

# Effects of drying method on the extraction yields and quality of oils from quebec sea buckthorn (*Hippophaë rhamnoides* L.) seeds and pulp

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## Abstract

The effects of air-drying and freeze-drying on the extraction yields and quality of oils from Quebec sea buckthorn (*cv. Indian-summer*) seeds and pulp were studied. Oil extractions were carried out using hexane. Air-dried (ADS) and freeze-dried (FDS) seeds, gave a similar extraction yields ( $\sim 12\%$  w/w), whereas those of air-dried (ADP) and freeze-dried (FDP) pulps were significantly different ( $35.9 \pm 0.8$  vs.  $17.1 \pm 0.6\%$  w/w). Fatty acid analysis revealed that  $\alpha$ -linolenic (37.2–39.6%), linoleic (32.4–34.2%) and oleic (13.1%) acids were the main fatty acids in seed oils, while pulp oils were rich in palmitoleic (39.9%), palmitic (35.4%) and linoleic (10.6%) acids. Lipid fractionation of crude oils, obtained by solid phase extraction (SPE), yielded mainly neutral lipids (93.9–95.8%). The peroxide values of seed and pulp oils were ca. 1.8 meq/kg and between 3.0 and 5.4 meq/kg, respectively. The melting behavior of seed and pulp oils showed multiple endothermic transitions, as observed normally in vegetable oils.

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## 1. Introduction

Sea buckthorn berries are rich in vitamins, carotenoids, flavonoids, proteins, antioxidants, amino acids, essential fatty acids, and phytosterols (Beveridge, Li, Oomah, & Smith, 1999). The most valuable components of the berries are their oils. Both seeds and berry pulp have high total lipid content, including tocopherols, tocotrienols, carotenoids, as well as  $\omega$ -3 and  $\omega$ -6 fatty acid families (Yang & Kallio, 2002a). The composition of the sea buckthorn seed and pulp oils vary according to the subspecies, origins, cultivation activities, harvesting time of the berries, and the extraction method (Yang & Kallio, 2002b). The seed oil is highly unsaturated, with proportions of linoleic (C18:2n-6) and  $\alpha$ -linolenic (C18:3n-3) acids between 30–

40% and 20–35%, respectively, whereas the pulp oil is more saturated containing high amounts of palmitoleic (C16:1n-7, 16–54%) and palmitic acids (C16:0, 17–47%) (Yang & Kallio, 2002a).

Solvent extraction using hexane and supercritical CO<sub>2</sub> extraction are the main methods used for sea buckthorn oil extraction, both in industrial and laboratory scale. Aqueous extraction and pressing are also used, mainly in laboratory scale. Recently, Yakimishen, Cenkowski, and Muir (2005) reported for sea buckthorn berries *cv. Indian-summer*, seed oil recoveries of 7.2% and 4.5% using supercritical CO<sub>2</sub> extraction and screw pressing, respectively, and a pulp-flake oil recovery of 17% for supercritical CO<sub>2</sub> extraction. Moreover, low recovery of pulp oil (1.2%) was obtained by aqueous extraction.

Oil extraction involves various preliminary operations, such as cleaning, dehulling, drying and grinding. However, the total amount of extracted oil depends mainly on the extraction time and temperature, moisture content and

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particle size of the oil-bearing materials. The greatest amount of oil is extracted during the first 20 min of extraction, and as moisture content decreases, oil recovery increases (Bernardini, 1982).

Drying methods have different effects on microstructure and quality of dehydrated products. Freeze-dried food materials remain a benchmark quality because of the structure preservation during removing water (Aguilera, Chiralt, & Fito, 2003), contrary to the significant structural changes caused by air-drying. Oomah, Liang, Godfrey, and Mazza (1998) studied the effects of microwave-drying and air-drying on the physical and chemical properties of oil from grape seeds. They found that microwave drying increased oil yield, viscosity, peroxide value and conjugated dienes, whereas *p*-anisidine and saponification values were reduced. Other studies have shown that drying methods have significant effects on oil extraction efficiency, physical properties and chemical compositions of aromatic plants (Raghavan, Rao, Singh, & Abraham, 1997; Omidbaigi, Sefidkon, & Kazemi, 2004; Hsu, Chen, Weng, & Tseng, 2003).

The effects of dehydration method, as a pretreatment operation to oil extraction, on extraction yields and quality from sea buckthorn berries have hardly been investigated. Therefore, this research aimed to evaluate these effects for two drying methods: air-drying and freeze-drying.

## 2. Materials and methods

### 2.1. Berries

Sea buckthorn berries (*Hippophaë rhamnoides* L. cv. *Indian-Summer*) from Sainte-Anne-de-Beaupré (Québec, Canada) were hand-cleaned and stored at  $-30\text{ }^{\circ}\text{C}$  in sealed plastic bags until the beginning of the experiments. Before drying, batches of 400 g of frozen berries were ground only for 60 s to avoid possible seed damage, using a blender (model PR200B, General Electric, USA).

### 2.2. Air-drying experiments

Frozen ground berries were placed in a perforated drying tray. Starting with approximately 900 g berries, air-drying experiments were conducted at  $50\text{ }^{\circ}\text{C}$  and 1.0 m/s air velocity for 24 h, using a laboratory tray drier (Model UOP8-G, Armfield, Hampshire England). The dried product was crushed in a mortar to facilitate the separation of seeds from the pulp. Special caution was exercised in order to avoid seeds damage. A 10 mesh (2 mm) sieve (Canadian Standard Sieve Series, W.S. Tyler, St. Catharines, ON, Canada) was used to separate most seeds from dried pulp. Pulp residues adhered to seeds, were hand-separated and crushed in a mortar. Air-dried seeds were cleaned and subsequently milled using a disc-grinder (Type C/11/1, Glen Mills Inc., Clifton, NJ, USA). Particle size distribution was measured using a sieve shaker (model 41314, Retsch Inc., Newtown, PA, USA). Both air-dried pulp (ADP)

and seeds (ADS) were stored at  $-20\text{ }^{\circ}\text{C}$  in plastic containers until oil extraction.

