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Physicochemical and Fatty Acids Composition of *Barberry* Integerrima Seed

Atefe Tavakoli[1], Mohammad Ali Sahari[1]*, Mohsen Barzegar[1], Mohsen Asghari Ghajari[1]

1. Department of Food Science and Technology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.

Abstract:

In this study, chemical composition of *Barberry integerrima* seed and physicochemical properties and fatty acids composition of its oil were determined. The seeds contained 11.4 % oil and protein, moisture, crude fiber and ash contents were 17.0%, 19.1%, 48.8% and 2.9%, respectively. The main fatty acids recognized by gas chromatography were linolenic (ω-3), linoleic (ω-6) and oleic (ω-9) acid (38.3, 37.0, 15.5%, respectively). The density and refractive index of the extracted barberry seed oil were 0.821 and 1.4780, respectively. FFA (% as oleic acid), acid value (mg KOH/g oil), iodine value (g I_2/100 g oil), saponification number (mg KOH/ g oil), and unsaponifiable matter content (%) of the extracted oil from *Barberry integerrima* seeds were 0.70, 1.4, 180.0, 197.2, and 2.3, respectively. Color of extracted crude oil exhibited red unit 4.9, yellow unit 18.6 and blue unit 1.9, respectively. Total phenolics compounds (mg Gallic acid/kg oil), total tocopherols (mg/kg oil) and total sterols (mg/100g oil), were 323.0, 111.1 and 762.3, respectively. Specific extinctions at 232 nm (K_{232}) and 270 nm (K_{270}) and R-value (K_{232}/K_{270}) were 3.9, 2.2 and 1.8, respectively. The present study revealed that *Barberry integerrima* seed oil can be a valuable source of oil for food, pharmaceutics and cosmetics uses.

Corresponding Author: Mohammad Ali Sahari, Department of Food Science and Technology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran. P. O. Box: 14115-336, Tehran, Iran Tel: +98-21-48292328, Fax: +98-21-48292200, E-mail Addresses: sahari@madares.ac.ir or m.alisahari@gmail.com

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Introduction:

The healthful effects of minor components available in vegetable oils have become a topic of renewed interest in recent years (1). Nowadays, especially valuable vegetable oils (such as berry seed oils) are gaining attention owing to their health properties which are linked to their high value of antioxidants and polyunsaturated fatty acids (PUFAs).

Different types of barberry are popular around the world for various benefits such as medical, ornamental and food uses (2). The genus Berberis belongs to Berberidaceae family (3). Barberry is an evergreen and self-fertile plant (4) which is represented by about 12 genera and 600 species. *Berberis* L. is the major group of this family with around 500 species (3,5). However, many of them may be equivalent (6). Distribution of Barberries perhaps appeared before Palaeocene in Eastern Asia (7). The Asia (or Eurasia) and South America are two centers of variety of the genus (6,8,9). Popular barberry has Asian origin, that presumably relates to Western and Middle Asiatic mountains (10).

Iranian barberries are classified, according to their botany, into five wild species including Berberis Orthobotrys Bienert ex Schneid, *Berberis vulgaris* L., Berberis integerrima Bunge, Berberis crataegina DC., Berberis khorasanica Browicz and Zielinski (11-13).

The precedent of zereshk slew in Iran dates back to two centuries ago (14). In commercial scale, Iran is the only country that generates seedless crop (15,16). *B. integerrima* (abi) and *B. vulgaris* (poloei) are two important barberry species in Iran (17). Integrifolious barberry (Zereshk-e Abi in Persian syn. *B. integerrima*) is a very wide distribution in Iran (13,18). *B. Integerrima* is a spiny shrub with brittle branches to a altitude of 1 to 3 meters. This type of barberry has small, red and ellipsoid fruits, with a mild sour taste and 7-10 mm long and 3-4 mm diameter. There are two, three or even five small fusiform seeds inside the fruit (17).

