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Supercritical CO_2 Extraction of Whole Berry Oil from Sea Buckthorn (*Hippophaë rhamnoides* var. *sp*) Fruit

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Abstract The whole berry, pulp, and seed of sea buckthorn fruit were extracted with supercritical CO_2 to produce edible oils. The effects of extraction pressure, temperature, and CO_2 flow rate on the oil yield and extraction rate were investigated, and the fatty acid composition, tocopherol, and carotenoid contents of the oils were compared. The results showed that the extraction rate was affected by pressure, temperature, and CO_2 flow rate and, in general, the yield increased with a rise in any of the 3 variables. Fatty acids in the whole berry and pulp oil were dominated by monounsaturated fatty acids (>64%), followed by saturated fatty acids (about 30%). In contrast, fatty acids in the seed oil consisted mainly of polyunsaturated (>60%) and monounsaturated fatty acids (>24%). The seed oil had a slightly higher content of tocopherols, but a much lower content of carotenoids, compared with the whole berry or pulp oil.

Key words: supercritical CO_2 extraction, sea buckthorn, whole berry oil

Introduction

Sea buckthorn (*Hippophaë rhamnoides* L.) is widely distributed in Asia, Europe, and North America (1). As a well known hardy plant, it can grow very well in very poor soils and under extremely low rainfall conditions, and hence play an important role in soil and water conservation (2). In China, sea buckthorn (*Hippophaë rhamnoides* var. *sp*) has been planted in many regions with a hush and dry climate for desert reforestation or preventing soil erosion (3). The plant bears a fruit which has long been used in Chinese traditional medicine (4,5). Some researchers have reported that the fruit is rich in a number of bioactive substances such as phenolics, ascorbic acid, tocopherols, and carotenoids (6-8). Both the pulp and seed of sea buckthorn berries are rich in lipids which is rare in the plant kingdom. The seed oil is characterized by high levels of linoleic (C18:2, *n*-6) and α-linolenic (C18:3, *n*-3) acids, while the pulp oil contains a high content of monounsaturated fatty acids (MUFA) including palmitoleic, vaccenic, and oleic acids (4).

Supercritical CO_2 ($SC\text{-}CO_2$) extraction has attracted considerable attention in recent years as a promising technology for extracting oils and other food ingredients. The technology offers a number of advantages over conventional solvent extraction and mechanical pressing, including non-solvent residues and better retention of aromatic compounds (9). Supercritical CO_2 technology has been used for extracting edible oils from the seeds of tomato (10), cherry (11), pumpkin (12), grape (13), and amaranth (14), and also from hazelnut (15), wheat plumule (16), and palm kernel (17). A few studies have also explored the possibility of extracting oils from the seed and pulp of sea buckthorn fruit with $SC\text{-}CO_2$. Yakilmishen *et al.* (18) compared oil recoveries from the seed and pulp of sea buckhorn with $SC\text{-}CO_2$, screw pressing, and aqueous extraction. Stastova *et al.* (19) investigated the effect of $SC\text{-}CO_2$ extraction conditions on the solubility of seed and pulp oils and their mass transfer rate from the solid phase to the extraction medium. However, few studies have been reported on the extraction of oil from the whole berries of sea buckthorn fruit with $SC\text{-}CO_2$. From a practical standpoint, the whole berry might be a better source for oil extraction as it would not require a processing step to separate the pulp and seed. Furthermore, due to the intensive labor required for the separation of the pulp and seed, the berries are frequently left on the tree, therefore wasting a very useful resource.

The objectives of the current study were to explore the feasibility of extracting oil from the whole berry of sea buckthorn fruit with supercritical CO_2, investigate the effect of extraction parameters on the extraction rate and compare the nutritional composition of the oil obtained from different parts of the sea buckthorn fruit.

