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ARTICLE · JANUARY 2006

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Quality of extracted sea buckthorn seed and pulp oil

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*[1]Department of Biosystems Engineering, University of Manitoba, Winnipeg, Manitoba R3T 5V6, Canada; and [2]Department of Chemistry and Biochemistry, University of Lethbridge, Lethbridge, Alberta T1K 3M4, Canada. *Email: stefan_cenkowski@umanitoba.ca*

Cenkowski, S., Yakimishen, R., Przybylski, R. and Muir, W.E. 2006. **Quality of extracted sea buckthorn seed and pulp oil**. Canadian Biosystems Engineering/Le génie des biosystèmes au Canada **48**: 3.9-3.16. The effects of four oil-extraction techniques (solvent extraction using petroleum-ether, supercritical fluid extraction using carbon dioxide (SCFE CO_2), screw pressing, and aqueous extraction) on the nutritional quality of sea buckthorn seed and pulp oil were evaluated by quantifying fatty acids, tocopherols and tocotrienols, total carotenoids, and sterols. The extracted quantities were compared against solvent extraction using chloroform/methanol as reference. Seeds and pulp-flakes were obtained by pilot-scale processing and separation. Juice was first extracted from berries on a bladder press and then wet pulp cake was dried at 50ºC for 24 h in a forced-convection drying oven. The dried seeds and pulp-flakes were separated using an industrial mixer and a vibratory screen separator. Processed seeds and pulp-flakes were then subjected to the four oil-extraction techniques. The concentration of fatty acids in oil extracted from seeds and pulp-flakes was similar in all tested extraction techniques. The predominant fatty acids were linoleic (35.3-36.3%) and linolenic (35.9-38.5%) acids in seed oil, and palmitic (34.4-35.5%) and palmitoleic (34.4-38.5%) acids in pulp oil. Alpha-tocopherol (vitamin E) was the major tocopherol identified in the seed oil (43 to 53% of total tocopherols) and pulp oil (74 to 85% of total tocopherols) and the extracted quantity depended on the extraction technique used. Petroleum-ether extraction gave the highest total carotenoid concentration of 22 mg/100 g in seed oil and 527.8 mg/100 g in pulp oil. The lowest carotenoid concentrations were obtained with 3h-SCFE CO_2 (6.2 and 122.3 mg/100g of oil from seed and pulp, respectively). Beta-sitosterol (prostate treatment natural compound) was the predominant sterol identified in the seed oil (97% range of total sterols for all extraction techniques) and pulp oil (96-98% of total sterols for extraction techniques tested). Petroleum-ether extraction consistently recovered oils having higher amounts of all analyzed nutritional components. Aqueous extraction and screw pressing methods were limited by the type of material which could be processed. No oil was recovered from sea buckthorn seeds by aqueous extraction and no oil was recovered from pulp-flakes by screw pressing. The SCFE CO_2 method was flexible in extracting both seed and pulp oils having relatively high concentrations of all identified nutritional compounds. **Keywords**: sea buckthorn, oil, quality, supercritical fluid extraction, screw pressing, solvent extraction, aqueous extraction, pulp, pulp-flakes, seeds, nutraceutical compounds.

Les effets de quatre techniques d'extraction d'huiles (extraction par solvant utilisant l'éther de pétrole, extraction par fluide supercritique utilisant le dioxide de carbone (EFSC CO_2), extraction avec une presse à vis, extraction aqueuse) sur la qualité nutritionnelle des graines et de l'huile de pulpe de l'argousier ont été évalués en quantifiant les acides gras, les tocophérols et les tocotriénols, les caroténoïdes totaux et les stérols. Les quantités extraites ont été comparées à celles obtenues lors de l'extraction par solvant utilisant du chloroforme/méthanol, celle-ci étant utilisée comme méthode de référence. Les graines et les flocons de pulpe ont été obtenus par transformation et séparation à l'échelle du laboratoire. Premièrement, le jus était extrait des baies à l'aide d'un pressoir pneumatique et ensuite la pulpe humide était séchée à 50ºC pour 24 h dans un four à convection forcée. Les graines séchées et les flocons de pulpe ont été séparés en utilisant un mélangeur industriel et un crible vibrant. Les graines et les flocons de pulpe ainsi traités ont été alors soumis aux quatre techniques d'extraction d'huile. La concentration en acides gras dans l'huile extraite des graines et des flocons de pulpe était comparable pour chacune des techniques d'extraction testées. Les acides gras prédominants étaient les acides linoléïques (35,3-36,3%) et linoléniques (35,9-38,5%) dans les graines et les acides palmitiques (34,4-35,5%) et palmitoléïques (34,4-38,5%) dans l'huile provenant de la pulpe. L'alpha-tocophérol (vitamine E) était le principal tacophérol identifié dans l'huile des graines (43 à 53% du total des tocophérols) et dans l'huile provenant de la pulpe (74 à 85% du total des tocophérols) et la quantité extraite était dépendante de la technique d'extraction utilisée. L'extraction à l'éther de pétrole a procuré la plus grande concentration de caroténoïdes totaux avec 22 mg/100 g dans l'huile des graines et 527,8 mg/100 g dans l'huile de la pulpe. Les plus petites concentrations de caroténoïdes ont été obtenues avec EFSC CO_2 -3h (6,2 et 122,3 mg/100g d'huile provenant respectivement des graines et de la pulpe). Le bêta-sitostérol (composé naturel pour le traitement de la prostate) était le principal stérol identifié dans l'huile de graines (environ 97% des stérols totaux pour chacune des techniques d'extraction) et dans l'huile de pulpe (96-98% des stérols totaux pour les techniques d'extraction testées). L'extraction par l'éther de pétrole a, de manière constante, extrait des huiles possédant les quantités les plus grandes de tous les composés nutritifs analysés. Les méthodes d'extraction aqueuse et utilisant une presse à vis étaient restreintes quant au type de produit qu'elles pouvaient transformer. Aucune huile n'a été extraite des graines d'argousier par l'extraction aqueuse, situation similaire avec les flocons de pulpe en utilisant la presse à vis. La méthode EFSC CO_2 était flexible en extrayant des huiles des graines et de la pulpe qui avaient des concentrations élevées de tous les composés nutritifs identifiés. **Mots clés**: argousier, huile, qualité, extraction par fluide supercritique, pulpe, flocons de pulpe, graines, composés nutraceutiques.