The barberry's fruits are used as food flavoring (19). A lot of *Berberis* L. species are used to diminish insomnia (20), bronchial diseases, and urinary and gastrointestinal inconveniences, liver disorder and as an antibacterial, antifungal (21), antipyretic, (22), antirheumatic (23), agent in traditional medication. Compounds including berberine, chloride, oxyacanthine, palmatine chloride, quaternary protoberberines, berbamine, and bisbenzylisoquinoline alkaloids are the major constituents of this plant (21, 24-26). Berberine has multitude pharmacological activities, such as its hypotensive, the immune system stimulation, and sedative attributes; it applies some positives effects on activities of central nervous system, including protective effect in depression, cerebral ischemia, Alzheimer's, anxiety, mental depressionand schizophrenia, via increasing the value of norepinephrine, serotonin, dopamine or in the brain (27-29).

The chemical composition for the most common vegetable oils is well known, while, the information is little concerning these compounds in berry seed oils.

In the present work, cold-pressed and filtered oils from *Barberry integerrima* seeds are studied and composition of the whole seed and physicochemical properties of the crude oil were determined. In addition, valuable compounds in the oils' potential as functional foods were identified such as the fatty acid composition, including EFAs, total phenolic content, unsaponifiable matter and tocopherols fraction.

Materials and Methods:

Materials

Berberis integrrima fruits were purchased from garden of Firoozkuh in North-East of Tehran. For the preparation of materials, all waste substance including stones, thorns, branches and leaves from fruits were carefully removed. Then purged fruits were packed in plastic bags and stored at -80°C freezer (JalTeb Lab Equipment, Type J D 300 L) until use for testing. For oil

extraction, we needed to remove the excess water from the fruit; therefore, to diminish effects of drying process on extracred oil, it was dried in the oven at 50°C for 48 hours (30). Seeds removed from dried fruit manually and were ground by Moulinex grinder (Type DPA 1, CMMF 800W, France).

All solvents and chemicals used in this study were purchased from Sigma Aldrich (St. Louis, MO), Merck (Darmstadt, Germany) and Rankem (New Delhi, India).

Proximate Analysis of Seeds

Crude oil (Soxhlet), moisture, crude protein (Kjeldahl), fiber and ash content of *B. integerrima* fruit seed were determined using the (31), methods Ba 4a-38, Ba 2a-38, Ba 3-38, Ba 5a-49 and Ba 6-84, respectively. All determinations were done in 3 repetitions.

Oil Extraction

Barberry seed oil was extracted by the solvent method. In summary, for separating the flesh of dried fruits and grinding the seeds, the solvent (petroleum benzene) was added to the barberry seed powder in a closed container and shaken continuously; the ratio of solids to solvent was 1:4, at 25 ˚C for 16 h. Then, the solution was filtered and the remaining solvent got evaporated by using a rotary evaporator below a temperature of 60 ˚C. The pure oil was transferred into small vials and maintained at -30 ˚C until used for testing. The oil content of barberry seeds (g oil/100 g barberry seed) was calculated (32- 34).

Physico-Chemical Analysis of Crude oil

The extracted oils were analyzed for density and refractive index following AOCS official methods Cc 10a–25 and 89, respectively (31). Refractive index (RI) was measured by a refractometer (RX-7000a; Atago Co., Japan). Free fatty acid content and acid value were determined by titration methods, Ca 5a-40 and Cd 3d-63, respectively, defined in AOCS (31) Official Methods. Saponification number and iodine value were carried out according to the (35) Official Methods 920.160 and 920.158 (Hanus method), respectively. Determination of the unsaponifiable matters was measured by AOCS Method Ca 6a-40 (31). The color of oil was determined by a Lovibond Tintometer (Tintometer Ltd., Salisbury, UK) using a 1-inch cell. Specific extinctions (E1% 1cm) at 232 nm (K_{232}) and 270 nm (K_{270}) were determined according to the AOCS official method Ch5-91(31) using a UV-Vis Spectrophotometer (Model UVS-2100Shinco, Southern Korea).