Materials and Methods

Materials Ripe berries of sea buckthorn (*Hippophaë rhamnoides* var. *sp*) fruit, which were naturally dried on the trees, were collected from the Xinjiang region, China and stored at −20°C until use. The berries were divided into 2 portions; the first portion was extracted for oil from the whole berries while the second portions of berries were separated manually into seeds and pulp to extract oils from the 2 parts of the fruit separately. Before extraction, the whole berries, seeds, and pulp were vacuum-dried to a moisture content of 6.0%, and then grounded to powder using a blender. The distribution of the particle sizes in the powder, as determined by passing it though 3 sieves of 20,

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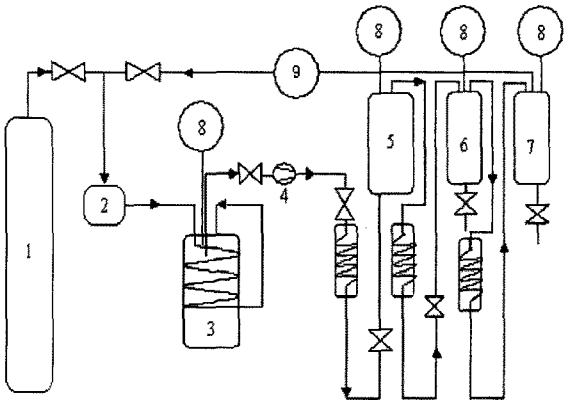

Fig. 1. Schematic diagram of SC-CO_2 extraction apparatus. 1, CO_2 feed tank; 2, filter; 3, cold bath; 4, pump; 5, extraction vessel; 6, 7, separators; 8, pressure gauge; 9, flow meter.

40, and 60 meshes, was as follows: <0.3 mm (9.7%), 0.3-0.6 mm (56.6%), 0.6-0.9 mm (30.5%), and >0.9 mm (3.2%). Moisture content was determined by drying samples (2 g) at 105±0.5°C until constant weight.

Chemicals Carbon dioxide (CO_2, 99.5%) was supplied by Pute Gas Co. (Beijing, China). Reference standards of fatty acids, α-, β-, γ-, δ-tocopherols, and β-carotene were purchased from Sigma-Aldrich (Shanghai, China). All the solvents (HPLC grade) were purchased from Merck (Damstadt, Germany) and the other chemicals (analytical grade) from Beijing Chemical Co. (Beijing, China), unless otherwise stated.

Supercritical CO_2 extraction apparatus and procedure The supercritical fluid extraction system (1,000 mL sample capacity) used in this study was purchased from Nantong Hua'an Co., Ltd. (model HA220-50-06; Nantong, Jiangsu, China). Figure 1 shows a schematic diagram of the system. Samples (300 g) of ground whole berries, seeds or pulp of sea buckthorn fruit were placed into the extraction vessel. After an initial air purge, liquefied CO_2 was pumped into the extraction vessel by a high pressure pump to a given pressure, and the temperature inside the vessel was raised to, and maintained at the desired level, by a heating jacket encasing the vessel. The pressure and temperature were controlled to an accuracy of ±0.5 MPa and ±0.5°C, respectively. The flow rate of the CO_2 was regulated by adjusting the length of the pump stroke. To investigate the effect of extraction conditions on the extraction rate, pressure was varied from 15 to 45 MPa (50°C and 15 L/hr), temperature from 30 to 70°C (30 MPa and 15 L/hr), flow rate from 10 to 20 L/hr (50°C and 30 MPa) and extraction time from 10 to 60 min. After each extraction, the oil was collected in the first separator (set at 60°C and 7 MPa) while water was recovered in the second one (set at 40°C and 4-6 MPa). The weight of the oil was measured to obtain the yield, and the fatty acid composition and the content of tocopherols and carotenoids of the oil were determined.

The oil content of the berries, seeds, and pulp of the sea buckthorn fruit was determined by Soxhlet extraction with *n*-hexane. The content of tocopherols and carotenoids in the oils obtained by the Soxhlet extraction was analyzed and compared with the oils extracted by SC-CO_2.

Analysis of fatty acid methyl esters (FAME) FAME were prepared and analyzed according to the method described by Yu *et al.* (12). The analysis of FAME was performed on an Agilent 6890N gas chromatography-flame ionization detector (GC-FID) equipped with a fused silica capillary column (30 m×0.25 mm i.d., 0.32 mm film thickness (J&W Scientific, Folsom, CA, USA). The sample (1 μL) was injected with a split ratio of 100 : 1 and the inlet temperature was set at 280°C. The oven temperature was initially set at 170°C for 14 min, then increased to 250°C at a rate of 10°C/min and kept at that temperature for 8 min. The detector temperature was set at 300°C. Nitrogen was used as the carrier gas with a linear velocity of 1.0 mL/min. Identification of FAME was achieved by comparing their retention times with those of authentic compounds (Sigma-Aldrich) analyzed under exactly the same conditions. Each sample was analyzed in triplicate. The relative concentrations of the fatty acids were calculated from their peak areas.