INTRODUCTION

The functional food and nutraceutical markets, collectively estimated as a multi-billion dollar global industry (up to $86 billion, 5 to 7.5% annual increase), has been gaining popularity (Oomah and Mazza 1999; Hardy 2000; Menrad 2003). However, there is major concern that these unprecedented growth rates are likely to- and have already attracted

irresponsible market entrants distributing products that do not deliver on quality (Hardy 2000; Datamonitor 2005). Berries from sea buckthorn (*Hippophae rhamnoides* L.), a shrub still virtually unknown in North America are currently being incorporated as a functional food and into nutraceutical products in Europe and Asia. (Li and Schroeder 1999). Traditional products from the berries include juices, liqueurs, wine, jams, candy, and ice-cream. However, the berry's unique chemical and nutritional composition has offered economic potential as a health food in North America (Oomah and Mazza 1999; Storey 2000).

Sea buckthorn seed and pulp oils are considered the most valuable components of the berries comprising a unique fatty acid composition, fat-soluble vitamins, and plant sterols (Yang and Kallio 2002). The seed oil, defined as being highly unsaturated, comprises two essential fatty acids, α-linolenic (C18:3*n*-3) (the parent substance of omega-3 fatty acid which helps to prevent chronic diseases such as heart disease and arthritis) and linoleic (C18:2*n*-6) acids (omega-6 fatty acid; unsaturated fatty acid considered essential to the human diet). The contributions of α-linolenic and linoleic acids are commonly at the 20 to 35 and 30 to 40% range, respectively (Yang and Kallio 2002). Oil from the pulp contains more saturated fatty acids than from the seeds and comprises primarily palmitic acid (C16:0; most common saturated fatty acid), palmitoleic acid (C16:1*n*-7, unsaturated fatty acid), and lower concentrations of polyunsaturated acids (Kallio et al. 2002). Seed oil contains α-, β-, γ-, and δ-tocopherol isomers which comprise 93 to 98% (84 to 318 mg/kg of berries) of the total tocopherols (fat soluble alcohols that behave similar to vitamin E, an important antioxidant that neutralizes free radicals in the body). Alpha-tocopherol (vitamin E) constitutes 76 to 89% of tocopherols in whole berries (Kallio et al. 2002). Among pigments, β-carotene (important anti-oxidant in the food industry) is the most abundant in the pulp oil and constitutes 15 to 55% of total amount of carotenoids (Yang and Kallio 2002). Important sterols include cholesterol, phytosterols and steroid hormones, and their contents in seeds, pulp, and fresh whole berries (ssp. *Rhamnoides* and *sinensis*) are 1200-1800, 240-400, and 340-520 mg/kg of berries, respectively (Yang et al. 2001).

The process used to separate oil from oil-bearing materials has a direct effect on the extractability and quality of oil (Bargale et al. 1999). Oil quality is also affected by the contamination of other oils and foreign material, colour fixation from increased processing temperatures, increases in free fatty acids, and oxidation (Burkhalter 1976). Four extraction techniques (solvent extraction using petroleum-ether, supercritical fluid extraction using carbon dioxide - SCFE, screw pressing, and an aqueous extraction) were evaluated by Yakimishen et al. (2005) for their extraction efficiencies (percentage of oil recovery). Seed oil recoveries were 65.1 and 41.2% for SCFE CO_2 (45ºC and 35 MPa) and screw pressing, respectively. No oil was recovered from seeds by aqueous extraction. Pulp-flake oil recovery was 86.3% for SCFE CO_2. The aqueous extraction yielded only 6% of pulp oil. No oil was recovered from pulp flakes by screw pressing. Aqueous extraction produced pulp oil characterized as being visually attractive (dark red and clear), having a pleasant fruity smell, and remaining as a liquid at room temperature for over a year. Unfiltered seed oil after screw pressing was yellowish-brown and cloudy due to seed particulates in the oil. Extracted pulp-flake oil in SCFE CO_2 was red and existed as a semi-solid at room temperature. Temperatures above 30ºC returned the semisolid oil to liquid. Seed oil was a clear, yellow-brown liquid at room temperature. Seed oil from petroleum ether extraction was bright yellow and existed as a clear liquid at room temperature, while the pulp oil was clear, bright-red, and solidified when cooled to room temperature. Each extraction technique affected the oil, but the effects of extraction technique on specific compounds in the extracted oil were not reported.