Fatty acid composition

To prepare fatty acid methyl esters 0.1 g oil placed in a screw cap vial, and 1 mL hexane and 5 mL methanolic NaOH 0.5 N were added. After heating vial for 10 min in boiling water bath, it was allowed to cool to about the room temperature. Then, 2.175 mL of boron trifluoride as catalyst was added, and the vial was heated for 3minutes in boiling water bath. After that vial was cooled, 1 mL of saturated sodium chloride solution and 1mL hexane was added and was shaken completely. The vial was settled for 5 minutes and then aliquot (0.2 mL) of the top hexane layer was injected into the gas chromatograph (GC; Unicam 4600, UK) and was analyzed using the conditions defined by Metcalf *et al.,* (36).

Fatty acid profile of barberry seed oil was specified by GC equipped with a flame ionization detector. A BPX70 fused-silica capillary column with 30 m * 0.25 mm i.d * 0.22 μm film thickness, from SGE, Melbourne, Australia was used, with helium as the carrier gas (20 psi) to separate the fatty acids. A split injector at 250 ˚C and a FID at 270 ˚C were also used during the separation. The initial temperature of column was 160 ˚C for 6 min and rose temperature to 180 ˚C at a rate of 6˚C/min. After 9 minutes, heating rate enhanced to 20˚C/min until reaching to 200 ˚C. Fatty acids quantification was determined by the internal standard method (using C15 as an internal standard).

Total Phenol Contents

The determination of total phenolic content in *B. integerrima* seed oil was based on Fuentes *et al.,* (37)

method. Oil sample (2.5 g) was weighed, dissolved in 5 mL hexane. Phenolic compounds extracted with 3 mL of 60% (v/v) methanol/ water by vortex system for 2 minutes. Phases were separated by centrifugation (for 10 min at 3,500 rpm), and the hexane phase was extracted in the identical way, again. The methanolic extracts were pooled. An aliquot (0.2 mL) of the methanolic phase was removed and the final volume of its reached to 2.5 mL with water and then 0.25 mL of Folin–Ciocalteu reagent was added to solution and the solution was settled for 3 minutes at room temperature, then it was added by 0.5 mL sodium carbonate solution (35%, wt/vol). The solution was finally stirred and diluted with water to 5 mL. The resulted solution was maintained at room temperature for 2 hours. The absorbance of the final solution was measured at a wavelength of 725 nm. The calibration curve was prepared using standard solutions of Gallic acid within the range of 2–200 mg l^{-1}.

Determination of Sterols

Phytosterols were extracted from saponified oil sample as described by Dutta and Normén (38). Approximately 250 mg of oil and 100 mL α-cholestanol (1 mg/mL, internal standard) were refluxed with 5 mL of ethanolic KOH (6 g/100 mL) for 1 hr. The unsaponifiable material was extracted three times with 10 mL of hexane and the solvent was completely evaporated from the combined hexane fractions under a moderateflow of nitrogen. Trimethylsilyl (TMS) derivatives of the sterols were prepared by mixing 100 µL each of BSTFA+ 1% TMCS and pyridine, and heating at 60˚C for 30-60 min. After derivatization, 1 mL aliquots were manually injected into the gas chromatography. GC analysis was carried out on Agilent (Little Falls, DE, USA) gas chromatograph system equipped with an FID, a split-splitless injector and a Supelco TYM-5 (30m×0.25mm×0.25µm) capillary column. The velocity of the helium, as the carrier gas, was 20 cm/s. The temperatures of the detector and injector were 290 and 270 ◦C, respectively. The initial column temperature was 250 ˚C and increased at a rate of 2 ˚C/min to 300 ˚C and then held for 12 min.

Determination of Tocopherols

The tocopherol composition of barberry seed oil was measured based on the AOCS (31) Official Method Ce 8–89. Tocopherols were determined by using HPLC system (Shimadzu, Kyoto, Japan). The chromatographic system included a UV–Vis detector and a normal phase column (250* 46 mm i.d. and 5 µm particle size, Partisil Si, Hichrom, UK). Separation of all tocopherols was based on isocratic elution when the solvent flow rate was maintained at 1 mL/min. The solvent system selected for tocopherol elution was n-hexane/propanol (1:2, v/v) with detection at 292 nm. Prior to HPLC analysis, 2 g of seed oil was diluted with 100 mL of hexane, filtered (0.45 mm nylon syringe filter) and 20 mL sample was injected.