Analysis of tocopherols Tocopherols were determined by high performance liquid chromatography (HPLC) according to the procedure described by Ranjith *et al.* (20) with slight modifications. HPLC was performed on an Agilent 1100 HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a diode array detector. Separation of tocopherols was carried out with an Agilent NH_2 column (5.0 mm, 250×4.6 mm i.d.) protected by a 10 mm guard column maintained at 30°C. The mobile phase was a 95 : 5 (v/v) mixture of *n*-hexane and isopropanol with a flow rate of 0.9 mL/min throughout, and the peaks were detected at 292 nm. Identification of tocopherol peaks was made by comparing their retention times with those of standards. The concentration of tocopherols was calculated from the calibration curves prepared for each individual isomer, analyzed under the same conditions. The analysis was carried out in triplicate.

Assay of carotenoids Total carotenoids were assayed as described by Gao *et al.* (21) with β-carotene used as a standard. Total carotenoids were expressed as mg β-carotene equivalent /100 g oil and the analysis was performed in triplicate.

Results and Discussion

Effect of SC-CO_2 extraction parameters on the yield of sea buckthorn oils Figure 2 presents the oil yield of sea buckthorn whole berries at different extraction pressures as a function of extraction time. When the extraction pressure was increased from 15 to 30 MPa, the oil yield was more than doubled after 120 min of extraction. However, further increasing the pressure to 37.5 and 45 MPa had little positive effect on the oil yield and extraction rate (time required to reach maximum yield). Maximum yields were achieved after about 40 min of extraction at 30, 37.5, and 45 MPa, but it took 120 min to reach maximum yield at 22.5 MPa. When the extraction was conducted at 15 MPa, only about 50% of the maximum yield at 45 MPa was achieved after 120 min of extraction (Fig. 2).

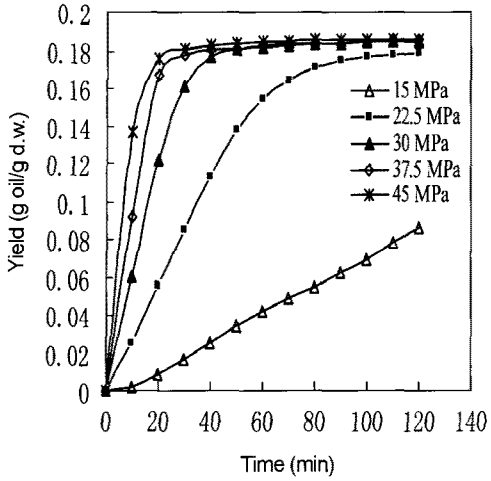

Fig. 2. Effect of extraction pressure on the oil yield of ground whole berries at 50°C and 15 L/hr.

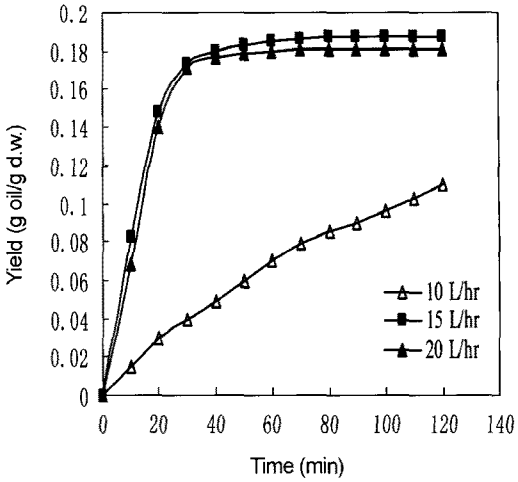

Fig. 4. Effect of CO_2 flow rate on the oil yield of ground whole berries at 50°C and 30 MPa.