### 2.3. Freeze-drying experiments

Frozen ground berries were laid in layers of approximately 2-cm thickness in aluminum trays covered with perforated aluminum paper. Freeze-drying experiments were carried out in a freeze-drier (model Ultra 25 LE, Virtis, Gardiner, NY, USA) at constant temperature of  $50\text{ }^{\circ}\text{C}$  for 24 h, under vacuum pressure of 0.0145 kPa and a condenser temperature of  $-48\text{ }^{\circ}\text{C}$ . After freeze-drying, the samples were immediately stored under vacuum in plastic bags, using a vacuum apparatus (Model 350, SIPROMAC Inc., St-Germain, QC, Canada), until sieving under an anhydrous atmosphere in an Atmos-bag (model Z106089-1EA, Aldrich Atmosbag, Sigma-Aldrich, Oakville, ON, Canada). Freeze-dried samples were sieved using a 10 mesh screen allowing the separation of seeds from the pulp and obtaining uniform size pulp particles. Freeze-dried seeds were cleaned manually and subsequently milled, sieved and stored as described above for air-dried seeds.

### 2.4. Total lipid content of starting materials

Total lipids were extracted from ground materials using the chloroform-methanol extraction procedure adapted from Christie (1982). Dried samples were homogenized for 5 min with chloroform/methanol (1:1, v/v) in proportion 1/10 (m/v). The mixture was filtered, and the obtained solid residue homogenized with chloroform in proportion 1/5 (m/v) for 5 min. The filtrate was transferred into a separatory funnel and the solid was extracted once again under the same conditions, and filtered. Distilled water (about one quarter of the total volume of the filtrate) was added to the combined filtrates, and the resultant mixture was thoroughly shaken and settled overnight. The lower layer containing the lipids was removed from the funnel, and subsequently, the solvent was evaporated using a rotary film evaporator (Büchi R-205, Cole-Parmer, Vernon Hills, IL, USA). The obtained crude lipid extracts were collected, evaporated under nitrogen, weighed, and stored in sealed amber glass vials at  $-20\text{ }^{\circ}\text{C}$  until lipid analysis.

### 2.5. Oil extraction with solvent

Oil extraction was conducted at 30, 40, 50 and  $68\text{ }^{\circ}\text{C}$  using hexane as solvent and three solid to solvent ratios (*R*): 1/4, 1/7 and 1/14 (m/v). A Soxhlet extractor was used for the extractions carried out at  $68\text{ }^{\circ}\text{C}$ , whereas extractions at 30, 40 and  $50\text{ }^{\circ}\text{C}$  were achieved in sealed Erlenmeyer flasks under continuous agitation (400 rpm) for 24 h, using a thermoregulated bath. After the extraction process, the flask contents were filtered under vacuum, and the liquid fraction containing lipid extract and solvent was poured into a 500-mL flask of a rotary film evaporator to remove the solvent. The extracts were treated as described above

for total lipid content. For each dried product, the oil extraction yield (%w/w) and the extraction efficiency were calculated using:

$$\text{Oil extraction yield(\%)} = \frac{\text{Mass of extracted oil(g)}}{\text{Mass of oil-bearing material(g)}} \times 100 \quad (1)$$

$$\text{Extraction efficiency} = \frac{\text{Oil extraction yield}}{\text{Total lipid content}} \quad (2)$$

## 2.6. Analytical methods

### 2.6.1. Melting profiles

Melting profiles of the seed and pulp oils were determined using a differential scanning calorimeter (Pyris 1 DSC, Perkin-Elmer, Norwalk, CT, USA) equipped with an intracooler II (Perkin-Elmer, Norwalk, CT, USA). The system was purged during analysis with nitrogen at 30 mL/min. DSC melting curves were performed within the temperature ranges of  $-60$  to  $30$  °C and  $-50$  to  $50$  °C for seed and pulp oils, respectively. Samples (10–20 mg) were cooled and held at the lowest temperatures for 5 min and then heated at  $4$  °C/min. An empty pan was used as inert reference to balance the heat capacity of the sample pan. Calibration of DSC was carried out using indium (m.p. =  $156.6$  °C,  $\Delta H_f = 28.71$  J/g). Data were analyzed using thermal analysis software (Pyris 1 Version 3.5, Perkin-Elmer). The solid fat index (SFI) was determined from the DSC melting curves by sequential integration of peak areas (Deroanne, 1977; Lambelet, 1983).

### 2.7. Lipid fractionation

Separation of individual lipid fractions was achieved using solid-phase extraction (SPE) cartridges (Bakerbond SPE amino [NH<sub>2</sub>] disposable extraction columns, J.T. Baker Inc., Phillipsburg, NJ, USA) as described by Oomah, Ladet, Godfrey, Liang, and Girard (2000). The cartridges were preconditioned with 2-mL methanol, 2-mL chloroform, and 4-mL hexane before use. Lipid fractions were recovered by sequential elution under vacuum with 4-mL each of chloroform/isopropanol (2/1, v/v), diethyl ether/acetic acid (95/5, v/v), and methanol, to separate neutral lipids, free fatty acids and phospholipids, respectively. The collected eluted fractions were evaporated under nitrogen, weighed, and stored at  $-20$  °C for subsequent fatty acid analysis.

### 2.8. Fatty acid composition

The fatty acid composition of the oil extracts was determined by GC. Seed and pulp oils were converted into their methyl esters (FAME) and analyzed on a 5890 series II gas chromatograph (Hewlett-Packard, Palo Alto, CA). The oven temperature was programmed as follows: from  $60$  °C (isothermal for 1 min) to  $190$  °C at  $20$  °C/min, and isothermal period of 30 min at  $190$  °C. The injector and

detector temperatures were set at  $250$  °C. Hydrogen was used as carrier gas. GC separation peaks was performed on a BPX-70 capillary column ( $60$  m  $\times$   $0.25$  mm i.d.  $\times$   $0.25$   $\mu$ m film thickness; SGE, Melbourne, Australia). Fatty acids were identified by comparing their retention times with those of the FAME standards purchased from Nu Chek Prep (Elysian, MN, USA) under the same conditions. Peaks were integrated using Hewlett-Packard ChemStation software.