Determination of β-Carotene Content

The β-carotene content of the oil sample was analyzed according to the method of Lianhe *et al*., (39). Briefly, 5 mL of acetone-hexane solution (4:6, v/v) was added to 200 mg of the oil and vigorously shaken for 1 min. After that, the absorbance of the solution was determined at 453, 505, 645 and 663 nm. The amount of β-carotene in *B. integerrima* seed oil was calculated according to the following equation:

$$\beta\text{-carotene (mg/100 mL)} = 0.216 * A663 - 1.220 * A645 - 0.304 * A505 + 0.452 * A453$$

Statistical Analysis

The data presented are means of three replicates ± standard deviation. The data were analyzed using a one factor analysis of variance (ANOVA) and Means were compared by the LSD test with the Statistical Analysis System (SAS) software. Significance was accepted at 5% level. ($p < 0.05$).

Result and Discussion

Chemical Composition of Dried Barberry Seeds

The results of chemical characteristics of the

dried Barberry seeds are summarized in Table 1. There were no reports on physicochemical properties of *B. integerrima* seed prior to this research; therefore we had to compare these results with different barberry varieties. The oil content was found to be 11.4±0.7% (Table 1). This value is in the range reported for different *berry* species (4.9-32.8%) but it was lower than that reported for the Finnish berries (12-16%) (40). It has been claimed that different climatic conditions and genetic variation could be differences in the oil content (41). The seeds contained 19.1±0.1% of moisture. The moisture content for raspberry seed was reported 13.6% (42). The protein content 17.00 ± 0.1% was found in this study (Table 1). This amount in for *B. integerrima* seed less, whole *B. integerrima and B. vulgaris* were reported 1.4 %, 0.5 % and 0.1% respectively (17). It should be noted that these results are related to the barberry fruit (not their seeds). There are no reports on protein content of Barberry seed. Proteins are a major source of energy, essential amino-acids and for the main structural constituents of many natural foods (43). Total ash content (2.7 ± 0.0%) of *B. integerrima* seeds was higher than that obtained for whole *B. integerrima* and *B. vulgaris* (17). Ash content designation is important because it is an indicator of the quality of feeding substances used by animal feed manufacturer for poultry and cattle feeding (44). The composition of ash depends on agro weather and environmental conditions (45). Crud fiber content (48.8 ± 2.5) higher compared to 12.1% and 2.6% for whole *B. integerrima* and *B. vulgaris,* respectively (17). The high level of crude fiber can be due to the woody tissue of barberry seeds. Dietary fibers have effective physiological effects, such lethargy, cholesterol and blood glucose reduction. The beneficial effects of fibers on lipid metabolism are known (46).

Fatty Acids Composition of Barberry Seed Oil

Table 2 shows the fatty acid composition of the barberry seed oil, which can be used to determine its nutritional quality and stability. A higher level of unsaturation fatty acid (more than 91%) makes it more susceptible to oxidation. On the other hand, there are substantial data to recommend a diminution in saturated and a moderate increase in mono and poly unsaturated (n-3 and n-6) fatty acids in human feeding in order to prevent some diseases such as coronary heart disease (47). The fatty acids composition varies depending on various factors including variety, growing region, climate and maturity (48). According to the Table 2, three major fatty acids, namely, linolenic, linoleic and oleic were found in the *B. integerrima* seed oil. This unsaturated fatty acid constituted more than 91% of the total amount. Since the barberry seed oil has large amounts of linolenic and linoleic, it is a rich source of ω-3 and ω-6 fatty acid. n-6/n-3ratiointhisoilisclose to1 that is important from a nutritional point of view. According to other studies, linolenic, linoleic and oleic are the main fatty acids in other berry seed oil (49) but the amount of them is various with the oil studied here In oils studied linoleic acid was the most prevalent fatty acid (ranging from

Table 1. Physicochemical properties of *B. integerrima* seed.

Properties	Content* (%)
Oil	11.4±0.7
Moisture	19.1±0.1
Protein	17.0±0.1
Ash	2.7±0.0
Fiber	48.8±2.5

*Mean± standard deviation.