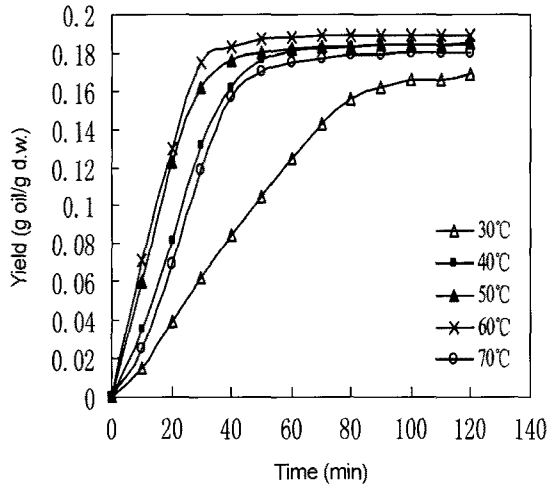

Fig. 3. Effect of extraction temperature on the oil yield of ground whole berries at 30 MPa and 15 L/hr.

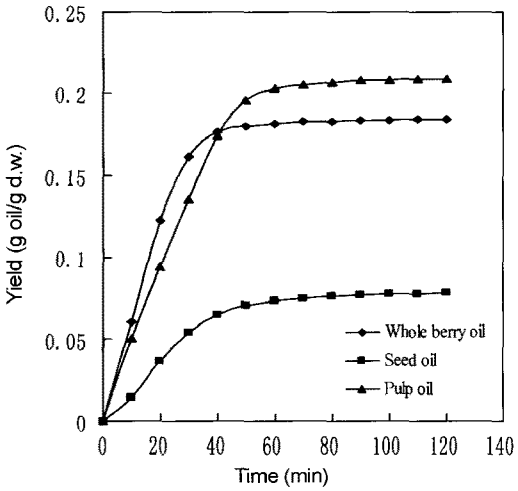

Fig. 5. Oil yield from the whole berry, pulp and seed of sea buckthorn fruit extracted with supercritical CO_2 extraction at 45 MPa, 60°C and a flow rate of 15 L/hr.

The oil yield and extraction rate were also influenced by extraction temperature (Fig. 3). When the extraction temperature was increased from 30 to 50°C, a substantial increase in the rate of extraction was observed and the oil yield increased by 9.7%. Further increasing the temperature to 60 or 70°C, however, achieved little improvement on the extraction rate or oil yield. In general, as temperature increases at a given pressure, the vapor pressure of the solute increases resulting in an increase in solubility, while the density of CO_2 decreases with a resultant lowering of the solute's solubility. The effect of increasing temperature on oil yield is therefore determined by which of the 2 opposing influences was more dominant. It appears that 55°C was a crossover temperature for oil yield. Maximum yield was achieved after about 50 min of extraction at 40-70°C, but it was not reached even after 120 min at 30°C.

Figure 4 shows that the oil yield and extraction rate was also influenced by the flow rate of CO_2. When the flow rate was increased from 10 to 15 L/hr, a marked increase in the extraction rate was observed and the oil yield after 120 min of extraction increased by 70.4%. Increasing the flow rate further to 20 L/hr, however, did not improve the extraction rate or oil yield any further. The maximum oil yield was achieved after about 40 min at a flow rate of 15 or 20 L/hr.

SC-CO_2 of sea buckthorn oil from whole berry, pulp, and seed Figure 5 shows the oil yield of the whole berries, pulp, and seeds of sea buckhorn extracted with supercritical CO_2 at 45 MPa, 60°C and a flow rate of 15 L/hr. The highest yield was obtained from the pulp, followed closely by whole berry while the seed gave the lowest yield. The maximum yield was achieved essentially after about 20 min of extraction for all 3 materials. The recovery of oil was 80.1% for the whole berry, 82.1% for the pulp, and 81.8% for the seed.

Results obtained in this study have shown that supercritical CO_2 technology can be successfully employed for extracting oils from the whole berry, pulp, and seed of sea buckthorn fruit. Under favorable conditions (e.g., 45 MPa, 60°C, and a CO_2 flow rate of 15 L/hr), oil recoveries of over 80% can be achieved in 1 extraction run. As expected, the extraction

rate increased with a rise in pressure, temperature, and CO_2 flow rate. However, a plateau in extraction rate was reached at 30 MPa, 50°C, and 15 L/hr, and further increases in the pressure, temperature, or flow rate achieved little improvement in extraction rate or oil yield. These results are in general agreement with the findings reported by previous researchers for cherry and pumpkin seed oils (11,12).

