SUPPLEMENTARY MATERIAL

Evaluation of chemical constitute, fatty acids and antioxidant activity of the fruit and seed of sea buckthorn (*Hippophae rhamnoides* L.) grown wild in Iran

Keramatollah Saeidi^{a*}, Abolfazl Alirezalu^b & Zahra Akbari^c

^a *Department of Horticultural Science, Shahrekord University, Shahrekord, Iran. Tel:*

+984424401-8 Fax: +984424401-8

^b *Department of Medicinal & Industrial plants, Institute of Biotechnology, Urmia University, Urmia, Iran.*

^c *Department of Horticulture, Science and Research Branch, Islamic Azad University, Tehran, Iran*

* Corresponding author: E-mail addresses: saeidi@agr.sku.ac.ir & Ka.saeedi@gmail.com

Abstract

In this investigation the chemical compositions of berries from sea buckthorn were studied. The amount of ascorbic acid and β -carotene determined by HPLC were 170 mg/100 g FW and 0.20 mg/g FW, respectively. Total phenols, anthocyanins, acidity and TSS contents were 247 mg GAE/100g FW, 3 mg/L (cyanidin-3-glucoside), 5.32% and 13.8%, respectively. Fruit antioxidant activity determined by FRAP method was 24.85 mM Fe /100g FW. Results confirmed the presence of six dominant fatty acids (determined by GC) in fruit including linoleic (34.2%), palmitoleic (21.37%), palmitic (17.2%), oleic (12.8%), linolenic (5.37%) and stearic acid (1.67%). Five dominant fatty acids of the seeds were linoleic (42.36%), linolenic (21.27%), oleic (21.34%), palmitic (6.54%) and stearic acid (2.54%). The nitrogen content was 3.96%. The P, K, Ca, Mg, Fe, Zn, Mn, Cu, Cd and Cl contents of fruit were 491, 1674, 1290, 990, 291, 29.77, 108.37, 17.87, 0.021 and 2.18 mg/kg DW, respectively.

Keywords: Ascorbic acid, Fatty acid, Fruit, Sea buckthorn, Seed.

Experimental

Collection of fruit sample

Fruits collected at the ripening stage from Talegan region (semi-wet and cold climate , 36° 09' N, 50° 42' E and 1720 m elevation) on September 2013. Voucher specimen (number MPH-2237) has been deposited at the Herbarium of the Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran. The samples kept in the cooled bags for transferring to the laboratory and then stored in polyethylene bags at -20 °C prior to analysis.

Extraction and Determination of ascorbic acid and β -carotene

Extraction and determination of ascorbic acid and beta-carotene content was carried out by high performance liquid chromatography (HPLC, Knaure, Germany) using the method described by Daood et al. (1994) and Hudsan et al. (1997), respectively. C18 sep-pak cartridge (Merck) was used for ascorbic acid purification before injection to HPLC. Ascorbic acid analyses were performed by HPLC using Vertex Column, Prontosil 120- 3 C18 AQ (250×46 mm; dp=3 μ m), Knauer, Germany. The mobile phase was 15 mM KH₂PO₄ /Methanol (95/5 pH=2) at a flow rate of 1 ml/min, and the injection volume was 20 μ L. Separation was carried out at room temperature by UV–VIS detector (Knauer, model K2500) at 242 nm for ascorbic acid (calibration equation of standard for ascorbic acid was $Y=0.043x-1.718$, $R^2=0.999$).

β -carotene analyses were performed by using of Vertex Column, Eurospher 100-5 C18, (250 $\times$ 4 mm; dp = 3 μ m), Knauer, Germany. The mobile phase was acetonitrile/methanol/dichloromethane/n-hexane (50:40:5:5 V:V:V:V) at a flow rate of 1 ml/min. The injection volume was 20 μ L. Separation was carried out at room temperature by UV–VIS detector (Knauer, model K2500) at 450 nm (calibration equation of standard for β -carotene was $Y = 0.1568x + 0.057$, $R^2 = 0.997$).

Determination of total phenolic content, total anthocyanins, total acidity and TSS.

