Fatty Acid Composition of Lipids in Sea Buckthorn (*Hippophaë rhamnoides* L.) Berries of Different Origins

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The oil content and fatty acid composition of berries from two subspecies of sea buckthorn (*Hippophaë rhamnoides* L.) were investigated. The berries of subsp. *rhamnoides* contained a higher proportion of oil in seeds (11.3% vs 7.3%, $p < 0.01$), berries (3.5% vs 2.1%, $p < 0.001$), and seedless parts (2.8% vs 1.7%, $p < 0.01$) than the berries of subsp. *sinensis.* Linoleic (18:2n-6) and α-linolenic acids (18:3n-3) comprised about 70% of seed oil fatty acids. Palmitoleic acid (16:1n-7), practically absent in the seed oil, comprised 12.1−39.0% of oil in pulp/peel and 8.9−31.0% of that in the whole berries. More linoleic acid (40.9% vs 39.1%) and less α-linolenic acid (26.6% vs 30.6%) was found in the seed oil of subsp. *sinensis* than in the seed oil of subsp. *rhamnoides* ($p < 0.05$). The proportion of palmitoleic acid was higher in the oil of berries of subsp. *rhamnoides* than the berries of subsp. *sinensis* (26.0% vs 21.5%, $0.05 < p < 0.1$), but was vice versa with α-linolenic acid (8.8% vs 11.2%, $0.05 < p < 0.1$). The proportions of α-linolenic acid correlated inversely with oleic and linoleic acids in the seed oil. In the oil of whole berries, the proportion of palmitoleic acid correlated negatively with the proportions of linoleic and α-linolenic acids.

Keywords: *Sea buckthorn; Hippophaë rhamnoides L.; subspecies; origins; oil content; fatty acid composition; triacylglycerols; glycerophospholipids*

INTRODUCTION

Sea buckthorn (*Hippophaë rhamnoides* L.) is a hardy bush of the *Elaeagnaceae* family, with delicious, nutritious berries. It grows wild in Central Asia, from China through Mongolia and southern Siberia, to upland areas in eastern Afghanistan and eastern Uzbekistan (*1*). It also grows in a distinct area from the Elburz Mountains in Persia to Caucasia and eastern Turkey. In Europe, the species is distributed from the Black Sea coast to the Alps; along the seashores of Northwestern Europe, particularly the English Channel, North Sea, Baltic Sea, and the Atlantic coast of Norway (*1*). The species is classified into nine subspecies (*1*), two of which (*Hippophaë rhamnoides* L. subsp. *sinensis* Rousi and *Hippophaë rhamnoides* L. subsp. *rhamnoides*) are most commonly used for commercial purposes. Nowadays, sea buckthorn is cultivated, in addition to the Middle and Nothern European locations noted above, also in Canada and in the U.S. (*2*).

In recent years, sea buckthorn has become an important raw material of health products and cosmetics, especially in China and Russia. This exploitation is based on more than one thousand years' application in Tibetan, Mongolian, and Chinese traditional medicines (*3, 4*). Seed oil has been shown to lower the risk of cardiovascular and cerebrovascular diseases, regulate immunofunctions, and attenuate inflammation (*5*−*10*). Sea buckthorn oils are becoming more and more popular as special food supplements and ingredients in Japan, Europe, and North America at a time when information on the effects of oils in clinical nutrition is also increasing in the west (*11*−*13*).

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Recently, the nutritional value of the berries has also been recognized in the western world because of their special chemical composition. The oil content is generally high in both seeds (up to 15% d.w.) and soft parts (up to 34% d.w.) of the berries (*14*−*18*). Seed oil is rich in the two essential fatty acids linoleic (18:2n-6, up to 42%) and α-linolenic (18:3n-3, up to 39%) acids (*15, 16, 19*−*22*). Both the seeds and soft parts are rich in oleic acid (18:1n-9) (*15, 16, 20, 22, 23*).

Fruit pulp/peel oil contains a high level of palmitoleic acid (16:1n-7, up to 43%) (*15, 16, 20, 22, 23*), which is not common in the plant kingdom. The oil is attracting more and more attention because of the increasing interest in the physiological role of the monounsaturated fatty acids (*24*−*27*). The high content of carotenoids (up to 7 g/kg), tocopherols (up to 7 g/kg), and phytosterols (up to 20 g/kg) (*14, 28*−*31*) are special characteristics of the oil from pulp/peel of the berries. The existing knowledge shows that the composition of sea buckhtorn berries varies greatly according to their origin, and climatic and geological conditions of the growth areas (*14, 17, 19, 20*−*22, 32, 33*).

