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Radical Scavenging Activity of Sea Buckthorn Oils from Different Parts of Sea Buckthorn Berry

Xiang Xu and Yanxiang Gao*

College of Food Science and Nutritional Engineering, China Agricultural University, Haidian District, Beijing 100083, PR China

Abstract Antioxidant-rich oils were extracted from different parts of sea buckthorn berry with supercritical CO_2 (SC-CO_2) and *n*-hexane. The functional components were analyzed and the extracts were screened for their potential as radical scavengers in 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 2,2'-azinobis-3-ethylbenzotiazoline-6-sulphonic acid (ABTS), galvinoxyl systems. Minor differences were found in fatty acid composition of oils extracted by the two methods. Seed oil contains the highest content of tocopherols while pulp oil and whole berry oil possessed higher concentration of carotenoids. Whole berry oil, pulp oil, and seed oil extracted by SC-CO_2 showed 91.7, 90.9, and 93.5% radical scavenging activity (RSA) at 6 mg/mL towards DPPH and 74.3, 54.3, and 74.8% towards galvanoxyl radical at 10 mg/mL. The hexane-extracted oils showed similar scavenging ability. However, the oils obtained by hexane showed significantly higher RSA ($p<0.05$) than those obtained by SC-CO_2 while whole berry oil has the highest RSA towards ABTS among 3 oil samples.

Keywords: sea buckthorn, whole berry oil, pulp oil, seed oil, radical scavenging activity

Introduction

Increasing attention has been paid to free radical biology and pathophysiology over the last few decades (1-3). Oxygen- and nitrogen-derived free radicals are generated during cellular metabolism and mitochondrial energy production. Meanwhile, the cell conserves highly specific antioxidant mechanisms to counteract the effects of free radicals and oxidants. Once the balance was broken, free radicals may damage important biological components in tissues and thus induce a series of health problems (4).

It has been a continuous effort to develop natural antioxidants for their potential applications in correcting the oxidative imbalance and preventing human illnesses. Natural antioxidants from nutraceuticals and phytoceuticals include carotenoids, vitamins, phenolic compounds, etc. Functional oils such as tea seed oil (5) and olive oil (6,7) containing those antioxidants have shown radical scavenging activities to protect the biological systems and they were proved to have the potential of lowering incidence of a series of diseases (8).

Sea buckthorn (*Hippophaë rhamnoides* L.) is a well known hardy plant widely distributed in Asia, Europe, and North America (9). The berries of sea buckthorn have been used as a raw material for foods and traditional medicines for a long time (10). Recently, the nutritional importance of the extract from the sea buckthorn berries has been increased in North America and Europe (11). Both seeds and the soft parts of sea buckthorn berry fruits are good sources of lipids and natural antioxidants including carotenoids, tocopherols, sterols, flavonoids, lipids, ascorbic acid, tanins, etc (12). These compounds possess biological activity including antioxidant, antitumoral, hepato-protective, and immunumodulatory properties (12-14). The tests expressing antioxidant potency can be categorized into *in vitro* and *in vivo*. Previous studies have showed that sea buckthorn oils extracted from the berries could slow down the oxidation process, stabilize membrane structure, and protect against chemically induced liver damage in animal models (14). The model of scavenging stable free radicals *in vitro* is widely used to evaluate the antioxidant properties in a relatively short time, as compared to test the ability to inhibit lipid oxidation under accelerated conditions (6,7).

Currently, supercritical CO_2 (SC-CO_2) extraction of plant oils received much attention as an alternative to the traditional solvent extraction. Although the differences in the fatty acid compositions of plant oils solvent and SC-CO_2 were minor (15,16), SC-CO_2 extraction of plant oils has so many advantages over traditional extraction methods, such as shorter extraction time, higher selectivity, and no solvent residue. So far, the free radical scavenging activity of sea buckthorn oils isolated from different parts of the sea buckthorn berries using different extraction methods has not been investigated.

Therefore, the aim of the present work is to evaluate the antioxidant properties of SC-CO_2 extracted and *n*-hexane extracted sea buckthorn oils in radical scavenging activity *in* different *vitro* tests including DPPH, ABTS, and galvinoxyl systems. The specific antioxidant activity associated with the radical scavenging ability will be tested.