Total phenolic content of fruit was determined using the method described by Meda et al. (2005). The fruits were packaged in polypropylene plastic containers, and frozen at -20 °C for 24 h. The frozen samples were put in the freeze-drier (Scanvac, Denmark) for three days until they were completely dried (temperature < 0.01 °C and pressure < 4.6 mmHg). Briefly, 0.1 g of lyophilized powder of sample was dissolved in 1 ml of deionized water. The solution (0.1 ml) was mixed with 2.8 ml of deionized water, 2 ml of 2% sodium carbonate (Na_2CO_3), and 0.1 ml of 50% Folin-Ciocalteu reagent. After incubation at room temperature for 30 min, absorbance of the mixture was measured at 750 nm against a deionized water blank on a spectrophotometer (Jenway, 6505). Gallic acid was chosen as a standard. Results expressed as mg gallic acid equivalents (GAE) g^{-1} FW. For extraction of anthocyanin, samples were extracted using 1% (v/v) HCl in methanol and shaking in a shaker at 4 °C for 24 hours, and total anthocyanin content measured using the pH differential method. Absorbance of the extracts was measured at 510 nm and 700 nm in buffers at pH 1.0 and 4.5 with a molar extinction coefficient of cyanidine-3-glucoside. Results were expressed as mg L^{-1} cyanidin 3-glucoside (Lee et al. 2005). Total acidity measured by titration method. Total soluble solid of the samples (TSS) were determined by a digital refractometer (KRUS Co. Germany, HR Series) at 22 °C (AOAC 1995).

Antioxidant activity

Reagents: Solution (A): 0.3 M acetate buffer, pH 3.6 (3.1 g sodium acetate 3 H_2O and 16 ml acetic acid in 1000 ml buffer solution. Solution (B): 0.01 M 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCL. Solution (C): 0.02 M $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$ in distilled water.

FRAP working solution: 25 ml of solution (A), 2.5 ml of solution (B) and 2.5 ml of solution (C). The working solution was prepared freshly. For calibration, the aqueous solution of known amount of Fe (II) was used.

300 ml freshly prepared FRAP reagent was warmed at 37 °C and a reagent blank reading was taken at 593 nm; 10 ml of sample was then added, along with 30 ml H₂O. The reaction was monitored up to 5 min at 594 nm using a spectrophotometer (UV-VIS Biorad, USA), at 37 °C. The Fe (II) standard solution was tested in a parallel process (200 to 2000 µmol L⁻¹). Calculations were made by a calibration curve (Benzie and Strain 1996).

Determination of nitrogen and mineral elements

Total N was determined by the Kjeldahl method. To determine the mineral composition of the fruits, samples were burned with a nitric acid solution, on a hot plate, at 200 °C. Then, the absorbance of the extract was measured by an atomic absorption spectrophotometer (Shimadzu, aa-670). Phosphorus content was analyzed by determining the absorbance of yellow color, obtained from the Barton reaction, using a spectrophotometer (Jenway, 6505) at 680 nm, and comparing the results to standard curve (James 1995).

Oil Extraction

Oil extracted from 20 g seeds and fruits using hexane solvent in a Soxhlet apparatus for 6-7 h then solvent removed by a rotary evaporator (Heidolph, Hei-VAP Value; pressure <10 mb bar and temperature 40 °C) (AOAC 1989).

Determination of fatty acid composition by gas chromatography

The extracted seeds and fruits oil were methylated in the common procedure by refluxing with a methanolic sodium hydroxide solution, boron trifluoride reagent and heptane (AOAC 1995). The fatty acid composition of the esterified oil was characterized and quantified using Agilent 7890A Chromatograph. For this purpose BPX 70 (120m×0.25 mm) column was used. The injection volume was 10 µl. The injector and detector temperature were 220 °C and 250 °C, respectively.

Results

Table S1. Some chemical and mineral composition of sea buckthorn fruit

Ascorbic acid (mg/100 ml)	Beta-carotene (mg/g FW)	Total phenols content (mg GAE/g DW)	Total anthocyanin content (mg/L cyanidin-3-glucoside)	Antioxidant activity (mmol Fe ²⁺ /100g)	TSS (Brix)	Total acidity (%)
170 ± 1.3	0.20 ± 0.2	247 ± 1.91	7.1 ± 0.1	24.85 ± 0.35	13.8 ± 0.3	5.32 ± 0.09

Table S2. Mineral content of sea buckthorn fruit

N (%)	P (mg/kg)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Cd (mg/kg)	Cl (mg/kg)
3.96 ± 0.07	491 ± 4.8	1674 ± 10.1	1290 ± 7.4	990 ± 4.93	291 ± 1.8	29.77 ± 0.8	108.37 ± 1.41	17.87 ± 0.6	0.021 ± 0.01	2.18 ± 0.11

Table S3. Fatty acid composition of sea buckthorn fruit and seed

Sample	Palmitic (%)	Palmitoleic (%)	Stearic (%)	Oleic (%)	Linoleic (%)	Linolenic (%)
Fruit	17.2 ± 0.2	21.37 ± 0.6	1.67 ± 0.03	12.8 ± 0.09	34.2 ± 0.9	5.37 ± 0.31
Seed	6.54 ± 0.1	-	2.54 ± 0.05	21.34 ± 0.4	42.36 ± 0.9	21.28 ± 0.7

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