### 2.9. Triacylglycerol composition

A gas chromatography (GC) method was used for the determination of the triacylglycerol (TAG) composition of the oil extracts. Seed and pulp oils were dissolved in octane and analyzed on a 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA). The oven temperature was programmed as follows: from  $250$  °C to  $350$  °C at  $1.5$  °C/min, from  $350$  °C to  $360$  °C at  $0.5$  °C/min, and isothermal period of 10 min at  $360$  °C. Injector and detector temperatures were set at  $400$  °C. Hydrogen was used as carrier gas. The GC separation method was performed on a RTX-65TG capillary column ( $30$  m  $\times$   $0.25$  mm i.d.  $\times$   $0.1$   $\mu$ m film thickness; Resteck, Bellefonte, PA, USA). The identification of the peaks was achieved by comparing their retention times with those of the standards purchased from Nu Chek Prep (Elysian, MN, USA) and Sigma-Aldrich (Sigma, St. Louis, MO, USA) under the same conditions. Peaks were integrated using Hewlett-Packard ChemStation software.

### 2.10. Peroxide value

Peroxide values of the extracted oils were determined according to Official method Cd 8b-90 of the AOCS (1993).

### 2.11. Scanning electron micrographs

Microstructure observations of ADP and FDP were carried out using scanning electron microscopy (JEOL, Model JSM-6360LV, Tokyo, Japan) operated under vacuum at an accelerating voltage of 30 kV.

### 2.12. Statistical analysis

All assays were carried out at least in duplicate. Analysis of variance by the general linear models (GLM) procedure and mean comparisons by the least significant difference (LSD) test were performed using the statistical analysis system (SAS Institute, 2000).

## 3. Results and discussion

### 3.1. Moisture content of the sea buckthorn materials

Moisture content of fresh pulp and seeds was  $87.6 \pm 0.0$  and  $5.5 \pm 0.2\%$ , respectively. After drying operations the

average moisture content was  $2.6 \pm 0.2$  and  $1.5 \pm 0.3\%$  for ADP and FDP, respectively, while no significant effects were found on the moisture content of seeds, in comparison to their initial value.

### 3.2. Total lipid content of the sea buckthorn materials

Chloroform–methanol extraction was used to determine the total lipid content of the starting materials. Significant differences were found between the total lipid content of ADP and FDP ( $35.8 \pm 0.8$  vs.  $16.4 \pm 0.5\%$  (w/w),  $p < 0.001$ ), whereas no significant differences were observed for ADS and FDS ( $11.1 \pm 0.0$  vs.  $13.1 \pm 0.8\%$  (w/w)). Results of lipid content of seeds are in agreement with those reported by Yakimishen (2004) for the cultivar *Indian-summer*, and by Kallio, Yang, Peippo, Tahvonon, and Pan (2002) and Yang and Kallio (2001) for the subspecies *mongolica* and *rhamnoides*. The lipid content of FDP obtained in this work, was at the lower end of the expected range reported previously by Yang and Kallio (2002a) for different subspecies of *Hippophaë rhamnoides*, whereas lipid yields from ADP were at the higher up of these reported values. Differences in the lipid content of sea buckthorn berries may be attributed to the different subspecies, geographical and climate conditions, harvesting time of the berries, as well as the extraction method (Yang & Kallio, 2002b). Nevertheless, the marked difference between oil recovery from

ADP and FDP found in this work could be due to the drying effects on pulp cellular microstructure. Fig. 1a and b shows the scanning electron micrographs of air- and freeze-dried sea buckthorn pulps, respectively. As evidenced, air-drying (Fig. 1a) caused significant structural changes on pulp cells, contrary to freeze-drying (Fig. 1b). It can be observed that the size and shape of air-dried pulp cells were irregular while freeze-dried pulp cells exhibited a regular shape and size. Moreover, the diameter and perimeter of ADP cells were larger than FDP cells. Air-drying caused breakage and destruction of cell walls, and consequently large cavities and intercellular spaces were formed. On the other hand, no broken cell walls were observed in freeze-dried pulp. Since mass-transfer through the solid matrix is usually the rate-controlling step in food extractions (Aguilera & Stanley, 1999), and because of the almost negligible cellular damage of FDP, the resistance to oil diffusion is bigger than in ADP. This behavior could explain the differences found in oil recoveries from ADP and FDP.

### 3.3. Oil extraction using solvent

Sea buckthorn seed and pulp oils were bright yellow and dark red, respectively. Both oils were liquid at room temperature. The pulp oil was more viscous than seed oil. This could be ascribed to its more saturated nature, as it will be shown later.