Materials and Methods

Chemicals and reagents Stable free radical 1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 2,2'-azinobis-3-ethylbenzotiazoline-6-sulphonic acid (ABTS), galvinoxyl, standards of fatty acid methyl esters, tocopherols, β-carotene, and Trolox were purchased from Sigma-Aldrich (St. Louis, MO, USA). All the solvents (HPLC grade) were purchased from Merck and the other chemicals (analytical grade) from Beijing Chemical Co. (Beijing, China), unless otherwise stated.

*Corresponding author: Tel/Fax: +86-106-273-7034
E-mail: caugyx@126.com
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Preparation of oil samples Ripe berries of wildly grown sea buckthorn (*Hippophaë rhamnoides* L.), which were naturally dried on the trees, were collected from the Akeshu region in Xinjiang province, China and stored at –20ºC until used. The berries of sea buckthorn were divided into 2 portions: one portion was extracted for oil from the whole berries while the other portion of berries was separated manually into seeds and pulp to extract oils from the 2 parts of the fruit separately. Before extraction, the whole berries, pulp, and seeds were vacuum-dried to a moisture content of 6.0%, and then grounded to powder using a blender.

SC-CO_2 extraction was performed with the supercritical fluid extraction system (1-L sample capacity) purchased from Nantong Hua'an Co., Ltd. (Nantong, Jiangsu, China). Samples (300.0 g) were placed into the stainless steel tubular extractor with an internal diameter of 6.0 cm and a height of 35 cm. 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 ±0.5ºC, respectively. The flow rate of the CO_2 was regulated by adjusting the length of the pumping stroke. After each extraction, the oil was collected in the first separator while water and volatile components were recovered in the second one. The experiments were carried out at 45 MPa, 60ºC with CO_2 flow rate of 15 L/hr in 3 hr since longer extraction times did not significantly increase oil yield.

For Soxhlet extraction, 10 g of sample were weighted and dried at 103ºC. The dried sample was placed in a Soxhlet apparatus and then continuously run for 8 hr at 1 time using *n*-hexane (69ºC). After extraction, the solvent was evaporated by reduced pressure evaporation (30ºC) and the extract was dried at 103ºC to remove residual solvent until a constant weight.

Compositional analysis of crude oils Analysis of fatty acid methyl esters (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 μm film thickness, J&W Scientific, Folsom, CA, USA) according to the method described by Wenli *et al.* (17). The sample (1 μL) was injected with a split ratio of 100:1 and the inlet temperature was set at 280ºC. The oven temperature was initially set at 170ºC for 14 min, then increased to 250ºC at a rate of 10ºC/min and kept at this temperature for 8 min. The detector temperature was set at 300ºC. Nitrogen was used as the carrier gas with a flow rate of 1.0 mL/min. Identification of FAME was achieved by comparing their retention times with those of authentic compounds analyzed under exactly the same conditions. Tocopherols were determined by high performance liquid chromatography (HPLC) as described previously (18). Separation of tocopherols was carried out on HPLC system with an Agilent NH_2 column (5.0 μm, 250×4.6 mm i.d.) protected by a 10 mm guard column maintained at 30ºC. 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. Total carotenoids were assayed as described by Ranjith *et al.* (18) with β-carotene used as a standard. Total carotenoids were expressed as mg β-carotene equivalent/100 g oil. All the analysis was performed in triplicate.

DPPH radical scavenging effect Radical scavenging activity (RSA) and the presence of hydrogen donors in crude oils were examined by reduction of DPPH in toluene according to Ramadan *et al.* (19). For evaluation, 2 mL of crude oils (6 mg/mL) was mixed with 2 mL of toluenic solution of DPPH radicals and the mixture was vortexed for 20 sec at ambient temperature. Against a blank of pure toluene without DPPH, the decrease in absorption at 515 nm was measured after 1, 5, 10, 20, 30, and 60 min of mixing, using a UV-757 spectrophotometer (Shanghai Jingmi Equipment Co., Ltd., Shanghai, China). RSA toward DPPH radicals was estimated from the differences in absorbance of toluenic DPPH solution with or without sample.