A systemic mapping of the chemical composition of sea buckthorn berries of different origins is lacking. The natural population of subsp. *sinensis* in China constitutes a large proportion of the world's sea buckthorn resources available for large-scale commercial exploitation. Subsp. *rhamnoides* is the major subspecies in Europe. No comparison of compositional data of berries of subsp. *sinensis* and subsp. *rhamnoides* has been reported.

In the present study, we collected berries of subsp. *sinensis* from twelve different locations in China and compared the oil content and fatty acid composition of whole berries and seeds with those of nine respective samples of the European subspecies *rhamnoides* grow-

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Table 1. Samples of Sea Buckthorn Berries of Different Origins

codes	place of growth	subspecies
S1	Wenshui, Shanxi, China	*sinensis*
S2	Fuxian, Shanxi, China	*sinensis*
S3	Wangtao, Shanxi, China	*sinensis*
S4	Kelan, Shanxi, China	*sinensis*
S5	Youyu, Shanxi, China	*sinensis*
S6	Xixian, Shanxi, China	*sinensis*
S7	Heshun, Shanxi, China	*sinensis*
S8	Xunyi, Shaanxi, China	*sinensis*
S9	Wuzai, Shanxi, China	*sinensis*
S10	Youyu, Shanxi, China	*sinensis*
S11	Wutai, Shanxi, China	*sinensis*
S12	Yongshou, Shaanxi, China	*sinensis*
R1[a]	Satakunta, Finland	*rhamnoides*
R2[a]	Satakunta, Finland	*rhamnoides*
R3[a]	Satakunta, Finland	*rhamnoides*
R4	Pyhämaa, Finland	*rhamnoides*
R5	Hirsilahti, Pyhäranta, Finland	*rhamnoides*
R6	Siikajoki, Finland	*rhamnoides*
R7	Vaasa, Finland	*rhamnoides*
R8	Pyhämaa, Finland	*rhamnoides*
R9	Pyhämaa, Finland	*rhamnoides*

[a] R1, R2, and R3 are three cultivars: 74006003, 74006005, and S3003, respectively.

ing in Finland. Fatty acids in triacylglycerols (TAG) and glycerophospholipids (GPL) were also analyzed because of their major existence and nutritional importance.

MATERIALS AND METHODS

Berries. Wild Chinese berries of subsp. *sinensis* were collected from twelve different locations from the end of October to the end of November 1996 (Table 1). The areas ranged between longitudes 108°04′E − 113°40′E, latitudes 34°56′N − 40°03′N, and altitudes 1020−2800 m. Nine samples of berries of subsp. *rhamnoides* were picked in southwest Finland (Table 1). Six of these were wild berries growing on their natural sites (R4−R9) on the shores of the Gulf of Bothnia and were collected in September 1999. Three samples (S3003, 74006003, and 74006005) of cultivated berries were collected from test fields of the Finnish Agricultural Research Center, Horticultural Research Station Satakunta in September 1996. One of the samples (S3003) was a clone selected from seedlings of X-ray irradiated seeds of wild Finnish subsp. *rhamnoides* berries, and the other two samples (74006003 and 74006005) were derived from two individual seedlings of a cross between a wild Finnish male bush and a wild German female bush (subsp. *rhamnoides*). The areas for these Finnish samples ranged between longitudes 21°04′E − 24°24′E, latitudes 60°45′N − 64°47′N, and altitudes 0−50 m.

The berries were loosely frozen immediately after collection and stored at −20 °C until analyzed.

Isolation of Seeds and Freeze-Drying of Berries. Seeds were isolated from frozen berries by pressing the juice and rinsing the residue with distilled water. The residue was dried at room temperature, and the seeds were separated mechanically. For the whole berry analysis, frozen berries were freeze-dried (Dura-Top Bulk Tray Dryer, FTS System Inc., Stone Ridge, NY) to 15−30% of the original weight, depending on the berry composition.

Lipid Extraction and Fractionation. Samples (1 g) of seeds and freeze-dried whole berries were crushed in a mortar in liquid nitrogen, and the lipids were isolated using a methanol−chloroform extraction procedure (*34*). The sample was homogenized in methanol (10 mL) for 1 min in a blender, chloroform (20 mL) was added, and homogenization continued for a further 2 min. The mixture was filtered, and the solid residue was re-suspended in chloroform/methanol (2:1, v/v, 30 mL) and homogenized for three minutes. 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