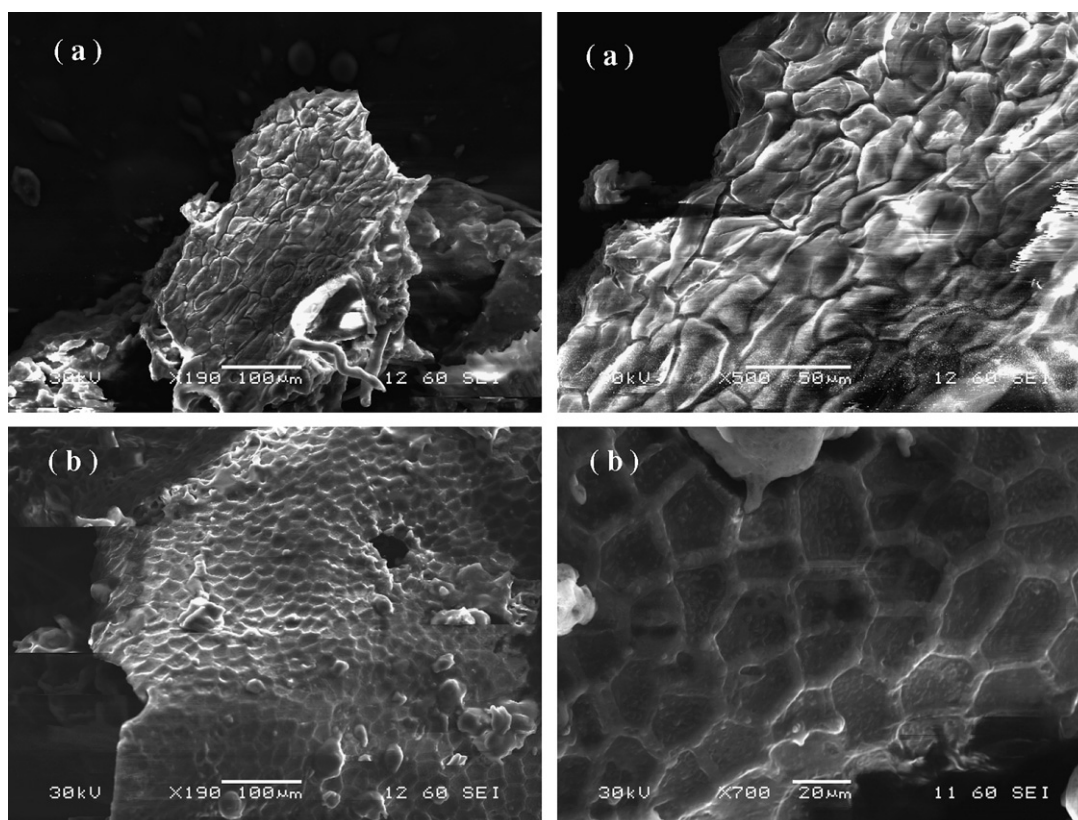


Fig. 1. Scanning electron micrographs of: (a) air-dried (ADP) and (b) freeze-dried (FDP) sea buckthorn pulps.



Extraction yields of oils from ground seeds and pulps were primarily monitored over time at 68 °C, using the Soxhlet extractor with a solid to solvent ratio  $R$  of 1/4. At these conditions, extractions reached equilibrium at about 4 h. Once again, the oil recovery from ADS and FDS was similar ( $11.2 \pm 0.1\%$  and  $12.1 \pm 0.6\%$  (w/w), respectively), whereas the oil extraction yield obtained from air-dried pulp was more than twofolds of that attained from freeze-dried pulp ( $35.9 \pm 3.9\%$  vs.  $16.4 \pm 0.7\%$  (w/w),  $p < 0.001$ ). The air-drying process introduced some structural changes in the shape of cells, and even destroyed them, allowing to the cellular substances, including lipids to be easily extracted. In contrast, the regular structure of FDP cells exerted a transfer resistance to an adequate diffusion of the solvent through the cell membrane. On the other hand, there was a slight increase in the extraction yield from ADS as the  $R$  ratio decreased from 1/4 to 1/14 g/mL ( $11.2 \pm 0.1\%$  vs.  $12.4 \pm 0.1\%$  (w/w),  $p < 0.05$ ), whereas this  $R$  ratio diminution did not show significant differences on oil extraction yields from FDS, FDP and ADP, where their average values were  $13.1 \pm 0.9\%$ ,  $17.1 \pm 0.6\%$  and  $35.9 \pm 0.8\%$  (w/w), respectively. These results did not differ significantly from those obtained with chloroform–methanol, indicating a low content of polar lipids, such as phospholipids, in the investigated sea buckthorn materials.

To investigate the temperature effect on the extraction efficiency of oils, equilibrium data were obtained at 30, 40 and 50 °C, after extraction times of ca. 24 h. For each temperature, the dried sea buckthorn materials were mixed continuously with hexane in sealed erlenmeyer flasks, using three levels of  $R$  (1/4, 1/7 and 1/14 g/mL). The results indicated that extraction efficiency of oils increased with the increase of temperature and with the decrease of  $R$ . However, the effect of  $R$  was more marked than the effect of temperature. When  $R$  values decreased, the effect of temperature became less important. Table 1 presents the effects of variation of temperature and solid-to-solvent ratio on the extraction efficiency of oils from air- and freeze-dried sea buckthorn seeds and pulps. As seen in this table, there was only an average 4%-increase in the oil extraction efficiency from ADP when the temperature increased from 30 to 50 °C, while this increase was about 15% ( $p < 0.05$ ) when  $R$  decreased from 1/4 to 1/14 g/mL. Thus, contrary to Soxhlet extraction, where the oil recoveries were practically not affected by  $R$  ratio because of the occurrence of

better mass transfer features during the extraction process, extractions carried out in erlenmeyer flasks were more dependent on  $R$  ratio than the temperature. Thus, to obtain high yields (greater than 90% of extraction efficiency), and prevent the negative effects of high temperatures on lipids and other valuable components, oil extractions from sea buckthorn seeds and pulp could be carried out at low temperatures, using low  $R$  ratios.

### 3.4. Fatty acid composition

The fatty acid composition of oils from air- and freeze-dried sea buckthorn seeds and pulps is summarized in Table 2. No significant differences were found in the fatty acid composition of pulp oils when comparing the two drying methods, where their main components are palmitoleic (Po), palmitic (P) and linoleic (L) acids ( $\sim 40\%$ ,  $\sim 35\%$  and  $\sim 10\%$ , respectively). Similar values were recently reported for sea buckthorn (cv. *Indian-summer*) pulp oil (Cenkowski, Yakimishen, Przybylski, & Muir, 2006), whereas Yang and Kallio (2001) reported lower concentrations of palmitic ( $\sim 27\%$ ) and palmitoleic acids (27.2–32.8%), and higher proportions of oleic acid ( $\sim 17\%$ ), in pulp oils from subspecies *sinensis* and *rhamnoides*. Linolenic and  $\alpha$ -linolenic acids were the major fatty acids in the sea buckthorn seed oils. Except for stearic and oleic acids, oils from ADS and FDS exhibited quasi-similar composition of fatty acid profiles (See Table 2). Oils from FDS were richer in  $\alpha$ -linolenic and linoleic acids (39.6% vs. 37.2%,  $p < 0.05$  and 34.2% vs. 32.4%  $p < 0.05$ , respectively), and poorer in palmitic and palmitoleic acids (6.5% vs. 8.0%,  $p < 0.05$  and 0.7% vs. 2.8%,  $p < 0.05$ , respectively) than oils from ADS. Oleic and stearic acids were 13% and 3%, respectively, in both oils from ADS and FDS. These values corresponded to those reported by Cenkowski et al. (2006) with slightly lower linoleic acid content for similar cultivar. However, compared to those of seed oils from subspecies *sinensis* and *rhamnoides* (Yang & Kallio, 2001), a lower content of oleic (O) and linoleic (L) acids (13% vs. 18% and  $\sim 33\%$  vs.  $\sim 39\%$ , respectively) and a higher amount of  $\alpha$ -linolenic (Ln) acid ( $\sim 38$  vs. 29%) were obtained in the present work.

