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Triacylglycerols, Glycerophospholipids, Tocopherols, and Tocotrienols in Berries and Seeds of Two Subspecies (ssp. *sinensis* and *mongolica*) of Sea Buckthorn (*Hippophaë rhamnoides*)

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Berries and seeds of two subspecies (ssp. *sinensis* and *mongolica*) of sea buckthorn (*Hippophaë rhamnoides* L.) were compared in terms of triacylglycerols, glycerophospholipids, tocopherols, and tocotrienols. The berries of ssp. *mongolica* contained less oleic acid (4.6 vs 20.2%, $p < 0.001$) and more palmitic (33.9 vs 27.4%, $p < 0.01$) and palmitoleic (32.8 vs 21.9%, $p < 0.05$) acids in triacylglycerols than those of ssp. *sinensis*. The proportions of linoleic acid (32.1 vs 22.2%, $p < 0.01$, in berries; 47.7 vs 42.7%, $p < 0.05$, in seeds) and palmitic acid (21.1 vs 16.4%, $p < 0.001$, in berries; 17.0 vs 14.1%, $p < 0.05$, in seeds) in glycerolphospholipids were higher in ssp. *mongolica* than in ssp. *sinensis*, and vice versa with oleic acid (4.3 vs 18.5% in berries, 10.0 vs 22.2% in seeds, $p < 0.001$). A higher proportion of α-linolenic acid was also found in the glycerophospholipids of ssp. *sinensis* berries (16.2 vs 10.1%, $p < 0.001$). α-, β-, γ-, and δ-tocopherols constituted 93−98% of total tocopherols and tocotrienols in seeds, and α-tocopherol alone constituted 76−89% in berries. The total contents of tocopherols and tocotrienols varied within the ranges of 84−318 and 56−140 mg kg^{-1} in seeds and whole berries, respectively. The seeds of ssp. *mongolica* were a better source of tocopherols and tocotrienols than those of ssp. *sinensis* (287 vs 122 mg kg^{-1}, $p < 0.001$). The compositional differences between the two subspecies should be considered when the berries are bred and exploited for nutritional purposes.

KEYWORDS: Sea buckthorn, *Hippophaë rhamnoides*; seeds; berries; fatty acids; tocopherols; tocotrienols; subspecies

INTRODUCTION

Sea buckthorn (*Hippophaë rhamnoides* L.) is a hardy bush with nutritious berries, naturally distributed in Central Asia and Europe (*1*). The species is classified into nine subspecies (*1*), of which ssp. *sinensis*, *mongolica*, and *rhamnoides* are most abundant and commercially interesting.

Sea buckthorn berries were originally used in Tibetan, Mongolian, and traditional Chinese medicines (*2*, *3*). In China and Russia, the berries have been used as a raw material for functional foods and medicines for decades (*2*, *3*). During the past few years, the nutritional importance of sea buckthorn berries has been increasingly recognized in Europe and North America (*4*−*9*). Sea buckthorn berry products are among the most popular functional foods in the United States, Canada, Finland, Germany, and some other European countries (*5*, *7*−*10*). The increasing industrial utilizations of the berries raise an urgent need for compositional information for selecting the best raw materials for food industry and for plant breeding.

One of the many special features of sea buckthorn is the exceptionally high oil content (10−40 g kg^{-1} of the fresh weight of the berry) in the fruit flesh (soft parts) surrounding the single seed in the berry (*5*, *11*−*14*). The oil content in seeds is ∼60−140 g kg^{-1} (*5*, *11*−*14*). The seed oil has been shown to lower plasma cholesterol levels, retard thrombus formation, and regulate immune functions (*2*, *3*, *15*, *16*). The oil from the whole berries and oil from the soft parts have been reported to inhibit platelet aggregation and promote the repair of injuries on skin and mucosa (*17*−*21*). Antioxidative (*22*) and anticarcinogenic (*23*) effects have also been reported for sea buckthorn seed oil.