Galvinoxyl radical scavenging effect The test was performed with an ER 200D-SRC electron spin resonance (ESR, Bruker, Berlin, Germany) according to Ramadan *et al.* (19). Experimental conditions were as follows: measurement at room temperature; microwave power, 10 db; centerfield, 3,385 G, sweep width, 200 G; receiver gain, 2×104; and modulation amplitude, 1 G. One mL of crude oils (10 mg/mL of toluene) was allowed to react with 1 mL of a toluenic solution of galvioxyl (0.25 mM).The mixture was shaken out on a vortex stirrer for 20 sec and then prepared for ESR analysis. The galvinoxyl signal of galvinoxyl radical was measured exactly 1, 5, 10, 20, 30, and 60 min after the addition of the galvinoxyl radical solution. The signal intensities were evaluated by the peak height of signals against a control. A quantitative estimation of the concentration of galvinoxyl was obtained by evaluating the decrease of the ESR signals during 60 min incubation.

ABTS radical scavenging effect The ABTS radical cation is a blue/green chromophore with characteristic absorption at 734 nm. It was performed as described previously (20) with some modifications. The ABTS radical cation was prepared by reacting a 7 mM aqueous solution of ABTS with 2.45 mM potassium persulfate (final concentration) and diluted in methanol to an absorbance of 0.70 (±0.02) at 734 nm. After addition of 3.0 mL of this solution to 1.0 mL aliquots of Trolox or oil samples (4 mg/mL of ethyl acetate), the absorbance was recorded at 20 min after initial mixing. Appropriate solvent blanks were run in each assay. The activities of sea buckthorn oils were expressed as mmol Trolox/100 g oil, the Trolox equivalent antioxidant capacity (TEAC), defined as the concentration (mmol/L) of Trolox having the equivalent antioxidant activity to a 1.0 mmol/L solution of the substance under investigation.

Statistical analysis To verity the statistical significance of the studied parameters, the values of mean±standard deviation (SD) were calculated. Where appropriate, the data were tested by two-way analysis of variance (ANOVA). A p value of less than 0.05 was considered as statistical significant.

Results and Discussion

Scavenging effect on DPPH radical Crude oils are usually more stable than their refined counterparts. A part of their oxidative stability depends on the fatty acid composition, the presence of minor fat-soluble bioactives. Antiradical properties of the crude oils in this study were compared by 3 selected methodologies using DPPH, galvinoxyl, and ABTS. Figure 1 and 2 show the absorbance of the DPPH radical with time in the presence of the oils tested. They show that sea buckthorn seed oil was the most reactive oil and thus had the highest RSA followed by whole berry oil and pulp oil. After 1 hr of incubation, 93.5% of DPPH radicals were quenched by the seed oil extracted by SC-CO_2, while whole berry oil and pulp oil extracted by SC-CO_2 were able to quench 91.7 and 90.9%, respectively.

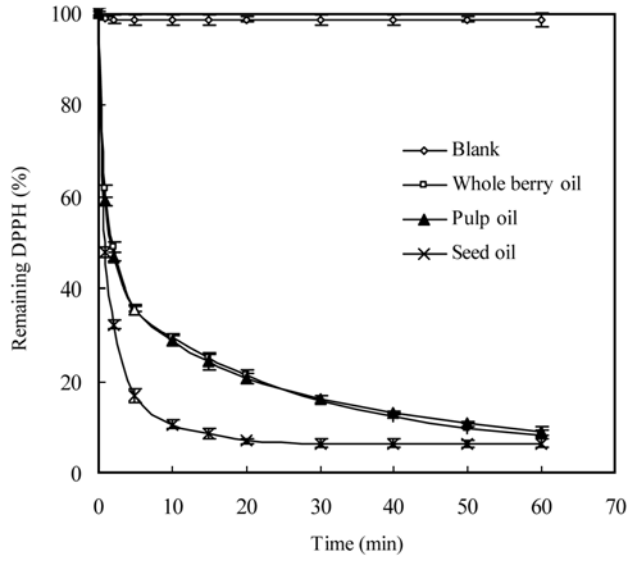

Fig. 1. Scavenging effect of whole berry oil, pulp oil, and seed oil extracted by SC-CO_2 on DPPH radical.