Table 2. Seed Content, Lyophilized Weight, and Oil Content of Sea Buckthorn Berries

		seeds			berries			soft parts (pulp/peel)		
origin/ variety/ cultivar		seed % in frozen berries	oil % in seeds	seed oil % in frozen berries	lyophilized weight % in frozen berries	oil % in lyophilized berries	berry oil % in frozen berries	lyophilized weight % in frozen berries	oil % in lyophilized soft parts	oil from soft parts % in frozen berries
S1		3.9	6.9	0.3	22.4	10.4	2.3	18.5	11.2	2.1
S2		5.8	7.3	0.4	24.6	10.8	2.6	18.8	11.8	2.2
S3		7.0	9.3	0.7	24.0	6.2	1.5	17.0	4.9	0.8
S4		6.5	6.4	0.4	26.1	5.8	1.5	19.6	5.6	1.1
S5		6.2	5.8	0.4	27.5	7.1	2.0	21.3	7.5	1.6
S6		7.1	8.9	0.6	27.6	8.2	2.3	20.5	8.0	1.6
S7		5.2	5.5	0.3	27.6	7.5	2.1	22.4	8.0	1.8
S8		9.0	8.3	0.7	31.0	10.9	3.4	22.0	12.0	2.6
S9		5.6	9.4	0.5	30.6	6.9	2.1	25.0	6.4	1.6
S10		6.8	6.5	0.4	27.4	5.7	1.6	20.6	5.5	1.1
S11		5.5	7.1	0.4	27.1	6.9	1.9	21.6	6.9	1.5
S12		4.7	6.4	0.3	25.8	7.7	2.0	21.1	8.0	1.7
R1		8.0	9.3	0.7	17.1	17.2	2.9	9.1	24.3	2.2
R2		5.6	8.2	0.5	18.7	19.4	3.6	13.1	24.1	3.2
R3		5.7	7.0	0.4	26.2	17.1	4.5	20.5	19.9	4.1
R4		5.4	12.6	0.7	23.7	17.2	4.1	18.3	18.5	3.4
R5		7.7	11.8	0.9	22.8	11.7	2.7	15.1	11.6	1.8
R6		5.7	14.2	0.8	17.0	14.6	2.5	11.2	14.8	1.7
R7		4.3	12.1	0.5	16.9	20.5	3.5	12.6	23.4	2.9
R8		5.6	13.1	0.7	22.6	17.9	4.0	17.0	19.4	3.3
R9		5.1	13.6	0.7	24.2	13.8	3.3	19.1	13.9	2.7
Comparison subsp. *sinensis* vs subsp. *rhamnoides*										
subsp. *sinensis*	mean	6.1	7.3	0.5	26.8[a]	7.9	2.1	20.7[a]	8.0	1.7
$n = 12$	std.	1.3	1.4	0.2	2.5	1.9	0.5	2.1	2.5	0.5
subsp. *rhamnoides*	mean	5.9	11.3[a]	0.7[a]	21.0	16.6[b]	3.5[b]	15.1	18.9[b]	2.8[a]
$n = 9$	std	1.2	2.5	0.2	3.6	2.8	0.7	3.9	4.7	0.8

[a] $p < 0.01$. [b] $p < 0.001$ between the subspecies.

Table 3. Fatty Acid Composition of Oils from Seeds, Whole Berries and Pulp/Peel of Sea Buckthorn Berries of Different Origins (Weight Percentages)