Oils from the seeds and soft parts have different fatty acid compositions. The seed oil is rich in linoleic (18:2*n*−6), α-linolenic (18:3*n*−3), oleic (18:1*n*−9), and palmitic (16:0) acids, whereas oil from the soft parts is more saturated, being rich in palmitic and palmitoleic (16:1*n*−7) acids and poor in α-linolenic acid (*5*, *11*−*14*). Triacylglycerols (TAG) and

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glycerophospholipids (GPL) together, constituting 85−95% of the lipophilic fraction, carry most of the fatty acids in oils from seed and berries (*24*−*26*). The fatty acid compositions of TAG and GPL of the berries from different sources have not been thoroughly investigated.

Tocopherols and tocotrienols are important bioactive components in the berries (*27*, *28*). The contents of these compounds are among the crucial criteria defining the quality of the seeds, berries, and oils.

In an earlier study (*14*), we found significant differences between ssp. *rhamnoides* and ssp. *sinensis* in the fatty acid compositions of seeds and berries. The aim of the present investigation was to compare the contents and fatty acid compositions of TAG and GPL, tocopherols, and tocotrienols in seeds and berries of ssp. *sinensis* from China and of five commercial cultivars of ssp. *mongolica* from Russia.

MATERIALS AND METHODS

Berries. Five samples of wild sea buckthorn (*Hippophaë rhamnoides* L. ssp. *sinensis*) berries were collected from different provinces (Shanxi, Qinghai, Ningxia, and Gansu) of China. Berry samples were also picked from five cultivars of *H. rhamnoides* L. ssp. *mongolica* (Ruet, Luchezarnaya, Dar Katuni, Vitaminaya, and Maslichnaya) grown in Novosibirsk, Russia. The berries were picked in the autumn of 1997 and loosely frozen in sealed plastic bags in order to avoid desiccation and external moisture condensation. Berries (500 g) of each sample were taken from a 5 kg lot using a sample partitioning procedure. The seeds were isolated from thawed berries by pressing the juice and rinsing the residue with distilled water. The press residue was dried at room temperature, and the seeds were separated mechanically. For whole berry analysis, frozen berries were freeze-dried (Dura-Top bulk tray dryer, FTS System Inc., Stone Ridge, NY) to 15−30% of their original weight, depending on the berry composition.

Lipid Extraction. Samples (1 g) of seeds and freeze-dried whole berries were crushed separately in a mortar in liquid nitrogen and the lipids isolated using a methanol/chloroform extraction procedure (*14*, *29*). The sample was homogenized in methanol (10 mL) for 1 min in a blender, chloroform (20 mL) added, and homogenization continued for a further 2 min. The mixture was filtered, and the solid residue was resuspended in chloroform/methanol (2:1, v/v, 30 mL) and homogenized for 3 min. The mixture was filtered again and washed with fresh solvent (chloroform/methanol, 2:1, v/v, 30 mL). The combined filtrates were transferred into a measuring cylinder, one-fourth of the total volume of 0.88% potassium chloride water solution was added, and the mixture was shaken thoroughly before being allowed to settle. The lower layer was removed and washed with one-fourth of its volume of methanol/water (1:1, v/v). The washing procedure was repeated, and the bottom layer containing the purified lipids was filtered before the solvent was removed on a rotary film evaporator. Lipids were stored in chloroform at −20 °C until analyzed.

Isolation of TAG and GPL from Extracted Total Lipids. The lipids were fractionated into different fractions on silica Sep-Pak cartridges (Waters Corp., Milford, MA) (*14*). Trinonadecanoylglycerol and dinonadecanoylphosphatidylcholine were added as internal standards before fractionation. After elution of hydrocarbons, steryl esters, and wax esters with hexane/methyl *tert*-butyl ether (MTBE) (200:2, v/v, 10 mL), the TAG were eluted with hexane/MTBE (96:4, v/v, 20 mL). The column was then acidified with hexane/acetic acid (100:0.2, v/v, 15 mL); free fatty acids, monoacylglycerols, and diacylglycerols were eluted with MTBE/acetic acid (100:0.2, v/v, 12 mL), and GPL was eluted with MTBE/methanol/ammonium acetate (5:8:2, v/v/v, 20 mL).