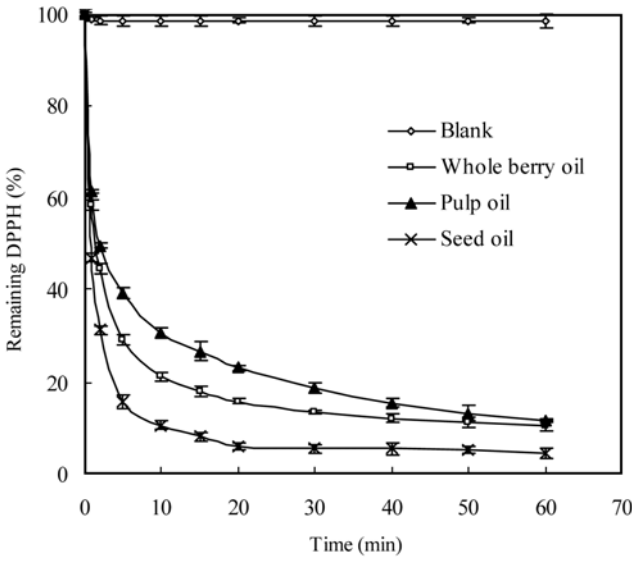

Fig. 2. Scavenging effect of whole berry oil, pulp oil, and seed oil extracted by *n*-hexane on DPPH radical.

The oils extracted by *n*-hexane also showed the same pattern, when seed, whole berry oil, and pulp oils quenched 95.5, 89.5, and 88.4% of DPPH radical, respectively. Scavenging effect on galvinoxyl radical Fig. 3 shows a typical sequence of ESR spectra measuring free RSAs. The intensity of the signal, measured in arbitrary units, decays with time and represents the radical-tissue reaction. Compared to DPPH spectrophotometric assays, ESR assays showed similar trends in the quenching of free radicals (Fig. 4 and 5). The whole berry oil, pulp oil, and seed oil extracted by SC-CO_2 showed 74.8, 74.3, and 54.3% towards galvanoxyl radical at 10 mg/mL. Whole berry oil possesses similar or slightly higher radical scavenging activity in the galvanoxyl test, while seed oil showed a remarkably higher scavenging activity either extracted by SC-CO_2 or *n*-hexane.

Scavenging effect on ABTS radical Table 1 shows the results obtained after measuring the antioxidant activity of different crude oil samples toward ABTS radical. The oils extracted by hexane showed significantly higher RSA ($p<0.05$) than those obtained by SC-CO_2. However, the data showed a different response for all samples compared to the other 2 radical reactions. It can be seen that whole berry oil has the highest value among the 3 kinds of oils, since different solvents were used to dissolve the crude oils and the free radicals which may cause differences in the antioxidant pattern between the groups of assays (21).

Antioxidant components in different oils The main functional components of the whole berry, pulp, and seed

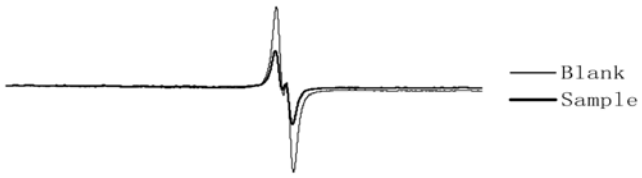

Fig. 3. ESR spectra with galvinoxyl radical.

