omega-3 fatty acids and childhood asthma. *Journal of Asthma*, *41*(3), 319−326.
Ogawa, K., Sakakibara, H., Iwata, R., Ishii, T., Sato, T., Goda, T., et al. (2008). Anthocyanin composition and antioxidant activity of the crowberry (*Empetrum nigrum*) and other berries. *Journal of Agricultural and Food Chemistry*, *56*(12), 4457−4462.
Parry, J., Su, L., Luther, M., Zhou, K. Q., Yurawecz, M. P., Whittaker, P., et al. (2005). Fatty acid composition and antioxidant properties of cold-pressed marionberry, boysenberry, red raspberry, and blueberry seed oils. *Journal of Agricultural and Food Chemistry*, *53*(3), 566−573.
Reiter, E., Jiang, Q., & Christen, S. (2007). Anti-inflammatory properties of alpha- and gamma-tocopherol. *Molecular aspects of medicine*, *28*(5–6), 668−691.
Seeram, N. P., Adams, L. S., Zhang, Y., Lee, R., Sand, D., Scheuller, H. S., et al. (2006). Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells in vitro. *Journal of Agricultural and Food Chemistry*, *54*(25), 9329−9339.
Simopoulos, A. P. (2000). Human requirement for n-3 polyunsaturated fatty acids. *Poultry science*, *79*(7), 961−970.
Simopoulos, A. P. (2001). n-3 Fatty acids and human health: Defining strategies for public policy. *Lipids*, *36*, S83−S89.
Simopoulos, A. P. (2008). The omega-6/omega-3 fatty acid ratio, genetic variation, and cardiovascular disease. *Asia Pacific Journal of Clinical Nutrition*, *17*, 131−134.
Stahl, E., Quirin, K. W., & Gerard, D. (1987). *Dense gases for extraction and refining* (pp. 237). Berlin: Springer-Verlag.
Stone, W. L., & Papas, A. (2003). Tocopherols and tocotrienols and vitamin E. In F. D. Gunstone (Ed.), *Lipids for functional foods and nutraceuticals* (pp. 53−72). Bridgwater, England: The Oily Press.
Traber, M. G., & Blatt, D. (2002). Vitamin E: Evidence for the 2:1 preference for RRR-Compared with all rac-α-tocopherol. In L. Packer, M. G. Traber, K. Kraemer, & B. Frei (Eds.), *The antioxidant vitamins* (pp. 161−170). Champaign, Illinois: AOCS Press.
Wagner, J. G., Harkema, J. R., Jiang, Q., Illek, B., Ames, B. N., & Peden, D. B. (2009). Gamma-tocopherol attenuates ozone-induced exacerbation of allergic rhinosinusitis in rats. *Toxicologic pathology*, *37*(4), 481−491.
Wagner, J. G., Jiang, Q., Harkema, J. R., Illek, B., Patel, D. D., Ames, B. N., et al. (2007). Ozone enhancement of lower airway allergic inflammation is prevented by gamma-tocopherol. *Free Radical Biology and Medicine*, *43*(8), 1176−1188.
Willcox, B. J., Curb, J. D., & Rodriguez, B. L. (2008). Antioxidants in cardiovascular health and disease: Key lessons from epidemiologic studies. *American Journal of Cardiology*, *101*(10A), 75D−86D.
Xing, J. F., Yang, B. R., Dong, Y. L., Wang, B. W., Wang, J. X., & Kallio, H. P. (2002). Effects of sea buckthorn (*Hippophaë rhamnoides* L.) seed and pulp oils on experimental models of gastric ulcer in rats. *Fitoterapia*, *73*(7–8), 644−650.
Yang, B., Kalimo, K. O., Tahvonen, R. L., Mattila, L. M., Katajisto, J. K., & Kallio, H. P. (2000). Effect of dietary supplementation with sea buckthorn (*Hippophaë rhamnoides*) seed and pulp oils on the fatty acid composition of skin glycerophospholipids of patients with atopic dermatitis. *Journal of Nutritional Biochemistry*, *11*(6), 338−340.
Yang, B., & Kallio, H. (2002). Composition and physiological effects of sea buckthorn (*Hippophaë*) lipids. *Trends in Food Science & Technology*, *13*(5), 160−167.
Yang, B., Karlsson, R. M., Oksman, P. H., & Kallio, H. P. (2001). Phytosterols in sea buckthorn (*Hippophaë rhamnoides* L.) berries: Identification and effects of different origins and harvesting times. *Journal of Agricultural and Food Chemistry*, *49*(11), 5620−5629.
Yang, B., Koponen, J., Tahvonen, R., & Kallio, H. (2003). Plant sterols in seeds of two species of Vaccinium (*Vaccinum myrtillus* and *Vaccinium vitis-idaea*) naturally distributed in Finland. *European Food Research and Technology*, *216*(1), 34−38.
Zhang, Y., Seeram, N. P., Lee, R., Feng, L., & Heber, D. (2008). Isolation and identification of strawberry phenolics with antioxidant and human cancer cell anti proliferative properties. *Journal of Agricultural and Food Chemistry*, *56*(3), 670−675.
Zheng, W., & Wang, S. Y. (2003). Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. *Journal of Agricultural and Food Chemistry*, *51*(2), 502−509.