Fatty Acid Analysis and Calculation of TAG and GPL Content in Seeds and Berries. TAG and GPL were transesterified into fatty acid methyl esters (FAME) with a sodium methoxide catalysis method (*30*). The FAME were analyzed with a Perkin-Elmer AutoSystem gas chromatograph (*14*). A silica capillary GC column NB-351 (stationary phase, nitroterephthalic acid modified polyethylene glycol, $L = 25$ m, i.d. $= 0.32$ mm, $d_f = 0.2$ μm) was used for GC analysis (HNU-Nordion Ltd., Helsinki, Finland). The flow rate of the carrier gas helium was 1.7 mL/min, and the split valve with a ratio of 1:40 was opened after 1 min. The temperature program was initially held for 2 min at 120 °C, then increased at a rate of 3 °C/min to a final temperature of 230 °C, and held for 20 min. The injector temperature was programmed from 170 to 250 °C at a rate of 200 °C/min and held for the rest of the run. The FID detector temperature was 270 °C. FAME were identified by comparison with a standard mixture of known composition (68D, NuChek Prep, Elysian, MN). The quantification of each fatty acid was carried out by comparing the peak of its methyl ester with that of methyl nonadecanoate without application of any correction factor. The fatty acid compositions of TAG and GPL were expressed as molar percentages of the fatty acids. The TAG and GPL contents in seeds and berries were calculated using an average molecular weight of fatty acids of the fraction in question. Phosphatidylcholine and phosphatidylethanolamine (3.5:1 in seeds and 2.5:1 in berries, according to our unpublished results) were the two GPL classes used in the calculation. The contents of TAG and GPL were expressed as weight percentages in seeds and frozen berries (based on fresh weight).

Table 1. Content of Seeds in Frozen Berries and Oil Content in Seeds and Frozen Berries (Grams per Kilogram of Fresh Weight)

		seed content in frozen berries		oil content in seeds		oil content in frozen berries	
ssp.	*n*	mean	SD	mean	SD	mean	SD
sinensis	5	85*[a]	29	97	2	41	28
mongolica	5	40	12	126*	23	59	27

[a] An asterisk indicates $p < 0.05$ between the two subspecies.

Analysis of Tocopherols and Tocotrienols. The oils from seeds (1 g) and freeze-dried whole berries (1 g) were dissolved in 3 mL of hexane, and D,L-tocol was added as internal standard (concentration in the final solution = 10 μg/mL). Tocopherols and tocotrienols were analyzed with normal-phase HPLC. The instrumentation was a Shimadzu LC-10AT equipped with a Shimadzu SIL-10A autoinjector, CTO-10A oven, and RF-530 fluorescence detector (Shimadzu Corp., Kyoto, Japan). The excitation wavelength was 295 nm and the emission wavelength 330 nm. The column was a Merck LiChroCART 250-4, Superspher Si 60 connected with a guard column Merck LiChroCART 4-4, Lichrospher Si 60. The sample injection volume was 20 μL. The tocopherols and tocotrienols were eluted at 30 °C with the eluting solvents programmed as follows: 0−5 min, 92% hexane/8% diisopropyl ether; 5−30 min, programmed change of eluting solvent from 92% hexane/8% diisopropyl ether to 83% hexane/17% diisopropyl ether; 30−35 min, 83% hexane/17% diisopropyl ether. The identification of individual peaks was carried out by co-injection with standard compounds. The quantification was carried out with internal standard tocol and corrected with specific correction factors

Statistical Analysis. Data analysis was carried out with SPSS 10.0 for Windows. An independent-samples *t* test was used to compare the differences between the two subspecies. Difference levels at $p < 0.05$ were taken as statistically significant.

RESULTS AND DISCUSSION

Seed and Oil Content in Berries. Table 1 presents the contents of seeds in berries and the oil content in seeds and berries [based on fresh weight (fw)]. The seed content in fresh berries varied from 50 to 129 g kg^{-1} in ssp. *sinensis* and from 26 to 59 g kg^{-1} in ssp. *mongolica*. The average value of ssp. *sinensis* (85 g kg^{-1} of fw) was higher ($p < 0.05$) than that of ssp. *mongolica* (40 g kg^{-1} of fw). The oil content in ssp. *mongolica* seeds (101−165 g kg^{-1}) was higher than that of ssp. *sinensis* (94−100 g kg^{-1}), the average values being 126 and 97 g kg^{-1}, respectively, in the two subspecies ($p < 0.05$). The ssp. *mongolica* seeds were bigger than the ssp. *sinensis* seeds (weight of 100 seeds, 2.2 vs 0.7 g, $p < 0.001$). Thus, the results deviate from the observed phenomenon in berries of the genera