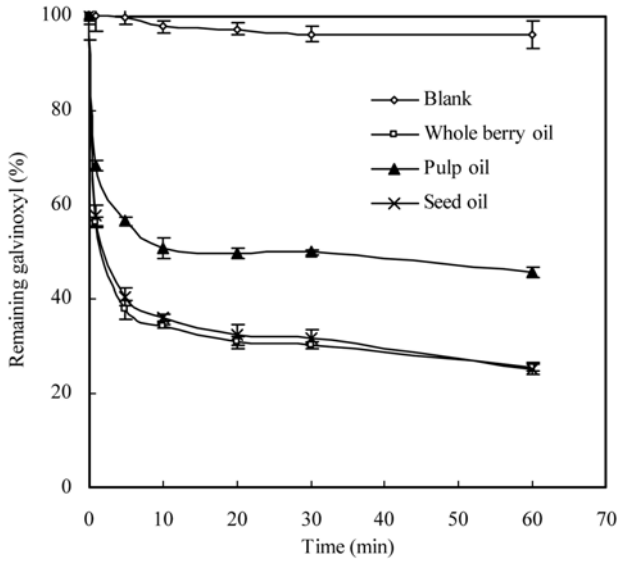

Fig. 4. Scavenging effect of whole berry oil, pulp oil, and seed oil extracted by SC-CO_2 on galvinoxyl radical.

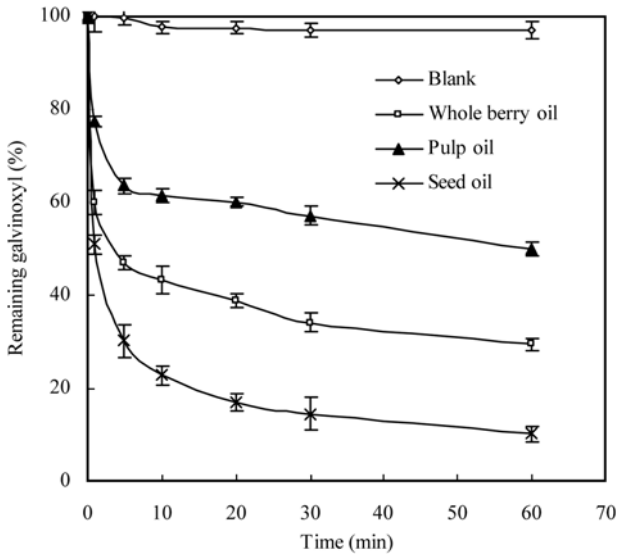

Fig. 5. Scavenging effect of whole berry oil, pulp oil, and seed oil extracted by *n*-hexane on galvinoxyl radical.

Table 1. TEAC values of whole berry, pulp, and seed oil extracted by different methods[1)]

	Whole berry oil	Pulp oil	Seed oil
SC-CO_2	1.614±0.079[c2)]	1.563±0.065[c]	1.204±0.043[d]
n-Hexane	2.199±0.155[a]	1.889±0.059[b]	1.482±0.064[c]

[1)](mmol Trolox/100 g oil), mean ±SD, n=3.
[2)][a-d]Means significantly difference among groups at $p<0.05$.

oil extracted by SC-CO_2 and *n*-hexane are reported in our previous study (22). No major differences were found in the fatty acid composition of the oils extracted by the 2 different methods. The fatty acid compositions of the whole berry oil and pulp oil were nearly the same to each other; however, they were quite different from the fatty acid composition of seed oil. The content of monounsaturated fatty acids was more than 64% of the total fatty acids, which dominated the fatty acids in the whole berry oil and pulp oil (22). Obviously, seed oil is more susceptible to oxidation. According to Ramadan *et al.* (19), the low RDA of niger seed oil was partly explained by the fact that niger seed oil has the highest level of polyunsaturated fatty acid. However, the results demonstrated that the effect of fatty acid profile of the oils in present study on the RSA was probably overwhelmed by the tocopherols and carotenoids scavenging effect.

It is accepted that both tocopherols and carotenoids can act as antioxidants by trapping free radicals. Moreover, combinations of carotenoids and tocopherols act synergistically (23). Different contents of them in the different oils lead to the diversity of their RSA.

From the above studies, it could be concluded that the whole berry oil, pulp oil, and seed oil of sea buckthorn either extracted by SC-CO_2 or hexane have radical scavenging ability in DPPH, ABTS, galvional model systems, and could be potential food radical scavengers. However, the results showed a different response to the ABTS radical for all the oil samples compared to the other 2 radical reactions. Natural antioxidants including tocopherols and carotenoids were abundant in those oils and acted as the main free radical trappers. Different contents of natural antioxidants in the oils extracted from different parts of whole berry lead to the diversity of their RSA. The results indicated that sea buckthorn oils from different parts of the whole berry could be exploited for their possible application as health supplements and neutraceuticals.

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