Polyunsaturated fatty acids (PUFA) of the seed oils amounted between 70% and 75% of the total fatty acids, while the monounsaturated (MUFA) and saturated (SFA) fatty acids were about 18% and 11%, respectively.

Table 1  
Temperature and  $R$  ratio effects on the extraction efficiency of oils extracted from air- and freeze-dried sea buckthorn seeds and pulps<sup>a</sup>

| $T$ (°C) | Extraction efficiency (%) <sup>b</sup> |                   |                   |                   | $R$ (g/mL) | Extraction efficiency (%) <sup>c</sup> |                   |                   |                   |
|----------|--|-------------------|-------------------|-------------------|------------|--|-------------------|-------------------|-------------------|
|          | FDS                                    | ADS               | FDP               | ADP               |            | FDS                                    | ADS               | FDP               | ADP               |
| 30       | 74.7 <sup>a</sup>                      | 80.2 <sup>a</sup> | 71.4 <sup>a</sup> | 81.6 <sup>a</sup> | 1/4        | 73.1 <sup>a</sup>                      | 74.5 <sup>a</sup> | 65.2 <sup>a</sup> | 76.6 <sup>a</sup> |
| 40       | 87.9 <sup>b</sup>                      | 80.4 <sup>a</sup> | 76.3 <sup>b</sup> | 84.1 <sup>a</sup> | 1/7        | 84.0 <sup>b</sup>                      | 85.8 <sup>b</sup> | 76.1 <sup>b</sup> | 83.2 <sup>b</sup> |
| 50       | 87.8 <sup>b</sup>                      | 91.0 <sup>b</sup> | 82.1 <sup>c</sup> | 85.3 <sup>a</sup> | 1/14       | 93.4 <sup>c</sup>                      | 91.4 <sup>c</sup> | 88.5 <sup>c</sup> | 91.2 <sup>c</sup> |

<sup>a</sup> Means in the same column followed by the same letter are not significantly different by LSD test at the 5% level.

<sup>b</sup> Data represent the average of the extraction efficiency values obtained using the three solid to solvent ratios ( $R$ ): 1/4, 1/7 and 1/14 g/mL.

<sup>c</sup> Data represent the average of the extraction efficiency values obtained at  $T = 30, 40$  and  $50$  °C.

Table 2  
Fatty acid composition of oils from air- and freeze-dried sea buckthorn seeds and pulps, extracted with hexane (30–68 °C)

| Fatty acid                       | PULP       |            | SEEDS                   |            |
|----------------------------------|------------|------------|-------------------------|------------|
|                                  | ADP        | FDP        | ADS                     | FDS        |
| C16:0 (Palmitic)                 | 35.3 ± 1.6 | 35.5 ± 0.5 | 8.0 ± 0.5 <sup>a</sup>  | 6.5 ± 0.2  |
| C16:1 (Palmitoleic)              | 40.1 ± 1.2 | 39.7 ± 1.2 | 2.8 ± 0.5 <sup>a</sup>  | 0.7 ± 0.2  |
| C17:0 (Margaric)                 | 1.8 ± 0.2  | 2.0 ± 0.3  | nd                      | nd         |
| C18:0 (Stearic)                  | 0.8 ± 0.0  | 0.9 ± 0.1  | 3.1 ± 0.5               | 2.9 ± 0.0  |
| C18:1 <i>n</i> -9 (Oleic)        | 3.2 ± 0.2  | 3.0 ± 0.4  | 13.1 ± 0.6              | 13.1 ± 0.2 |
| C18:1 <i>n</i> -7 (Vaccenic)     | 6.5 ± 0.5  | 6.3 ± 0.1  | 2.2 ± 0.1 <sup>a</sup>  | 1.9 ± 0.0  |
| C18:2 <i>n</i> -6 (Linoleic)     | 10.6 ± 0.6 | 10.6 ± 0.7 | 32.4 ± 1.0 <sup>a</sup> | 34.2 ± 0.3 |
| C:18:3 <i>n</i> -3 (α-Linolenic) | 0.9 ± 0.1  | 1.1 ± 0.2  | 37.2 ± 1.4 <sup>a</sup> | 39.6 ± 0.4 |

nd: Not detected.

<sup>a</sup>  $p < 0.05$  between the two drying methods.

These oils, characterized by high ratios of PUFA/MUFA and PUFA/SFA, are prone to the oxidation because of their high content in α-linolenic acid.

### 3.5. Triacylglycerol composition

Fig. 2a shows the triacylglycerol (TAG) composition of oils from ADP and FDP. Significant differences were found in the TAG profiles of pulp oils when comparing the two

drying methods. These differences were probably due to the more severe conditions of air-drying introducing some changes in the TAG molecules of ADP. However, pulp oils contain mainly TAG with acyl carbon numbers of 48 and 50, with 1–3 double bonds (C48:2, C48:1, C50:3, C50:2 and C48:3), representing approximately 85% of the total TAGs. Taking into account the fatty acid composition of pulp oils (Table 2), C48 molecules (representing more than 50% of the total TAGs) should largely correspond to TAG of three 16-carbon fatty acids. Most of these TAG molecules could mainly be PoPoP, PPPo and PoPoPo. Similarly, C50 molecules (representing about 40% of the total TAGs) would correspond to TAGs with two 16-carbon fatty acids and one 18-carbon fatty acid. In view of these results, the majority of TAGs could correspond principally to PPOl and PPL.

The TAG distribution of oils from ADS and FDS is showed in Fig. 2b. As can be seen in this figure, seed oils were mainly constituted by TAGs with acyl carbon numbers of 54 and 52, and minor amounts of C48 and C50 molecules, which comprise predominantly 18- and 16-carbon fatty acids. As shown in Table 2, oils from ADS were richer in palmitic and palmitoleic acids, and poorer in linoleic and α-linolenic acids than oils from FDS. These differences made that the proportions of C54 and C52 TAGs were higher in oils from FDS than in oils from ADS (66% vs. 56%,  $p < 0.01$  and 30% vs. 28%,  $p < 0.01$ , respectively), whereas concentrations of C48 and C50 molecules were higher in oils from ADS than in oils from FDS (8% vs. 1%,  $p < 0.01$  and 7% vs. 2%,  $p < 0.01$ , respectively). Regarding the fatty acid composition of seed oils, C54 and C52 TAGs would be mainly, LLL, LnLnO, OLLn, LLLn, LLO, LLP and PLLn. In general, the TAG compositions of seed and pulp oils obtained in this work were in accordance with those reported by Yang and Kallio (2006) for seeds and berries of sea buckthorn of different subspecies and origins.