Table 2. Contents of Triacylglycerols (TAG) and Glycerophospholipids (GPL) in Berries and Seeds of the Two Subspecies (Grams per Kilogram of Fresh Weight; $n = 5$ in Each Subspecies)

	berry				seed			
	ssp. *sinensis*		ssp. *mongolica*		ssp. *sinensis*		ssp. *mongolica*	
	mean	SD	mean	SD	mean	SD	mean	SD
TAG	32.2	22.0	47.9	21.7	83.5	12.8	83.1	32.4
GPL	2.5	1.3	1.8	0.5	9.5	2.1	9.8	1.2
TAG + GPL	34.7	23.3	49.7	22.2	93.0	14.0	92.9	31.6
GPL/TAG	0.08**[a]	0.02	0.04	0.01	0.11	0.02	0.13	0.06

[a] Two asterisks indicate $p < 0.01$ compared with ssp. *mongolica*.

Ribes, *Rubus*, and *Arctostaphylos*, according to which the smaller the seeds within one genus, the higher the oil content (*31*, *32*).

Oil content of the whole fresh fruits varied from 20 to 105 g kg^{-1} in the analyzed samples. Due to the high deviation, the two subspecies did not differ statistically, the average in ssp. *mongolica* being 59 g $kg^{-1}$and that in ssp. *sinensis* being 41 g kg^{-1} ($p = 0.346$).

TAG and GPL Contents in Seeds and Berries. The TAG and GPL contents in berries and seeds in the two subspecies are summarized in **Table 2**. The major lipid class in sea buckthorn berries and seeds was TAG, constituting ~80% of the whole berry oil. The value reported in the literature is within the range of 70−90% (*16*−*18*). The absolute content of TAG varied from 15 to 85 g kg^{-1} in the analyzed samples. The average TAG contents of ssp. *mongolica* (48 g kg^{-1}) and ssp. *sinensis* (32 g kg^{-1}) did not differ statistically ($p = 2.90$) (**Table 2**). The GPL content in the soft parts was extremely low, reflected as the low GPL level in the whole berries, 2.5 g kg^{-1} in ssp. *sinensis* and 1.8 g kg^{-1} in ssp. *mongolica*.

The TAG content in the seeds (58−125 g kg^{-1} in the analyzed samples) showed less deviation than in whole berries. The average TAG content of the seeds of ssp. *sinensis* and *mongolica* were practically identical, 84 and 83 g kg^{-1}. The GPL contents were also quite stable, 9.5 and 9.8 g kg^{-1} in ssp. *sinensis* and *mongolica* seeds, respectively.

The proportion of TAG in the lipophilic fraction in seeds was higher in ssp. *sinensis* (86%) than in ssp. *mongolica* (66%) ($p < 0.05$) according to the total oil content and TAG content in the present study. A statistically significant difference between the two subspecies was also found in the GPL/TAG ratio in the whole berries ($p < 0.01$), 0.08 in ssp. *sinensis* and 0.04 in ssp. *mongolica* (**Table 2**). These results may be regarded as a chemotaxonomic difference between the two subspecies.

Fatty Acids. The results of fatty acid analysis of TAG in the whole berries and seeds of both subspecies are shown in **Table 3**. A striking feature of the whole berries was the high relative level of the two $n-7$ monounsaturated fatty acids, palmitoleic and *cis*-vaccenic (18:1$n-7$) acids. Palmitoleic acid constituted 15−40% of the total fatty acids of whole berries. The relative content of oleic acid in ssp. *mongolica* berries was only 5%, whereas the corresponding value in ssp. *sinensis* was 20% ($p < 0.001$). The proportions of palmitic acid (34 vs 27%, $p < 0.01$) and palmitoleic acid (33 vs 22%, $p < 0.05$) were higher in the ssp. *mongolica* berries (**Table 3**).