Table 2. Fatty acid composition of the *B. itegerrima* seed oil

Fatty acid	Content* (%)
Capric acid (10: 0)	0.1±0.0
Lauric acid (12:0)	0.1±0.0
Myristic acid (14: 0)	0.1±0.0
Palmitic acid (16:0)	5.9±0.7
Palmitoleic acid (16:1)	0.1±0.0
Stearic acid (18:0)	1.9±0.2
Oleic acid (18:1)	15.5±0.2
Linoleic acid (18:2)	37.0±0.1
Linolenic acid (18:3)	38.3±0.2
Arashidic acid (20:0)	0.1±0.0
Behenic acid (22:0)	0.1±0.0
Erosic acid (22:1)	0.1±0.0
SAFAs	8.4
MUFAs	15.7
PUFAs	75.3
n6/n3	1

*Mean ± standard deviation

35% to 62.8%) while, the percentages of linolenic was lower (ranging from 1.1% to 36.5%). Kiwi oil seed solely contained 17.3% C18:2 but it had a very high content of C18:3 (57.1%) which was in agreement with the results of present study. (42,50-53). The barberry seed oil contained about 8% saturated fatty acids, with the mainly one being palmitic acid (5.9%) and followed by stearic acid (1.9%). Also, the amount of other fatty acids in the barberry seed oil was very low, like to the results reported in the literature (53).

Physicochemical characterization of barberry seed oil

Results of some Physicochemical properties of the barberry seed oil are presented in Table 3. Physical

Table 3. Physicochemical characteristics of the *B. integerrima* seed oil.

Parameter	Value*
State at room temperature	Liquid
Density (g/cm^3)	0.821±0.004
Refractive index (30 ºC)	1.4780±0.0001
Acid value (mg KOH/g oil)	1.4 ± 0.02
Free fatty acid content (% as oleic acid)	0.7 ± 0.01
Iodine value (g of I_2/100 g oil)	180.0 ± 0.9
Saponification value (mg KOH/g oil))	197.2 ±2.0
Unsaponifiable matters content (% of oil)	2.3 ± 0.2
Total phenolics content (mg Gallic acid/kg oil)	323.0 ± 0.5
Specific extinctions at 232 nm (K_{232})	3.9±0.2
Specific extinctions at 270 nm (K_{270})	2.2 ± 0.1
R-value (K_{232}/ K_{270})	1.8 ± 0.0
Color	
Red unit	4.9
Yellow unit	18.6
Blue unit	1.9

*Mean ± standard deviation

attributes of lipids are determined by chemical structures and functional groups and greatly impress the roles of lipids in foods and the procedures required for their manipulation and processing The barberry seed oil was yellowish-brown in color and the state to be liquid at room temperature (25 ± 1 °C), even in a refrigerator. Density of the oil was 0.821 ± 0.004 g/cm^3. This value fell in the range reported for olive (0.910-0.920), coconut (0.908-0.921), rapeseed (0.910 -0.920), and canola (0.914- 0.920) oils (54).

Refractive index is used to measure theunsaturation changesduringhydrogenation ofoil or fat. The refractive index of oils depends on several factors such as their fatty acid chain length, molecular weight, degree of conjugation, and degree of unsaturation (54, 55). The barberry seed oil showed a refractive index of 1.4780 ± 0.0001 at 25°C, which was higher than that reported for pumpkin (56), sunflower, soybean and corn (57), palm, palm kernel and coconut oils (54). Pure oils have specified ranges of density and refractive index; thus, the amount of variation of a typical oil from its correct values may demonstrate its relative purity.

K_{232} and K_{270} are simple and helpful parameters for evaluation of the oil oxidation. K_{232} is usually considered as a indicatorof the primary oxidation products, conjugated dienes. K_{270}measures the presence of conjugated trienes, as secondary products of the oil oxidation, such ketones and aldehydes and the primary oxidation products of linolenic acid (58). in addition, both measurements have been used to specify the addition of an oil to pure ones (59). As shown in Table 3, the K_{232}, K_{270}, and R-value (K_{232}/K_{270}) of barberry seed oil were 3.9 ± 0.2, 2.2 ± 0.1 and 1.8, respectively. The K_{232}, K_{270}, and R-value for the Iranian pumpkin seed oil were reported by Gohari Ardabili *et al,* (56) 4.8, 3.52 and 0.74, respectively. K_{232} and K_{270} of the oils obtained from olive, sunflower, and the pumpkin were found to be 3.3 and 0.7, 4.9 and 0.5, and 8.9 and 2.0 , respectively (60).