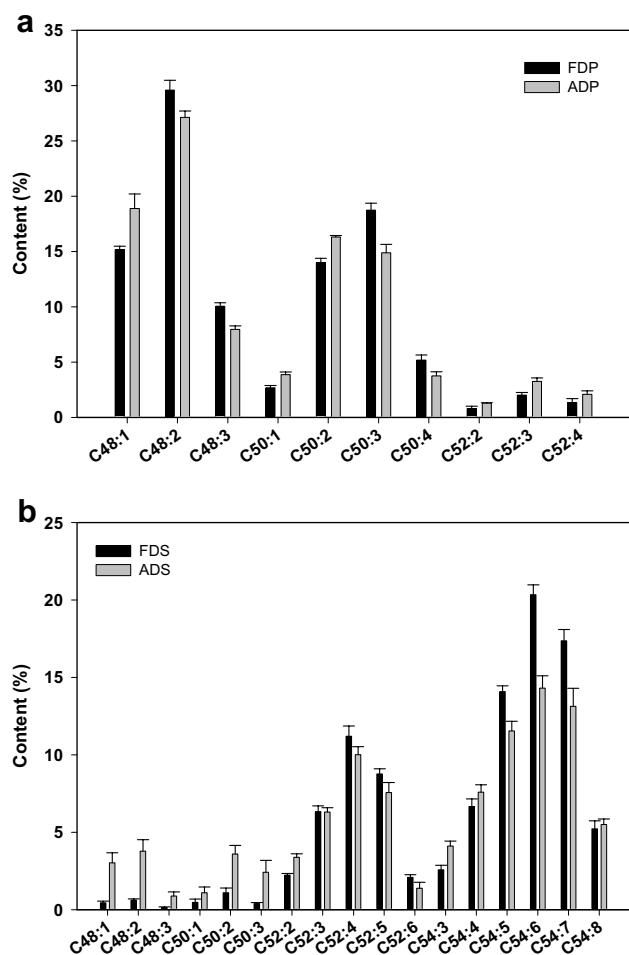


Fig. 2. Triacylglycerol composition of oils from: (a) air- and freeze-dried sea buckthorn pulps, and (b) air- and freeze-dried sea buckthorn seeds.

### 3.6. Lipid fractions obtained by SPE

Sea buckthorn seed and pulp oils consisted mainly of neutral lipids (NL) (~95%), with minor amounts of free fatty acids (FFA) (~2–4%) and phospholipids (PL)

Table 3  
Fatty acid composition in individual lipid fractions of oils from air- and freeze-dried sea buckthorn seeds and pulps, extracted with hexane using the Soxhlet extractor during 4 h

|                               | Oil from ADP            |                         |                         | Oil from FDP |            |            |
|-------------------------------|-------------------------|-------------------------|-------------------------|--------------|------------|------------|
|                               | NL                      | FFA                     | PL                      | NL           | FFA        | PL         |
| Composition (mass %)          | 95.8 ± 1.3              | 1.7 ± 0.8 <sup>a</sup>  | 2.2 ± 0.3               | 94.3 ± 0.1   | 4.0 ± 0.5  | 1.5 ± 0.4  |
| C16:0 (Palmitic)              | 35.8 ± 0.1 <sup>a</sup> | 28.7 ± 1.0              | 25.0 ± 1.1 <sup>a</sup> | 35.2 ± 0.1   | 27.6 ± 2.2 | 22.7 ± 0.4 |
| C16:1 (Palmitoleic)           | 39.2 ± 0.1 <sup>a</sup> | 38.2 ± 0.5              | 34.8 ± 0.7              | 39.9 ± 0.1   | 39.4 ± 0.8 | 35.2 ± 0.7 |
| C17:0 (Margaric)              | 2.0 ± 0.0 <sup>a</sup>  | 2.9 ± 0.0               | 3.2 ± 0.3 <sup>a</sup>  | 2.2 ± 0.0    | 3.1 ± 0.3  | 3.9 ± 0.1  |
| C18:0 (Stearic)               | 0.9 ± 0.0               | nd                      | 1.6 ± 0.3               | 0.9 ± 0.0    | nd         | 1.2 ± 0.0  |
| C18:1 $n$ -9 (Oleic)          | 3.0 ± 0.0 <sup>a</sup>  | 2.4 ± 0.1               | 2.4 ± 0.9               | 2.7 ± 0.0    | 2.1 ± 0.1  | 1.4 ± 0.1  |
| C18:1 $n$ -7 (Vaccenic)       | 6.4 ± 0.0               | 7.6 ± 0.2               | 8.9 ± 0.3               | 6.3 ± 0.0    | 7.3 ± 0.3  | 8.9 ± 0.3  |
| C18:2 (Linoleic)              | 11.0 ± 0.0 <sup>a</sup> | 13.6 ± 0.3              | 18.4 ± 0.9 <sup>a</sup> | 10.8 ± 0.0   | 12.7 ± 0.5 | 20.5 ± 1.0 |
| C:18:3 ( $\alpha$ -Linolenic) | 0.9 ± 0.0               | 6.6 ± 1.0               | 5.8 ± 0.2               | 1.0 ± 0.0    | 6.9 ± 1.8  | 5.3 ± 0.2  |
|                               | Oil from ADS            |                         |                         | Oil from FDS |            |            |
| Composition (mass %)          | 94.3 ± 1.1              | 2.9 ± 0.2               | 1.2 ± 0.1               | 93.9 ± 0.6   | 2.7 ± 1.9  | 0.8 ± 0.2  |
| C16:0 (Palmitic)              | 8.2 ± 0.1 <sup>a</sup>  | 9.1 ± 0.7 <sup>b</sup>  | 12.2 ± 0.7 <sup>b</sup> | 6.9 ± 0.1    | 6.8 ± 0.0  | 10.0 ± 0.2 |
| C16:1 (Palmitoleic)           | 2.9 ± 0.1 <sup>a</sup>  | 3.1 ± 0.1 <sup>c</sup>  | 1.9 ± 0.6 <sup>a</sup>  | 1.4 ± 0.2    | 1.4 ± 0.0  | 0.6 ± 0.2  |
| C18:0 (Stearic)               | 2.9 ± 0.0               | 3.2 ± 0.2 <sup>a</sup>  | 6.5 ± 0.2 <sup>a</sup>  | 3.0 ± 0.1    | 3.0 ± 0.0  | 5.9 ± 0.2  |
| C18:1 $n$ -9 (Oleic)          | 12.7 ± 0.2              | 12.2 ± 0.4 <sup>b</sup> | 12.6 ± 0.2 <sup>c</sup> | 13.0 ± 0.2   | 13.0 ± 0.1 | 14.2 ± 0.2 |
| C18:1 $n$ -7 (Vaccenic)       | 2.2 ± 0.0 <sup>a</sup>  | 2.3 ± 0.1 <sup>a</sup>  | 4.9 ± 0.1 <sup>a</sup>  | 2.0 ± 0.0    | 2.0 ± 0.0  | 4.5 ± 0.1  |
| C18:2 (Linoleic)              | 31.9 ± 0.4 <sup>a</sup> | 30.9 ± 0.9 <sup>a</sup> | 44.4 ± 1.7              | 33.1 ± 0.5   | 32.9 ± 0.3 | 45.9 ± 0.9 |
| C:18:3 ( $\alpha$ -Linolenic) | 37.7 ± 0.4              | 35.8 ± 1.5 <sup>a</sup> | 15.2 ± 0.1 <sup>a</sup> | 38.7 ± 0.7   | 38.5 ± 0.4 | 17.4 ± 1.4 |