The major fatty acids in seed TAG were linoleic, α-linolenic, oleic, and palmitic acids. The proportion of palmitoleic acid was <0.5% in seed TAG. The only significant difference between the two subspecies was found in the proportion of a minor fatty acid, stearic acid (3 vs 2% in ssp. *mongolica* and *sinensis*, respectively, $p < 0.01$). Standard deviations in all major fatty acids were low in both subspecies, indicating the extreme genetic uniformity of the composition of the seed storage fat.

Table 3. Fatty Acid Composition of Triacylglycerols (TAG) in Berries and Seeds of the Two Subspecies (Molar Percentage; $n = 5$ in Each Subspecies)

	berry				seed			
fatty acid	ssp. *sinensis*		ssp. *mongolica*		ssp. *sinensis*		ssp. *mongolica*	
	mean	SD	mean	SD	mean	SD	mean	SD
16:0	27.4	3.1	33.9**[a]	2.1	9.0	0.7	8.6	0.9
16:1*n*−7	21.9	4.3	32.8*	6.0	tr[b]		tr	
18:0	1.5	0.4	1.2	0.2	2.2	0.4	3.3**	0.5
18:1*n*−9	20.2***	3.7	4.6	1.1	22.4****	3.7	17.9	3.2
18:1*n*−7	6.2	0.9	6.4	0.8	2.0	0.3	2.1	0.2
18:2*n*−6	13.2	5.3	15.5	3.0	35.4	3.0	38.6	3.9
18:3*n*−3	9.7****	3.8	5.6	1.8	29.0	3.2	29.1	4.1
total	100		100		100		100	

[a] Asterisks: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $0.05 < p < 0.1$ between the two subspecies. [b] tr, <0.5%.

Table 4. Fatty Acid Composition of Glycerophospholipids (GPL) in Seeds and Berries of the Two Subspecies (Molar Percentage; $n = 5$ in Each Subspecies)

	berry				seed			
fatty acid	ssp. *sinensis*		ssp. *mongolica*		ssp. *sinensis*		ssp. *mongolica*	
	mean	SD	mean	SD	mean	SD	mean	SD
16:0	16.4	0.6	21.1***[a]	0.7	14.1	0.5	17.0*	7.8
16:1*n*−7	15.9	2.3	21.5	7.0	tr[b]		tr	
18:0	1.6	0.5	2.4	0.8	3.3	0.7	6.0***	10.3
18:1*n*−9	18.5***	3.5	4.3	1.5	22.2***	3.4	10.0	1.3
18:1*n*−7	9.2	0.8	8.5	0.5	4.7	0.4	4.3	3.7
18:2*n*−6	22.2	3.8	32.1**	3.4	42.7	2.5	47.7*	3.4
18:3*n*−3	16.2***	1.5	10.1	1.8	13.0	1.7	14.8	23.0
total	100		100		100		100	

[a] Asterisks: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ between the two subspecies. [b] tr, <0.5%.

GPL fatty acids in the berries and seeds (**Table 4**) of the two subspecies differed significantly from each other. In the whole berries, oleic acid (19 vs 4%) and α-linolenic acid (16 vs 10%) were higher in the ssp. *sinensis* ($p < 0.001$), whereas palmitic acid (21 vs 16%, $p < 0.001$) and linoleic acid (32 vs 22%, $p < 0.01$) were more abundant in the cultivars of ssp. *mongolica*.

In seed GPL, palmitic (17 vs 14%, $p < 0.05$), stearic (6 vs 3%, $p < 0.001$), and linoleic (48 vs 43%, $p < 0.05$) acids were more abundant in ssp. *mongolica*, whereas oleic acid was more abundant in ssp. *sinensis* (22 vs 10%, $p < 0.001$). The proportion of palmitoleic was <0.5%.

Tocopherols and Tocotrienols in Seeds and Berries. Figure 1 presents example chromatograms of tocopherols and tocotrienols in whole berries and seeds. The berries and seeds showed clearly different profiles even though all four tocopherol isomers were always present. β-Tocotrienol was the major trienol both in berries and in seeds, whereas α- and γ-tocotrienols were detected only in trace amounts in whole berries.