Fatty acid composition of sea buckthorn oils extracted by SC-CO_2 and *n*-hexane Table 1 presents a comparison of the fatty acid composition of the sea buckthorn oils extracted by SC-CO_2 at 45 MPa, 60°C, and a flow rate of 15 L/hr and by the conventional Soxhlet method using *n*-hexane. No major differences were found in the fatty acid composition of the oils extracted by the two different methods. The fatty acid compositions of the whole berry and pulp oils were similar to each other; however, they were markedly different from the fatty acid composition of seed oil. The fatty acids in the whole berry and pulp oils were dominated by MUFA, which accounted for more than 64% of the total fatty acids. Saturated fatty acids (SFA) were the second most abundant at about 31% while the content of polyunsaturated fatty acids (PUFA) was very low in the whole berry and pulp oils, accounting for less than 5% of the total fatty acids. The main MUFA in the whole berry and pulp oils were palmitoleic (about 39%), oleic (about 16.5%), and vaccenic (about 8.5%) acid. The SFA in the whole berry and pulp oils were predominantly palmitic acid (about 29%) with small amounts of stearic and myristic acid. The PUFA in the oils consisted of small amounts of linoleic and linolenic acid.

In contrast, fatty acids in the seed oil of sea buckthorn fruit were dominated by PUFA, which accounted for more than 61% of the total fatty acids in the oil. MUFA was the second most abundant fatty acids in the oil, accounting for slightly over 24% while SFA was the lowest, accounting for only about 13% of the total fatty acids. The main PUFA in the seed oil was linoleic acid at about 36%, followed by linolenic acid at about 25%. The major MUFA was oleic (about 14%), palmitoleic (about 7%), and vaccenic acid (about 3%). The main SFA in the seed oil was palmitic acid (about 10%), followed by a small amount of stearic acid (about 2%). The results were generally consistent with those reported by Yang and Kallio (4).

Content of tocopherols and carotenoids in sea buckthorn oils extracted by SC-CO_2 and *n*-hexane Table 2 shows the content of tocopherols and carotenoids in the sea buckthorn oils obtained by SC-CO_2 extraction, together with corresponding data obtained by *n*-hexane extraction. The content of total tocopherols was the highest in the seed oil, followed by the whole berry oil, while the content was lowest in the pulp oil. The tocopherols were dominated by

Table 1. Fatty acid composition (%) of oils extraction from the whole berry, pulp oil, and seed of sea buckthorn fruit by SC-CO_2 and *n*-hexane[1)]

	Whole berry		Pulp		Seed	
	SC-CO_2	*n*-Hexane	SC-CO_2	*n*-Hexane	SC-CO_2	*n*-Hexane
Myristic acid	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.3±0.0	0.2±0.0
Palmitic acid	29.4±0.2	28.8±0.4	29.9±0.3	30.0±0.2	10.7±0.4	10.9±0.2
Palmitoleic acid	39.3±0.4	38.8±0.3	39.9±0.6	39.8±0.3	7.1±0.2	7.2±0.1
Stearic acid	1.2±0.1	1.3±0.1	1.2±0.1	1.2±0.0	2.3±0.1	2.2±0.0
Oleic acid	16. 5±0.4	16.4±0.5	17.0±0.5	16.6±0.3	14.0±0.1	14.3±0.2
Vaccenic acid	8.5±0.2	8.5±0.1	8.9±0.3	8.5±0.2	3.2±0.0	3.1±0.1
Linoleic acid	2.2±0.1	2.3±0.1	1.2±0.1	1.5±0.2	36.8±0.3	36.0±0.5
Linolenic acid	1.4±0.1	2.1±0.3	0.7±0.0	1.3±0.1	25.0±0.1	25.1±0.1
MUFA	64.4±0.2	63.8±0.5	65.8±0.3	64.9±0.4	24.3±0.2	24.6±0.2
PUFA	3.6±0.1	4.4±0.1	1.9±0.0	2.8±0.1	61.8±0.3	61.2±0.1
UFA	67.9±0.3	68.2±0.4	67.7±0.3	67.7±0.2	86.1±0.4	85.8±0.3
SFA	30.8±0.2	30.4±0.1	31.4±0.2	31.5±0.2	13.2±0.2	13.3±0.4

[1)]Values represent average of triplicates±SD; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; UFA, saturated fatty acids; SFA, unsaturated fatty acids.