nd: Not detected.

<sup>a</sup>  $p < 0.05$ .

<sup>b</sup>  $p < 0.01$ .

<sup>c</sup>  $p < 0.001$  between the two drying methods.

(1–2%) (Table 3). High levels of neutral lipids (92%) have been reported for sea buckthorn seed oil by Zadernowski, NowakPolakowska, Lossow, and Nesterowicz (1997), while similar values for FFA and PL fractions in oils from pumpkin seeds were recently reported (Yoshida, Shougaki, Hirakawa, Tomiyama, & Mizushina, 2004). The fatty acid profiles of the NL fractions were very close to the corresponding crude seed and pulp oils, because of the quantitative primacy of this fraction in the oils. However, when comparing the two drying methods, slight differences were found between the fatty acid compositions of oils from ADP and FDP. On the other hand, similar differences observed in the fatty acid profiles of crude seed oils were found in the NL fractions of ADS and FDS.

The PL fractions of pulp oils were poorer in palmitic and palmitoleic acids (~11% and ~4%, respectively), but much richer in linoleic and  $\alpha$ -linolenic acids (~8% and ~5%, respectively) than in crude oils (Table 3). Significant differences, that could be attributed to the alterations of the cell membrane during air drying, were found in the proportions of linoleic (18.4% vs. 20.5%,  $p < 0.05$ ) and palmitic (25% vs. 22.7%,  $p < 0.05$ ) acids, when comparing the PL fractions of oils from ADP and FDP, respectively. The PL fractions of seed oils were in general richer in linoleic (~13%), palmitic (~4%) and stearic (~3%) acids, and poorer in  $\alpha$ -linolenic acid (~20%), than in crude oils (Table 3). Except for linoleic acid, which amounted 45% of the total fatty acids in both oils from ADS and FDS, significant differences in the proportions of the other fatty acids were found in PL fractions when comparing air- and freeze drying methods.

In general, for both seed and pulp oils, the fatty acid profiles of PL fractions obtained in this work, are within the range reported for others subspecies of sea buckthorn (Kallio et al., 2002; Yang & Kallio, 2001). Nevertheless, the sea buckthorn (cv. *Indian-summer*) pulp oils would have a higher content in palmitoleic and palmitic acids, but a lower content in linoleic and  $\alpha$ -linolenic acids, compared with subspecies *mongolica*, *sinensis* and *rhamnoides*. Genetic differences among subspecies, as well as geographical conditions, cultivating activities and lipid extraction processes, could explain these differences (Yang & Kallio, 2002b).

The fatty acid profiles of the FFA fractions of seed and pulp oils were relatively similar to those of crude oils (Table 3). However, lower proportions of palmitic acid (~7%) and higher amounts of  $\alpha$ -linolenic acid (~6%), were observed in pulp oils. In the case of seed oils, once again the same differences found in the fatty acid compositions of crude oils were observed in the FFA fractions, when comparing the two investigated drying methods. As observed in Table 3, the different conditions of the drying methods used in this work introduced some changes in the fatty acid profiles of individual lipid fractions of the oils. Consequently, their TAG compositions were also affected, as showed in Figs. 2a and b.

### 3.7. Peroxide values of the oils

Fresh oils from air- and freeze-dried sea buckthorn seeds and pulps had a peroxide value (PV) of 1.8 and ~4 meq/kg, respectively, which are lower than those generally found in

commercial vegetable oils ( $\leq 10$  meq/kg). However, the PV of oils from ADP was higher than those from FDP ( $5.4 \pm 0.3$  vs.  $3.0 \pm 1.9$  meq/kg). The absence of oxygen during freeze-drying of pulp and the effects of the more severe conditions of air-drying could explain these results.

### 3.8. Melting profiles

The melting curves of seed oils showed four overlapping peaks as depicted in Fig. 3a. This figure shows one minor low-temperature endothermic transition below  $-40$  °C, followed by one prominent endothermic transition, and two high-temperature endothermic minor peaks at about  $-24$  °C and  $-15$  °C, respectively. The main endothermic transition, most likely corresponded to melting of the major TAG groups, C54 TAGs, such as LLL, LnLnO, OLLn, LLLn and LLO. As can be seen in Fig. 3a, oils from ADS did not show the two higher-temperature peaks as exhibited by FDS oils. Only, a single peak at about  $-21$  °C was depicted. Moreover, the lower-temperature endotherm was broader for oils from ADS than those from FDS. The slight differences between the melting curves

could be attributed mainly to the difference in the TAG composition of the oils obtained from air- and freeze-dried seeds. The melting enthalpies found for oils from ADS and FDS ( $70.3 \pm 5.8$  and  $64.7 \pm 4.7$  J/g, respectively) are in agreement with those reported by Tan and Che Man (2002) for various vegetable oils.