The cosmetic, nutraceutical, functional food, and pharmaceutical industries are very demanding and require products that meet stringent criteria of performance and composition (Kalustian 1985). Thus, processing to isolate oils should not affect the endogenous nutritional components originally present in plant oils (Puupponen-Pimiä et al. 2002). Increasing processing temperatures can improve oil yield but at the same time can negatively affect oil quality (Carr 1997). Oils expelled at temperatures below 60ºC are classified as "cold pressed", and have a positive market connotation (cold press means that oils are extracted at temperatures below 60ºC (Bockisch 1998).

The objective of this research was to determine the effects of four extraction techniques: solvent extraction using petroleum-ether, supercritical fluid extraction using CO_2, screw pressing, and aqueous extraction on the nutritional components in sea-buckthorn seed and pulp oils.

METHODOLOGY

Preparation of experimental material

Sea buckthorn berries (*Hippophae rhamnoides* L. ssp. *rhamnoides*, cv. Indian-Summer) were collected from a 15-year old orchard at Pearl Creek Farms in Melville, Saskatchewan. Berries were manually harvested in November 2002. Berries were hand cleaned to remove dry leaves, branches, and berries damaged by harvesting and then wind screened. Cleaned berries were double bagged in 50-kg portions, placed in cardboard boxes, frozen to approximately -15ºC, and shipped to the University of Manitoba via bus (approximately 10 h in transport). Upon arrival, the berries were stored in a walk-in freezer at -25ºC for approximately 2 to 3 months.

Oil was extracted from sea buckthorn seeds and pulp-flakes obtained by pilot-scale processing and separation. The pilot scale processing was conducted at the Food Development Centre (FDC) in Portage la Prairie, Manitoba. Juice was extracted from berries on a bladder press using 0.8 MPa maximum pressure for a 5-min extraction time. The wet pulp-cake was then dried at 50ºC for 24 h in a forced-convection drying oven. The dried seeds and pulp-flakes were separated using an industrial mixer (Hobart Cutter Mixer, Troy, OH) operated for short time intervals (approximately 3 s) repeatedly for 10 to 15 cycles to minimize seed damage, and a vibratory screen separator (SWECO, Toronto, ON) operated at 1200 rpm (Yakimishen et al. 2005). The seeds and pulp flakes were then stored at -5ºC until they were used in the oil extraction experiments, approximately 1 to 4 months later.

Oil extraction

Oils were isolated from sea buckthorn seeds and pulp-flakes by: (i) solvent extraction using petroleum-ether, (ii) supercritical

fluid extraction with carbon dioxide (SCFE CO_2 at 45ºC and 35 MPa), (iii) expelling with a screw press, and (iv) aqueous extraction.

Solvent extraction using petroleum-ether (boiling range 35-60ºC) was done in a Goldfisch laboratory oil extraction apparatus (Model 35001, Labconco Corporation, Kansas City, MO) following a standard method (AACC 2000). Prior to extraction, seeds were prepared by grinding them in two 15-s cycles in a rotary mill (Stein Mill, Model M-2, Stain labs Inc., Atchison, KS). During petroleum ether extraction, the oil temperature was maintained at 45 ±1ºC and measured using a T-type thermocouple connected to an Omega microprocessor thermometer (model HH23, Omega Engineering, Stamford, CT).

Supercritical fluid extraction was conducted using a supercritical fluid extraction screening system (Newport Scientific Inc., Jessup, MD). The major component of the SCFE system included a carbon dioxide source (compressed cylinder of liquefied CO_2 gas, 99% pure), a continuous compressor, a cylindrical stainless steel extraction vessel (300-mL capacity), a temperature controlled heating pad (silicone-rubber type, 180 W) external to the extraction vessel, a stainless steel cylindrical oil collection vessel (125-mL capacity), and a flow-rate indicator with a flow totalizer. Seeds were prepared by grinding following the method used in petroleum-ether extraction. A 140-g sample of ground seeds or a 70-g sample of pulp-flakes was loaded into a high-pressure extraction vessel of 381 mm inside diameter and 267 mm inside depth. Steel wool (grade #2, medium course) was placed at the bottom and top of the extraction vessel to prevent the sample particles from entering and clogging the supercritical CO_2 inlet and outlet. To prevent channeling of the supercritical CO_2 through a sample, steel wool was also inserted at a midway point between two equal layers of the sample. All samples (ground seeds or pulp-flakes) were gently compacted inside the extraction vessel to 0.6 kPa. Oil extraction from seeds and pulp-flakes was conducted at 45ºC and 35 MPa. Flow rate of CO_2 through the sample in the extraction vessel was maintained at approximately 4.5 L/min.