The amount of free fatty acids and acid value provided information about the status of the vegetable oils. Free fatty acids more than intact fatty acids in the triglyceride are susceptible to lipid oxidation, which lead to rancidity, decrease smoke point and production of off-odor (61). The FFA% (as oleic acid) and acid value for *B. integerrima* seed oil was measured 0.7 ± 0.01 % and 1.4 ± 0.02 mg KOH/g oil, respectively. So, this oil is suitable for edible aims, since the amount of free fatty acid in it was lower than 1.2% (62) and as its acid value did not exceed the maximum limit of 4.0 (mg KOH/g oil) according to the New Zealand Food Regulation (1984) and Codex Alimentarius Commission (1999) (63).

Van Hoed *et al.*, (53) have studied five berry species seed oil, they reported all oils had a %FFA between 0.5 - 1.5%, as oleic acid equivalents. Acid value for pumpkin and groundnut reported 1.6 (64) and 2.8 (65), respectively.

The iodine value is an important characteristic that determines unsaturation, but does not specify the specific fatty acids (66). The *B. integerrima* seed oil had an iodine value of 180.0 ± 0. 9 (Table3). The high iodine value in *B.integerrima* seed oil as compared to corn (55), cottonseed, canola, rapeseed (54), tea seed, sunflower seed, and olive oils (67) was an indicative of the presence of a higher unsaturated bonds number.

The saponification value (SV) is an indicator of the average length of the Fatty acid. It is inversely proportional to the molecular weight of the lipid. According to Table 3, The saponification value of *B. integerrima* seed oil was high and close to the saponification value of olive oil (184-196) and sunflower (188-194) (54) and it was in good agreement with the values for palm (190-209), Pumpkin seed (174-197) (54) and sunflower (197) oil. However, it was higher than those reported for Rapeseed, canola (54), grape seed (42) and tea seed oils (67) and lower than 248-269 range reported by Nichols and Sanderson, (54) for coconut oil. Unsaponifiable matters in the vegetable oils are various nonglyceridic bioactive

materials containing aldehydes, pigments, hydrocarbons, alcohols, ketones, fat-soluble vitamins, and sterols that may happen naturally or may be formed during the process or degradation of oils (68). As depicted in Table 3, the content of unsaponifiable matters was 2.3 ± 0.2% for oil studied. It was in a close agreement with the 2.4% reported by Milovanović and Pićurić-Jovanović, (69) for coconut oil. However, it was higher than the values reported for cottonseed (70), oil bean seeds (71) groundnut and melon seed oils (69), and it fell in the values reported for the pumpkin seeds, rice bran (56) and palm kernel oils (71).

Lately, there has been an increasing notice to studying phenolic compounds from oilseeds, because they stand for potentially health-promoting matters and have industrial applications (72). These naturally occurring compounds are known to prevent lipid oxidation and have a great affect on the stability, sensitivity and nutritional attributes of oil and its products (73) As shown in Table 3, *B. integerrima* seed oil is a very rich source of phenolic compounds since its level (323.0 0.5 ± mg Gallic acid/kg oil) is much higher than those reported for various vegetable oils (Table 4). This is within the range reported by Basu *et al.,* (74) for olive oil.Color of extracted crude oil exhibited red unit 4.9, yellow unit 18.6 and blue unit 1.9, respectively. The *B. integerrima* seed oil was yellowish brown in color.