In Fig. 3b, the DSC melting curves of oils from ADP and FDP indicate the presence of three major groups of triacylglycerols melting independently between  $-30$  and  $20$  °C. The more saturated nature of sea buckthorn pulp oils is highlighted in these thermograms. The first low-temperature endotherm (LTE), between  $-30$  and  $-6$  °C, could mainly correspond to the melting of unsaturated TAGs such as PPO<sub>L</sub>, PPL and PoPoPo, whereas the other two middle- and high-temperature endotherms (MTE and HTE), would represent the melting of more saturated TAGs, such as PoPoP and PPPo, representing about 50% of the total TAGs in sea buckthorn pulp oils. As depicted in Fig. 3b, the main peak of the middle-temperature endotherm was followed by two minor transitions prior to the melting of the third group of TAGs. Nevertheless, the melting temperature selected for this endotherm corresponded to the main peak. A similar criterion was used for the temperature attribution for the first low-temperature endotherm. Therefore, when comparing the two drying methods, there were no significant differences between the melting temperatures of endotherms, being their average values  $-22.5$ ,  $-4$  and  $10$  °C for the low-, middle- and high-temperature endotherms, respectively. The melting enthalpy of the low-temperature endotherm was lower in oils from ADP than in those from FDP ( $19.4$  vs.  $25.6$  J/g,  $p < 0.01$ ), whereas melting enthalpies for the middle- and high-temperature endotherms were about  $10$  and  $40$  J/g, respectively. The different TAG profiles observed in oils from ADP and FDP could explain the differences found between the melting enthalpies of the low-temperature endotherms. On the other hand, according to Timms (1980), the large area of the highest melting peak would partly due to the higher heat of melting of the TAG groups corresponding to this endotherm. Another reason which could explain this situation is connected to the presence of high melting triacylglycerols crystallizing together with lower melting triacylglycerols in a quasi-stable mixture.

The solid fat indices (SFI) of the sea buckthorn seeds and pulp oils, calculated from their corresponding thermograms by sequential peak integration areas, indicated that SFI decreased as temperature increased. Seed oils melted completely at temperatures around  $0$  °C, because of their high unsaturated nature, whereas complete melting of TAGs is achieved above  $20$  °C for pulp oils, confirming their more saturated nature.

## 4. Conclusions

Air-dried (ADS) and freeze-dried (FDS) sea buckthorn seeds, gave a similar extraction yield around 12% w/w,

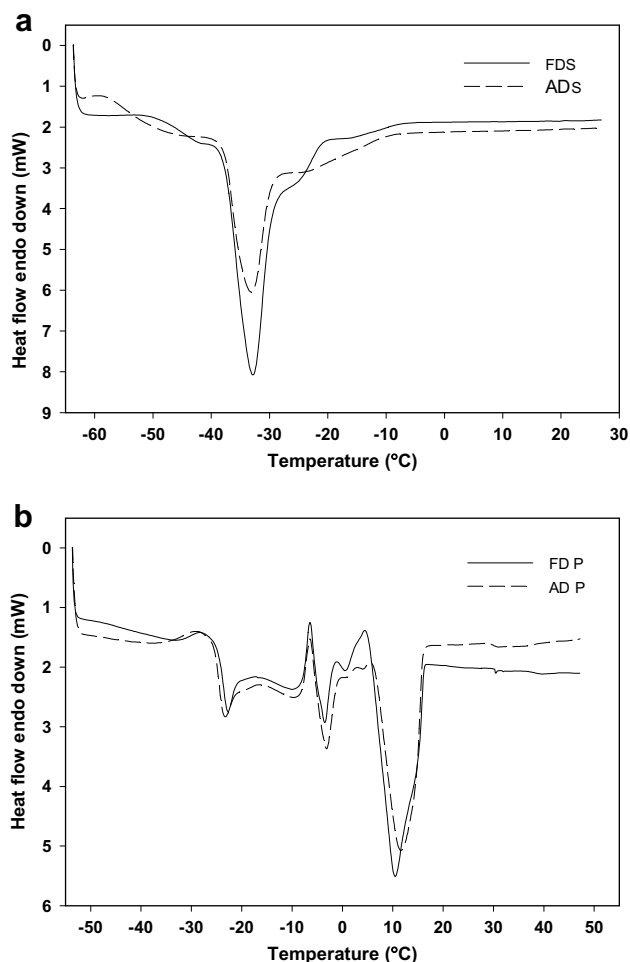


Fig. 3. DSC melting curves of oils from: (a) air- and freeze-dried sea buckthorn seeds, and (b) air- and freeze-dried sea buckthorn pulps.



whereas significant differences were found between extraction yields of air-dried (ADP) and freeze-dried (FDP) sea buckthorn pulps ( $35.9 \pm 0.8\%$  vs.  $17.1 \pm 0.6\%$  (w/w)). Fatty acid analysis revealed that  $\alpha$ -linolenic (37.2–39.6%), linoleic (32.4–34.2%) and oleic (13.1%) acids were the main fatty acids in seed oil extracts, while pulp extracts were rich in palmitoleic (39.9%), palmitic (35.4%) and linoleic (10.6%) acids. Lipid fractionation of crude seed and pulp oils, obtained by SPE, yielded mainly neutral lipids (93.9–95.8%). Sea buckthorn seed oils exhibited four thermal structural transitions between  $-50\text{ }^{\circ}\text{C}$  and  $0\text{ }^{\circ}\text{C}$ , whereas multiple transitions were observed in melting profiles of pulp extracts. The seed oils were characterized with peroxide values of 1.8 meq/kg, while those of pulp oils were between 3.0 and 5.4 meq/kg. Drying method did not have a marked effect on oil quality. However, it is worth mentioning that oils from freeze-dried pulps had a much lower peroxide value bearing out their enhanced quality.

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