Oil pressing was conducted using a Täby oil screw press with a 6-mm die (model Type-20, Skeppsta Maskin AB, Örebro, Sweden). A method adopted from Singh et al. (2002) was used for oil pressing trials. Prior to pressing, the screw was first allowed to heat for 20 min via a 120-W electrical resistance heating ring attached around the press head to raise its temperature to 95ºC. After heating, the whole seeds and pulp-flakes were pressed for 4 min to achieve steady flow of press cake and oil and then 300 g of seeds or pulp-flakes were fed into the screw press (Yakimishen et al. 2005). During screw pressing, a computer and data acquisition system (Omega MultiScan 1200, Omega Engineering, Stamford, CT) was used to monitor and record the temperature of the extracted oil stream. Extracted oil was collected in 25-mL vials which were wrapped with aluminum foil for protection from light (Kiritsakis et al. 1984). The oil samples in vials were flashed with nitrogen prior to storage at -25ºC and were analyzed 2 to 4 months later.

In aqueous extraction, seeds were prepared by grinding following the method used in petroleum-ether extraction. Ground seeds were added to 40% ethanol in the ratio of 1:2.5 per volume and heated for 2 h at 70ºC in a water bath stirred periodically. The mixture was centrifuged in 500-mL bottles for 10 min at 8275 × g (7000 rpm, Sorall RC-5C plus, rotor GS-3, Sorvall, Newton, CT) and at 20ºC. The liquid fraction was decanted and centrifuged again at room temperature for 10 min at 17200 × g (10000 rpm, rotor SS-34) to recover seed oil. To extract oil from the pulp, approximately 2 kg of berries were macerated using a blender (Osterizer, model LR47897, Sunbeam Corp, Delray Beach, FL) in approximately 100-g batches. The blades of the blender were covered with surgical tubing to prevent seed damage. The blender was operated in 'stir' mode for short time intervals (5 to 10 s) repeatedly for 10 cycles. The slurry was heated in a water bath for 1 h at 45ºC and then centrifuged in 250-mL bottles for 5 min at 7425 × g (7000 rpm, Sorvall RC Superspeed, model SV728211, rotor SLA-1500, Sorvall, Newton, CT). Centrifugation produced three layers: a top cream layer (an emulsion of pulp oil and suspended solids), a middle juice layer, and a solids layer (seeds and pulp fibre). The two first layers were decanted and mixed with 95% ethanol in the volume ratio of 2:1. The mixture was heated in a water bath for 2 h at 80ºC stirring periodically. The mixture was then centrifuged in 50-mL vials for 10 min at 7425 × g (7000 rpm) and 45ºC. The resulting oil layer was drawn from each vial using a Pasteur pipette, flashed with nitrogen, and used as the starting material for oil analysis.

Oil analysis

To evaluate the levels of extracted compounds in the oil by each of the four tested extraction techniques, chloroform/methanol extraction was conducted. This technique can extract all lipids from a sample and, therefore, served here as reference (Christie 1992).

Fatty acid analysis Composition of fatty acid was established by analysis of methyl esters prepared according to AOCS (2000) method Ce 1-62. The fatty acid methyl esters were analyzed using a Hewlett Packard gas chromatograph (GC) (Model 5890, Hewlett Packard, Palo Alto, CA) equipped with split/splitless injector and flame ionization detector. A fused silica capillary column DB-23 (30 m x 0.25 mm, with film thickness of 0.25 μm, J&W Scientific, Folsom, CA) was used. The linear velocity of the hydrogen carrier gas was 0.5 m/s. For injection, 1 μL of sample was used with a split ratio 1:80. Column temperature was programmed as follows: 155ºC held for 2 min, increased at a rate of 2ºC/min to 215ºC, and final temperature held for 1 min. The injector and detector temperatures were set at 250ºC. The fatty acid esters were identified by comparing retention data with a standard mixture (#461, NuChek Prep, Elysian, MN). The fatty acid composition was expressed as a mass percentage of the total amount of fatty acids.

Tocopherol and tocotrienol analysis Tocopherols and tocotrienols were analyzed following ISO (2004) procedure 9936. These components were separated using normal-phase High Performance Liquid Chromatography (HPLC) on Shimadzu 10AD apparatus with a Shimadzu SIL-10A autoinjector, and RF-10AXL flourescence detector (Shimadzu Corporation, Kyoto, Japan). The fluorescence detector excitation and emission wavelengths were set at 290 and

Table 1. Major fatty acid compositions in sea buckthorn seed and pulp oil, as mass percentage of total amount of fatty acid, extracted with different extraction techniques.