Table 2. Tocopherols (T) and carotenoids of the whole berry, pulp, and seed oil extracted by SC-CO_2 and *n*-hexane[1)]

	Whole berry		Pulp		Seed	
	SC-CO_2	*n*-Hexane	SC-CO_2	*n*-Hexane	SC-CO_2	*n*-Hexane
α-T	114.25±2.29	106.76±1.02	112.86±2.06	101.61±2.38	121.41±2.87	99.95±2.63
β-T	9.45±0.28	8.66±0.51	4.71±0.12	8.84±0.93	9.97±0.26	8.77±0.14
γ-T	6.36±0.21	6.47±0.48	3.10±0.06	4.06±0.12	28.22±1.35	24.66±1.95
δ-T	1.24±0.62	0.76±0.17	0.45±0.04	0.76±0.08	5.38±0.30	2.32±0.12
Total	131.29±2.26	122.65±1.37	121.12±2.22	115.28±3.11	164.97±4.56	135.70±2.99
Carotenoids	298.26±3.34	337.97±4.72	318.34±8.92	359.42±8.77	90.75±3.74	123.46±1.16

[1)]Values represent average of triplicates±SD (n=3).

α-tocopherol, irrespective of the source of the oil. For the whole berry and pulp oils, β-tocopherol was the second most abundant, followed by γ-tocopherol and small amounts of δ-tocopherol. For the seed oil, however, γ-tocopherol was the second most abundant, followed by β- and δ-tocopherols. In general, more tocopherols were found in oils extracted by SC-CO_2 except for the pulp oil, where more β-, γ-, and δ-tocopherols were extracted by hexane.

The content of caroteniods was the highest in the pulp oil, followed by the whole berry oil, while the seed oil had the lowest caroteniod content. More carotenoids were extracted by hexane than SC-CO_2, irrespective of the material used.

The health beneficial effects of sea buckthorn oil, such as preventing gastric ulcers, reducing the symptoms of atopic dermatitis, improving the immune functions, and the ability to lower low-density lipoprotein (LDL) cholesterol level and the risk of cardiovascular disease, have been well documented (6,23,24). Previous studies have focused on extracting oils from the seed and pulp of sea buckthorn fruit (18,19,22). The present study has shown that the whole berry of the sea buckthorn fruit contained more than double the amount of oil in the seed, and therefore is a much better source for extracting oil. Although the seed was low in oil yield, nutraceutical and pharmaceutical function of the oil appeared to be different from that of the whole berry and pulp oil. The fatty acids of the seed oil was dominated by PUFA (>60%), especially linoleic and linolenic acid, and was very low in SFA (about 13%). These results are generally consistent with those reported by Yang and Kallio (4) for the seed oil of sea buckthorn. On the other hand, the high PUFA content of the seed oil could make it unstable during processing and storage. The fatty acids of whole berry and pulp oil consisted mainly of MUFA (about 64%) and SFA (30%), which make them more stable although nutritionally inferior to the seed oil.

Although the content of tocopherols was slightly higher in the seed oil than in the whole berry and pulp oils, it may not be enough to protect the high concentration of PUFA in the oil from oxidation during long term storage. For long term storage, other protection methods such as addition of exogenous antioxidants may be necessary. The tocopherol profiles of the whole berry and pulp oils were similar to those reported for sea buckthorn (7). In contrast to the similar contents of tocopherols in the 3 different types of oils, the content of carotenoids in the pulp and whole berry oil was almost 3 times higher than that in the seed oil. This is not surprising as carotenoids, being the major pigments in many fruits, are mainly located in the pulp rather than the seed of the fruit. The much higher content of carotenoids in the whole berry oil would improve its nutritional value.

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