The proportions of individual tocopherols and tocotrienols in whole berries and seeds of the two subspecies are shown in **Figure 2**. In whole berries, α-tocopherol constituted 75.7−89.2% of the total analytes, the rest being γ-tocopherol (4.0−10.8%), β-tocopherol (2.4−12.2%), δ-tocopherol (0.3−2.4%), β-tocotrienol (0.4−4.8%), α-tocotrienol (0.4−3.2%), and γ-tocotrienol (0.6−2.5%). α-Tocopherol (17.2−66.1%), γ-tocopherol (25.3−55.8%), β-tocopherol (5.0−13.8%), δ-tocopherol (1.7−10.7%), and β-tocotrienol (1.9−7.6%) were the major compounds in seeds.

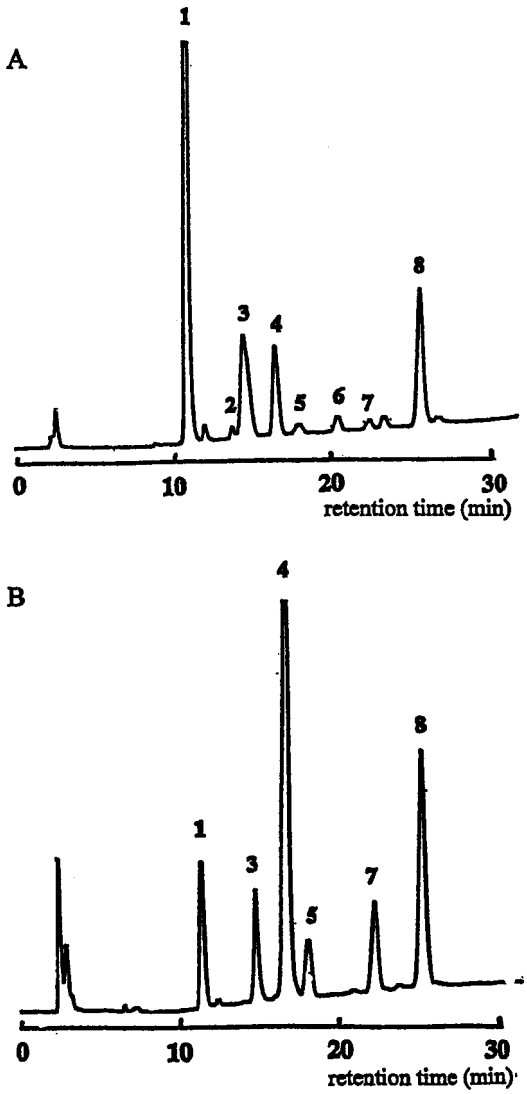

Figure 1. Chromatograms of tocopherols and tocotrienols in whole berries (A) and seeds (B) of sea buckthorn collected in Ningwu County, Shanxi Province, People's Republic of China (*H. rhamnoides* L. ssp. *sinensis*): 1, α-tocopherol, 10.95 min; 2, α-tocotrienol, 14.08 min; 3, β-tocopherol, 15.00 min; 4, γ-tocopherol, 16.83 min; 5, β-tocotrienol, 18.35 min; 6, γ-tocotrienol, 20.72 min; 7, δ-tocopherol, 22.66 min; 8, tocol (internal standard), 26.07 min. In the chromatogram of berries, the peak of β-tocopherol overlapped with an unknown peak.