Fatty acid (common name)	Reference	Extraction technique (seeds and pulp)			
	Chloroform/ methanol	Petroleum-ether	SCFE CO_2 3 h or 6 h	Screw press	Aqueous**
			Seeds*		
16:0 (Palmitic)	7.5 ± 0.1	7.0 ± 0.1	7.2 ± 0.3	6.7 ± 0.2	n/a
18:0 (Stearic)	2.8 ± 0.01	2.6 ± 0.04	2.4 ± 0.1	2.5 ± 0.01	
18:1*n*-9 (Oleic)	13.4 ± 0.03	13.6 ± 0.02	13.0 ± 0.3	13.6 ± 0.04	
18:1*n*-11 (11-Octadecanoic)	2.3 ± 0.02	2.1 ± 0.1	1.9 ± 0.01	1.9 ± 0.05	
18:2*n*-6 (Linoleic)	36.3 ± 0.2	35.5 ± 0.2	35.9 ± 0.1	35.3 ± 0.1	
18:3*n*-3 (Linolenic)	35.9 ± 0.2	37.4 ± 0.1	37.9 ± 0.1	38.5 ± 0.2	
			Pulp*		
16:0 (Palmitic)	34.8 ± 0.3	35.2 ± 0.1	35.5 ± 0.01	n/a	34.3 ± 0.01
16:1 (Palmitoeic)	34.4 ± 0.01	35.0 ± 0.03	36.3 ± 0.1		38.5 ± 0.01
18:0 (Stearic)	1.2 ± 0.01	1.2 ± 0.01	1.1 ± 0.0		1.1 ± 0.0
18:1*n*-9 (Oleic)	3.4 ± 0.03	3.3 ± 0.1	3.5 ± 0.03		3.2 ± 0.01
18:1*n*-7 (11-Octadecanoic)	7.1 ± 0.03	6.9 ± 0.01	6.9 ± 0.05		7.3 ± 0.02
18:2*n*-6 (Linoleic)	13.5 ± 0.1	12.8 ± 0.01	12.4 ± 0.1		13.0 ± 0.1
18:3*n*-3 (Linolenic)	2.0 ± 0.2	1.5 ± 0.01	1.2 ± 0.01		1.1 ± 0.01
24:1 (Nervonic)	1.1 ± 0.01	1.3 ± 0.1	0.9 ± 0.02		0.05 ± 0.01

* Standard deviations (n = 2)
** Non-processed pulp used
n/a = technology not applicable

335 nm, respectively. A Prodigy 5-µm silica column (250 mm x 3.20 mm; Phenomenex, Torrance, CA) was used for separation with 5% *tert*-butylmethyl ether in hexane as the mobile phase. The 10 µL sample (solution of oil in hexane) was injected for analysis. The identification of individual tocopherols was done by comparing retention data with standards (^{MJS}BioLynx, Brockville, ON; Cat# MT1072, MT1071, MT1073, and MT1790 for calibration of each tocopherol and diluted accordingly in hexane). Quantification is based on external calibration for all isomers of tocopherol and tocotrienol. Amounts of tocopherols and tocotrienols are expressed as a percentage of the total amounts of tocopherol and tocotrienol and in mg/100 g of oil.

Determination of carotenoids Total amounts of carotenoids were determined following the modified method by Gao et al. (2000). Solutions of oil in hexane (0.1 g/10 mL) were measured at 460 nm in the spectrophotometer (Spectronic, model 3000 ARRAY, Milton Roy, Ivyland, PA). Quantification of the amounts of carotenoids were based on calibration with β-carotene standard (type II: synthetic) and amounts of carotenoids were expressed in mg/100 g of oil.

Analysis of sterols Sterols were analyzed by GC following a method adapted from Yang et al. (2001). Oil samples were saponified at room temperature with 2M potassium hydroxide in ethanol for 24 h. After saponification, water was added to the sample and unsaponified matter was extracted three times with diethyl ether. Extracts were washed with water until neutral and the solvent was removed using a rotary evaporator under vacuum. Residue was dissolved in isooctane and analyzed for composition. Sterols were analyzed using Shimadzu GC model 17A (Shimadzu Corporation, Kyoto, Japan) on a DB-5 capillary column (30 m x 0.25 mm; phase thickness 0.25 µm, Restek, Bellefonte, PA). The column temperature was programmed as follows: held at 60ºC for 1 min, programmed at 40ºC/min to 240ºC, held for 1 min, and finally programmed to 300ºC at 2ºC/min, final temperature was held for 2 min. Hydrogen (2.2 mL/min) was used as a carrier gas. The injector and detector temperatures were set at 275 and 320ºC, respectively. Sterols were identified by comparison of retention data with standards and quantified using internal standard 5α-cholestane. Sterol amounts were expressed as a mass percentage of the total amount of sterols and in mg/100 g of oil.

Oil samples obtained from seeds, pulp (in aqueous extraction), and pulp-flakes using different extraction techniques were analyzed in duplicate.

RESULTS and DISCUSSION

Fatty acids

Three major fatty acids namely oleic, linoleic, and linolenic acid were in the seed control sample extracted with chloroform/methanol. These fatty acids comprised more than 85% of all fatty acids (Table 1). The latter two acids were the predominant fatty acids with contributions of 36.5 and 35.9%, respectively. Similar values were reported for European (ssp. *rhamnoides*) sea buckthorn seeds (Yang and Kallio 2002). Seed oil for subspecies *sinensis* and *rhamnoides* has been characterized as being high in unsaturated fatty acids (40% linoleic, 20% linolenic, and 17% oleic acids) and lower in

Table 2. Major tocopherol and tocotrienol concentrations (mg/100 g oil) in sea buckthorn seed and pulp oil obtained with different extraction techniques.