Sterols

Phytosterols, as natural matters of vegetable oils, have received special attention because of their ability to lower serum cholesterol contents in humans (75) as a result, the risk of heart diseases will be reduced. Phytosterols are also considered to have anti-inflammatory, anti-bacterial, antitumor and anti-ulcerative attributes (76) so their contribution to the value of *B.integerrima* containing products as a medicative nutraceutical is clear. The information is lacking about the content of phytosterols in *B.integerrima* seed oil. In this current study, the total sterol content was found to be 762.3 mg/100 g oil. The total sterol reported ranging from 404 – 692 mg/100 g oil content for five different berry seed oils (53) and phytosterols in cranberry seed oil was found 657 mg/100 g by Nawar (77). Compared with known vegetable oils, this value is higher than the sterol content of olive oil (102–206 mg/100 g) (78), sunflower seed oil (244– 455 mg/100 g) and soybean oil (184–409 mg/100 g), but lower than the amount in rice bran oil (1,055 mg/100 g) and corn oil (795–2,215mg/ 100g) (79).

As in most vegetable oils, the main sterol in this sample was β-sitosterol, (71.8%). In the *B. integerrima* seed oil, the other phytosterols were campesterol (15.8%), following the Δ5-avenasterol (7%) and stigmasterol (3.1%) (Table 5).

Tocopherols

The determination of tocopherol homologues in

Table 4. Comparison of phenolic content of *B. integerrima* seed oilwith other oils

Oil	Phenolic content*	Reference
B. integerrima seed	323.5	Present study
Soybean	14.8	(39)
Sunflower	24.8	(84)
Corn	12.6	(39)
Grape seed	34.4	(87)
Hemp	24.5	(39)
Pumpkin seed	24.6	(88)
Olive	300-500	(74)
Flax seed	11.4	(39)

*mg Gallic acid/kg oil

Table 5. Sterol content of *B. integerrima* seed oil

Phytosterol	Content (%)
β-sitosterol	71.8
Campesterol	15.8
Stigmasterol	3.1
Cholesterol	1.0
Sitostanol	0.7
Δ5-avenasterol	8.0
Δ7-avenasterol	0.4
Chlerosterol	0.8
Total sterol (mg/ 100 g oil)	762.3

the *B.integerrima* seed oil not only is important owing to their antioxidative effects, that provide some protection against oil oxidation by terminating free radicals, but also their positive nutritional effects in human metabolism as biological antioxidants (80). The total tocopherol content may depend on various growing, processing and storage situations, and consequently may vary significantly within a certain oil class (81). The major tocopherol in *B. integerrima* seed oil was the α isomer at 84.6% of the total tocopherol. As shown in Table 6, α- and γ-Tocopherol contents of the oil were 94.0 and 17.1 mg/100 g, respectively. β and δ-Tocopherol were not detected in the sample tested. The α-Tocopherol is the most important lipid-soluble antioxidant in human body (82). The content of α-tocopherol in *B. integerrima* seed oil was close to the level found in sea buckthorn seed oil and higher than the amount found in the raspberry, blackcurrant (49), blackberry, blueberry, cranberry, red raspberry seed oils (53), however, the level of γ-tocopherol in *B. integerrima* seed oil was lower than those reported for berry seed oils except crowberry and cranberry seed oils (49, 53).

β-Carotene is profitable for long term storage of oils since it is a secondary or preventive antioxidant acting as a singlet oxygen quencher (83). Furthermore, β-carotene has been shown to be an antioxidant and a precursor of retinoic acid, to enhance gap junction intercellular communication, and to increase the immunological function (74). In this study the amount of β -carotene content was 48.9 ± 0.2 mg/kg oil which is lower than those reported for palm seed (39), tea seed (84), olive and sesame seed oils (85, 86).

Conclusion

There is a growing consumer consciousness for

Table 6. Tocopherols content of *B. integerrima* seed oil

Composition	Content* (mg/kg)
α-Tocopherol	94.0 ± 0.8
β-Tocopherol	ND
γ-Tocopherol	17.1 ± 0.6
δ-Tocopherol	ND
β- carotene	48.9 ± 0.2

*Mean ± standard deviation

ND= not detected

healthy food products. The unique fatty acid profile, high phytochemical content and other worthwhile physicochemical characteristics reveal potential uses of *B. integerrima* seed oil in food, pharmaceutics and cosmetics industries. The oil of *B. integerrima* is rich in ω-3 and ω-6, phytosterols and phenolic compounds that is significant in nutritional aspect and can be considered as a renewable resource.

Reference

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