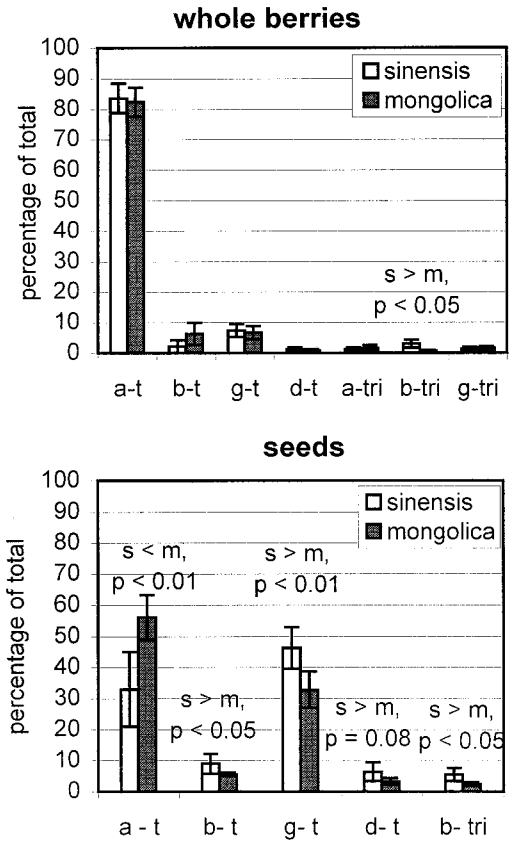

Figure 2. Proportions (mean ± standard deviation) of individual compounds of total tocopherols and tocotrienols in whole berries and seeds of the two subspecies: a-t, α-tocopherol; b-t, β-tocopherol; g-t, γ-tocopherol; d-t, δ-tocopherol; a-tri, α-tocotrienol; b-tri, β-tocotrienol; g-tri, γ-tocotrienol; s, ssp. *sinensis*; m, ssp. *mongolica*.

The only significant difference between the whole berries of the two subspecies was found in the proportion of β-tocotrienol (3.0% in ssp. *sinensis* vs 0.7% in ssp. *mongolica*, $p < 0.05$). The proportion of α-tocopherol of all tocopherols and tocotrienols in seeds was significantly higher in ssp. *mongolica* than in ssp. *sinensis* (56.1 vs 32.9%, $p < 0.01$), and vice versa with all of the other compounds (**Figure 2**).

Figure 3 presents the absolute levels of tocopherols and tocotrienols in fresh whole berries and seeds. Their total contents varied within the ranges of 56−140 mg kg^{-1} in whole berries and 84−318 mg kg^{-1} in seeds. The levels of α-, β-, and γ-tocopherols as well as total tocopherols and tocotrienols were significantly higher in seeds of ssp. *mongolica* compared with those of ssp. *sinensis* (160 vs 42 mg kg^{-1}, $p < 0.001$; 16 vs 10 mg kg^{-1}, $p < 0.01$; 95 vs 56 mg kg^{-1}, $p < 0.05$; 287 vs 122 mg kg^{-1}, $p < 0.001$; respectively) (**Figure 3**). Seed oil contained 872−2910 mg kg^{-1} tocopherols and tocotrienols, the average level in oil of ssp. *mongolica* being significantly higher than that of ssp. *sinensis* (2310 vs 1300 mg kg^{-1}, $p < 0.01$). The content of α-tocopherol in seed oil of ssp. *mongolica* (1294 mg kg^{-1}) was also higher ($p < 0.001$) compared with that of ssp. *sinensis* (444 mg kg^{-1}).

In whole berries, the level of the major compound, α-tocopherol, varied from 43 to 116 mg kg^{-1} with high deviation within both subspecies. The only significant difference found between the two subspecies was in the level of β-tocotrienol (2.3 mg kg^{-1} in ssp. *sinensis* vs 0.7 mg kg^{-1} in ssp. *mongolica*, $p < 0.01$). In the oil of whole berries, the total contents of tocopherols and tocotrienols were 2330 and 1640 mg kg^{-1} in ssp. *sinensis* and *mongolica*, respectively, the difference being close to statistically significant ($p = 0.08$). Analogously, the level of α-tocopherol in berry oil of ssp. *sinensis* was higher than that of ssp. *mongolica* (1940 vs 1360 mg kg^{-1}, $p = 0.06$).

Tocopherols and tocotrienols, commonly known as vitamin E, are the major lipid-soluble, membrane-localized antioxidants in humans. Deficiency of these compounds affects many tissues in mammalian and bird models (*33*). Vitamin E deficiency in man causes defects in the developing nervous system of children and hemolysis in man (*34*). Epidemiologic studies suggest that people with lower vitamin E and other antioxidant intake and plasma levels may be at increased risk for certain types of cancer and for atherosclerosis (*35*−*37*). It is also suggested that supplementation with antioxidants may decrease the risk of these and other degenerative processes (*38*).

α-Tocopherol is the most efficient antioxidant of these compounds. β-Tocopherol has 25−50% of the antioxidative activity of α-tocopherol, γ-tocopherol 10−35%, and α-tocotrienol ∼30% (*39*).