Compound	Reference	Extraction technique (seeds and pulp)				
	Chloroform/ methanol	Petroleum-ether	SCFE CO_2		Screw press	Aqueous**
			3 h	6 h		
			Seeds*			
α-tocopherol	121 0 ± 6.2	223.4 ± 11.8	170.5 ± 36.9	196.7 ± 18.3	147.8 ± 4.4	n/a
β-tocopherol	9.5 ± 0.6	11.8 ± 0.1	11.2 ± 0.3	12.1 ± 0.3	8.1 ± 0.02	
γ-tocopherol	130.0 ± 5.4	177.4 ± 4.5	154.2 ± 18.4	176 ± 10.2	127.0 ± 4.1	
δ-tocopherol	6.4 ± 0.9	8.0 ± 0.1	8.8 ± 0.3	8.6 ± 0.2	5.3 ± 0.1	
β-tocotrienol	6.7 ± 0.3	9.7 ± 0.3	7.6 ± 1.5	9.2 ± 0.6	7.2 ± 0.4	
Plastochromanol-8	2.9	n/d	n/d	n/d	n/d	
			Pulp*			
α-tocopherol	220.8 ± 0.0	143.7 ± 7.9	101.1 ± 16.2	113.0 ± 12.1	n/a	138.4 ± 11.4
β-tocopherol	21.1 ± 0.2	14.5 ± 0.1	11.3 ± 0.01	12.6 ± 0.5		9.4 ± 1.5
γ-tocopherol	11.1 ± 0.3	7.2 ± 0.4	6.7 ± 0.1	7.0 ± 0.2		3.0 ± 0.2
δ-tocopherol	6.5 ± 0.3	5.3 ± 1.2	6.0 ± 0.03	6.2 ± 0.2		n/d
γ-tocotrienol	1.7 ± 1.2	2.3 ± 0.6	2.3 ± 0.01	2.5 ± 1.0		2.9 ± 0.2
Plastochromanol-8	13.2 ± 0.2	8.1 ± 0.2	1.6 ± 0.4	1.9 ± 1.1		8.3 ± 1.0

* Standard deviations (n = 2)
** Non-processed pulp used
n/a = technology not applicable
n/d = not detected

saturated fatty acids (13% palmitic and 8% steric acids) (Li and Beveridge 2003). Seed oil from these subspecies has a high concentration of two essential fatty acids, namely linoleic (42%) and linolenic (39%) acid. Higher concentrations of linolenic acid (37.4 – 38.5%) were found when solvent SCFE CO_2 or screw press were used in comparison to chloroform/methanol extraction as control (35.9% for seeds). The variation in the concentration of fatty acids in the four tested techniques was negligible.

There was no difference in fatty acid composition in seed or pulp-flake oil extracted by SCFE CO_2 conducted for 3 h versus 6 h. Therefore, Table 1 does not distinguish the extraction duration for SCFE CO_2.

Palmitic and palmitoleic acid were the predominant fatty acids in the sea buckthorn pulp-flake oil. Contributions of both acids to the pulp-flakes in the reference sample were 34.4 and 34.8%, respectively (Table 1). Lipids from the pulp of subspecies *sinensis* and *rhamnoides* have been characterized (Yang and Kallio 2002) as having high concentrations (up to 47%) of saturated fatty acids comprised primarily of palmitic acid and lower concentrations of unsaturated fatty acids such as palmitoleic (28%), oleic (18%), linoleic (4%), and linolenic (2%). Palmitoleic acid concentration in oil extracted from pulp by aqueous extraction was the highest (38.5%) among the four techniques tested. This could be attributed to the fact that in aqueous extraction unprocessed pulp was used. In all other extraction techniques pulp-flakes were produced by drying at 50ºC for 24 h, which may have reduced the concentration of palmitoleic acid in the oil.

Tocopherols and tocotrienols

Control sample concentrations (chloroform/methanol extracted) comprising 273.6 mg/100 g of oil of tocopherols and tocotrienols in seed and 261.2 mg/100 g of oil in the pulp oil are shown in Table 2. Seed oil was comprised primarily of α-tocopherol (vitamin E) and γ-tocopherol, which were more than 90% of the total amount of analyzed chromanols.

Concentrations of α-tocopherol, the predominant tocopherol in the seed oil, fluctuated with extraction method (Table 2). The concentration of α-tocopherol was highest in the petroleum-ether extracted oil (223.4 mg/100 g oil), followed by SCFE CO_2 (170.5 to 196.7 mg/100 g oil), and then screw pressing (147.8 mg/100 g oil). Concentrations of α-, β- and γ-tocopherols tended to increase with the SCFE CO_2 duration (3 versus 6 h extraction time). Screw pressed oil contained the lowest concentration of tocopherols and tocotrienols. Generation of friction during screw pressing, resulting in temperatures exceeding 60ºC, may have caused thermal degradation of tocopherol and tocotrienols (Bockisch 1998).

Predominant tocopherols in the pulp oil were α-tocopherol and β-tocopherol, with α-tocopherol constituting 85% of the total tocopherols and tocotrienols identified (Table 2). Processing of pulp which included, thawing berries, macerating, extracting juice, pressing cake, drying, threshing, and sieving had a deleterious effect, decreasing α-tocopherol concentrations in pulp-flake oil. Pulp oil extracted by petroleum-ether contained the highest concentration of α-tocopherol (143.7 mg/100 g oil), followed by aqueous (138.4 mg/100 g oil), and SCFE CO_2 (101.1 mg/100 g for 3 h extraction; to 113.0 mg/100 g oil for 6 h extraction).

Table 3. Total carotenoids sea buckthorn seeds and pulp oil obtained with different oil extraction techniques. Total carotenoids are expressed in mg/100g of expressed oil.

Material	Reference	Extraction technique*				
	Chloroform/ methanol	Petroleum-ether	SCFE CO_2		Screw press	Aqueous**
			3 h	6 h		
Seeds	17.2 ± 0.2	22.2 ± 0.7	6.2 ± 2.6	11.7 ± 0.1	15.3 ± 0.1	n/a
Pulp	347.1 ± 48.2	527.8 ± 14.9	122.3 ± 3.7	148.4 ± 11.7	n/a	292.4 ± 16.1

* Standard deviations (n = 2)
** Non-processed pulp used
n/a = technology not applicable

Table 4. Sterol concentrations (mg/100 g oil) in sea buckthorn seed and pulp oil obtained with different extraction techniques.

Compound	Reference	Extraction technique (processed seeds and pulp-flakes)				
	Chloroform/ methanol	Petroleum-ether	SCFE CO_2		Screw press	Aqueous**
			3 h	6 h		
			Seeds*			
Cholesterol	n/d	3.7 ± 1.0	n/d	n/d	n/d	n/a
Campesterol	17.2 ± 0.5	22.4 ± 0.5	19.9 ± 0.1	22.5 ± 0.7	18 ± 10.7	
Stigmasterol	n/d	n/d	n/d	n/d	2.7[a]	
β-sistosterol	598.9 ± 6.3	746.3 ± 22.8	667.8 ± 20.8	748.1 ± 5.1	635 ± 43.9	
			Pulp*			
Cholesterol	4.6 ± 0	4.5 ± 1.4	n/d	n/d	n/a	n/d
Campesterol	9.7 ± 0.1	12.4 ± 0.6	10.9 ± 0.04	10.9 ± 0.2		6.6 ± 0.6
Stigmasterol	n/d	6.6 ± 2.5	n/d	10.8 ± 3.4		n/d
β-sistosterol	522.06.8	576.9 ± 32.3	525.0 ± 13.5	525.7 ± 5.2		288.6 ± 8.6

* Standard deviations (n = 2)
** Non-processed pulp used
[a] n = 1
n/a = technology not applicable
n/d = not detected

Total carotenoids

Total carotenoid concentration in oil obtained from seeds from the reference sample was 17.2 mg/100 g oil (Table 3). Beveridge et al. (1999) reported trace amounts of total carotenoids in seed oil of some sea buckthorn varieties ranging between 50 and 85 mg/100 g oil. Beveridge (2003) reported a total carotenoid concentration of 41.1 mg/100 g in seed oil of cv. Indian-Summer, however, the method of oil extraction was not mentioned. It seems that in our experiments separation of seeds from pulp involving thawing berries, macerating, extracting juice, pressing cake, drying, threshing, and sieving negatively affected the total concentration of carotenoids. A comparison of total carotenoids in seed oil showed substantial variation in the carotenoid content between solvent extraction (22.2 mg/100 g oil), SCFE CO_2 (6.2 to 11.7 mg/100 g oil), and screw press (15.3 mg/100 g oil). Concentration of total carotenoids increased with extraction time (3 to 6 h). Screw pressed oil contained higher amounts of total carotenoids compared to the SCFE CO_2 technique.

Total carotenoids in chloroform/methanol extracted oil from pulp-flakes were 347.1 mg/100 g oil. Beveridge (2003) reported a wide range of total carotenoids from 330 to 1000 mg/100 g oil, depending on plant subspecies or cultivar. Petroleum-ether extracted pulp oil (Table 3) had the highest concentration of total amounts of carotenoids (527.8 mg/100 g oil) followed by, aqueous extraction (292.4 mg/100 g oil), and SCFE CO_2 extracted oil (148.4 mg/100 g oil, 6 h extraction). The concentration of total carotenoids from SCFE CO_2 pulp-flake oil increased with extraction duration from 122.3 mg/100 g oil after 3 h to 148.4 mg/100 g oil after 6 h extraction.

Sterols

Presence of cholesterol, campesterol, stigmasterol, and β-sitosterol in seed oil extracted with chloroform/methanol as reference and the four tested techniques are shown in Table 4. Seed oil was comprised primarily of β-sitosterol (97%) with trace amounts of campesterol (2%). Yang et al. (2001) reported

Table 5. Effect of extraction methods employed on changes of nutritional components in oils (order of increasing concentration: low < high < highest).