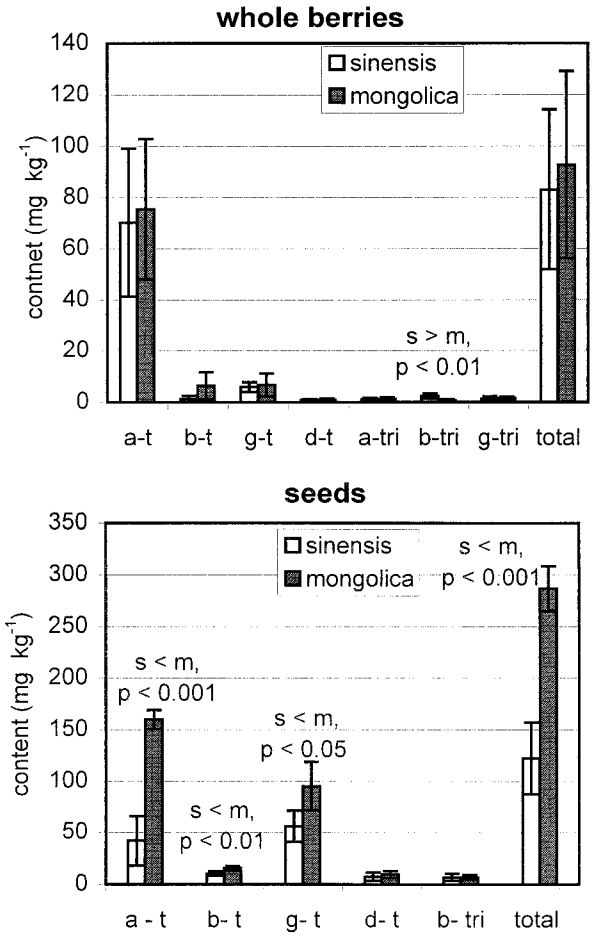

Figure 3. Content (mean ± standard deviation, mg kg^{-1}) of tocopherols and tocotrienols in whole berries and seeds of two subspecies of sea buckthorn (based on fresh weight): a-t, α-tocopherol; b-t, β-tocopherol; g-t, γ-tocopherol; d-t, δ-tocopherol; a-tri, α-tocotrienol; b-tri, β-tocotrienol; g-tri, γ-tocotrienol; s, ssp. *sinensis*; m, ssp. *mongolica*.

The results of the present study showed that whole berries of both subspecies, that is, the seedless parts, were better sources of α-tocopherol than seeds, considering the small proportion of seeds in berries (2−6% in the analyzed samples). This was especially clear in ssp. *sinensis* berries. The content of tocopherols and tocotrienols in seeds showed significant difference between the two subspecies, seeds of ssp. *mongolica* being the better source for α-tocopherol and its isomers. The total vitamin E content was also significantly higher in ssp. *mongolica*. Compared with levels in seeds, greater variation was found in the levels of α-tocopherol in whole frozen berries among the individual samples. Interestingly, the proportions of individual compounds remained stable. These results provide useful information for the industrial application of sea buckthorn seeds and berries.

In the samples of the present study, the total content of tocotrienols varied from 1.5 to 8.1 mg kg^{-1} in berries and from 43 to 188 mg kg^{-1} in berry oil. Even though the levels of these compounds in the berries did not differ between the two subspecies, the berry oil of ssp. *sinensis* was richer in tocotrienols compared with that of ssp. *mongolica* (126 vs 63 mg kg^{-1}, $p < 0.05$). In seeds the level of the only tocotrienol isomer, β-tocotrienol, was 3−11 mg kg^{-1}, the corresponding value in seed oil being 30−120 mg kg^{-1}. No significant difference was found between the seeds of the two subspecies. Whole berries were a better source of total tocotrienols even though the seeds contained more β-tocotrienol.

Recent investigations have revealed many beneficial effects of tocotrienols on humans and animals, among which the hypocholesterolemic, antitumor and skin-protecting effects are most commonly claimed (*40*−*43*). Tocotrienols at the levels found in the seeds and whole berries of sea buckthorn may be of nutritional importance in the application of the berries.

ABBREVIATIONS USED

TAG, triacylglycerols; GPL, glycerophospholipids; MTBE, methyl *tert*-butyl ether; FAME, fatty acid methyl esters.

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