	Oil components	Extraction method			
		Petroleum-ether	SCFE CO_2	Screw press	Aqueous
Seed oil	Major fatty acids	Similar concentrations for most fatty acids			
	Major tocopherols and tocotrienols	high	highest	low	n/a
	Total carotenoids	high	highest	low	n/a
	Major sterols	high	highest	high	n/a
Pulp oil	Major fatty acids	Similar concentrations for most fatty acids			
	Major tocopherols and tocotrienols	high	low	n/a	high
	Total carotenoids	highest	low	n/a	high
	Major sterols	highest	high	n/a	low

n/a = not applicable, no oil extracted

that β-sitosterol is the major sterol in seeds of ssp. *sinensis*. Cholesterol and stigmasterol were not detected in seed oil samples.

The concentration of β-sitosterol in seed oil changed with extraction method, namely solvent extraction (746.3 mg/100 g oil), SCFE CO_2 (667.8 to 748.1 mg/100 g oil), and screw pressing (635.0 mg/100 g oil) (Table 4). A similar trend was evident with campesterol concentrations. The amount of β-sitosterol and campesterol increased by more than 10% when extraction time was increased from 3 to 6 h. Beta-sitosterol and campesterol concentrations in the seed oil were lower for screw pressed oil compared to solvent extraction and SCFE CO_2 extracted oils. The highest oil temperatures were evident in the screw pressing ranging from 63.0 to 69.6ºC. Frictional heat generated during screw pressing resulted in temperatures greater than 60ºC that may have caused thermal degradation of these compounds. Analysis of the screw press residues would be needed to verify the above statement. Cholesterol was found only in the solvent extracted seed oil at a concentration of 3.7 mg/100 g oil.

Campesterol, stigmasterol, and β-sitosterol were present in the pulp oil with the latter having the highest contribution (97%). The petroleum-ether technique extracted similar quantities of cholesterol (4.5 mg/100 g oil) as was extracted by the chloroform/methanol method. This technique also extracted the highest amount of β-sitosterol (576.9 mg/100 g oil) as compared to SCFE CO_2 (525.0 mL/100 g oil) and aqueous extraction methods (288.6 mg/100 g oil). The duration of SCFE CO_2 extraction had no effect on concentrations of sterols. However, after a 6 h extraction, stigmasterol (10.8 mg/100 g oil) was detected in the pulp oil.

Nutritional quality summary

The information gathered in this research on nutritional components such as fatty acids, tocopherols and tocotrienols, carotenoids, and sterols in sea buckthorn seed and pulp oils showed the effects of processing and extraction on final product quality. Table 5 qualitatively summarizes relative concentrations of fatty acids, tocopherols and tocotrienols, total carotenoids, and sterols associated with the extraction technologies studied. Petroleum-ether extraction consistently recovered oils having higher amounts of all analyzed nutritional components. Yang and Kallio (2002) suggested that solvent extraction is not suitable for sea buckthorn oil extraction because harmful solvent residues can be left behind in extracted oil and adds to environmental pollution. Aqueous extraction and screw pressing methods are limited by the type of material (seeds vs pulp) which can be processed (Yakimishen et al. 2005). The SCFE CO_2 method was flexible in extracting both seed and pulp oils having relatively high concentrations of all identified nutritional compounds. The addition of co-solvents with CO_2 may enhance selectivity for extracting additional nutritional components. Data presented on the concentrations of nutritional components associated with a Canadian sea buckthorn cultivar may be used as a guideline for processing and extracting to achieve oils having high nutritional value.

CONCLUSIONS

The nutritional qualities of seed and pulp oils from sea buckthorn berries were evaluated after oil extraction was conducted using four oil isolation techniques. These were: solvent extraction using petroleum-ether, supercritical fluid extraction using carbon dioxide (SCFE CO_2), screw pressing, and aqueous extraction. The extracted nutritional quantities were compared against solvent extraction using chloroform/methanol as reference. In general, aqueous extraction was unsuccessful in extracting oil from sea buckthorn seeds and screw pressing was unsuccessful in extracting oil from pulp-flakes.

The concentration of fatty acids in oil extracted from seeds and pulp-flakes was similar in all tested extraction techniques.

Petroleum-ether extraction recovered oils having the highest concentrations of tocopherols (420.6 mg/100 g seed oil and 170.7 mg/100 g pulp oil) and tocotrienols (9.7 mg/100 g seed oil and 7.6 mg/100 g pulp oil), carotenoids (22.2 mg/100 g seed oil and 527.8 mg/100 g pulp oil), and sterols (772.4 mg/100 g seed oil and 600.4 mg/100 g pulp oil), followed by SCFE CO_2. Screw pressed and aqueous extracted oils contained the lowest amounts of nutritionally important components.

Concentrations of α-, β-, γ-tocopherols, and total carotenoids extracted with oil from seeds and pulp-flakes, and

β-sitosterol and campesterol extracted with oil from seeds increased with the SCFE CO_2 duration (3 versus 6 h extraction time); but the duration of SCFE CO_2 extraction had no effect on concentrations of sterols extracted from pulp-flake oil.

ACKNOWLEDGEMENTS

The authors thank the Natural Sciences and Engineering Research Council (NSERC), Manitoba Hydro, and the Manitoba Rural Adaptation Council (MRAC).

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