fatty acid	origin/variety/cultivar																				
	S1	S3	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	R1	R2	R3	R4	R5	R6	R7	R8	R9
											seed										
16:0	9.3	9.6	8.0	9.1	7.7	8.4	9.0	8.6	9.2	7.8	8.7	9.4	7.6	7.6	8.2	7.4	7.7	6.7	7.4	7.3	7.0
18:0	2.1	2.5	2.5	2.5	2.1	2.4	2.7	2.4	2.4	2.6	3.3	2.2	3.8	4.1	3.3	2.6	2.3	2.7	2.7	2.9	2.9
18:1n-9	20.3	21.2	21.2	26.1	12.9	16.9	18.7	23.2	18.7	15.2	22.1	16.3	17.9	20.0	17.8	17.4	18.1	16.8	14.5	18.3	13.7
18:1n-7	2.5	2.2	2.1	2.2	2.5	2.1	2.1	2.1	2.2	2.0	1.9	2.2	1.8	2.1	3.1	3.7	2.6	2.6	2.7	3.8	2.7
18:2n-6	38.2	42.9	42.8	40.3	38.9	41.4	40.2	41.7	39.5	40.4	41.5	43.6	39.6	41.1	38.3	37.0	38.9	43.0	36.7	37.2	40.3
18:3n-3	28.1	21.9	23.7	20.2	36.3	29.2	27.6	22.4	28.3	32.3	22.9	26.7	29.7	25.4	29.8	32.0	30.4	28.3	36.0	30.4	33.5
total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
											berry										
16:0	29.2	28.0	14.1	16.2	22.5	25.4	27.6	22.9	20.8	19.7	23.4	24.4	25.5	24.2	28.1	26.5	20.7	18.8	18.5	25.7	24.5
16:1n-7	28.8	27.1	9.7	8.9	22.2	20.7	27.6	20.6	23.1	18.2	25.0	26.3	24.7	28.0	28.9	26.0	21.4	22.8	23.0	28.6	31.0
18:0	1.0	1.4	2.0	2.0	1.4	1.4	1.3	1.7	1.4	1.8	1.7	1.0	1.4	1.2	1.3	1.2	1.3	1.3	1.3	1.1	1.0
18:1n-9	14.4	14.4	17.5	23.2	12.6	16.3	16.0	24.8	17.9	17.5	18.2	18.6	18.9	18.4	17.4	18.2	18.0	18.2	18.3	14.3	13.4
18:1n-7	7.1	6.7	4.7	5.1	6.8	6.2	5.9	8.0	8.0	6.9	7.2	8.3	6.6	7.9	8.1	8.2	6.8	8.1	8.1	7.7	8.5
18:2n-6	12.9	15.5	33.9	29.8	20.0	18.3	12.5	13.8	17.4	20.6	15.5	13.0	14.6	13.6	10.2	12.6	19.7	19.3	19.5	14.5	13.4
18:3n-3	6.5	6.9	18.1	14.8	14.5	11.7	9.1	8.2	11.5	15.2	9.0	8.4	8.3	6.7	6.2	7.3	12.1	11.1	11.2	8.1	8.3
total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
											pulp/peel										
16:0	31.8	31.6	19.1	18.9	25.8	32.0	30.6	27.0	24.7	24.3	27.2	27.1	31.5	26.6	30.0	30.4	27.4	24.8	20.4	29.8	29.0
16:1n-7	32.5	32.3	17.3	12.1	27.1	28.5	31.9	26.3	30.5	25.2	31.5	30.9	32.8	32.0	31.7	31.2	32.5	34.0	27.1	34.9	39.0
18:0	0.9	1.2	1.6	1.9	1.3	1.1	1.1	1.6	1.0	1.6	1.2	0.8	0.6	0.8	1.1	0.9	0.7	0.6	1.0	0.8	0.5
18:1n-9	13.6	13.1	14.7	22.2	12.5	16.1	15.6	25.3	17.7	18.4	17.2	19.0	19.3	18.1	17.3	18.3	18.0	18.8	19.0	13.4	13.3
18:1n-7	7.7	7.6	6.8	6.2	7.7	7.7	6.5	9.7	9.9	8.8	8.6	9.4	8.3	8.8	8.5	9.1	9.0	10.7	9.1	8.5	10.0
18:2n-6	9.7	10.2	27.0	25.9	15.8	9.7	8.2	6.0	10.2	13.0	8.8	7.6	6.3	9.7	7.5	7.7	9.8	7.7	16.5	9.5	6.5
18:3n-3	3.8	4.1	13.7	12.8	9.6	5.1	6.2	4.3	6.0	8.7	5.4	5.2	1.1	4.0	3.9	2.4	2.7	2.7	6.8	3.1	1.8
total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

	seed oil				berry oil				pulp/peel oil			
	subsp. *sinensis*		subsp. *rhamnoides*		subsp. *sinensis*		subsp. *rhamnoides*		subsp. *sinensis*		subsp. *rhamnoides*	
	mean	std.	mean	std.	mean	std.	mean	std.	mean	std.	mean	std.
16:0	8.7[c]	0.7	7.4	0.4	22.9	4.6	23.6	3.4	26.7	4.5	27.8	3.4
16:1n-7					21.5	6.5	26.0[a]	3.3	27.2	6.4	32.8[b]	3.2
18:0	2.5	0.3	3.0[b]	0.6	1.5[b]	0.3	1.2	0.1	1.3[c]	0.3	0.8	0.2
18:1n-9	19.4	3.7	17.1	2.0	17.6	3.5	17.2	2.0	17.1	3.8	17.3	2.3
18:1n-7	2.2	0.2	2.8[b]	0.7	6.7	1.1	7.8[b]	0.6	8.1	1.2	9.1[b]	0.8
18:2n-6	40.9[b]	1.7	39.1	2.1	18.6	6.8	15.3	3.4	12.7	6.9	9.0	3.1
18:3n-3	26.6	4.7	30.6[b]	3.0	11.2[a]	3.7	8.8	2.1	7.1[c]	3.4	3.2	1.6

[a] $0.05 < p < 0.1$. [b] $p < 0.05$. [c] $p < 0.01$ between the two subspecies.

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. The lipids were weighed, and the oil contents (percentages) in seeds and berries were calculated. Lipids were stored in chloroform at −20 °C until analyzed.

The lipids (20 mg), dissolved in 0.5 mL of hexane, were placed in a preconditioned (with 12 mL of hexane) silica Sep-Pak Catridge (Waters Corp., Milford, MA). After elution of hydrocarbons with hexane (10 mL), steryl esters and wax esters were eluted with hexane/methyl-*tert*-butyl ether (MTBE) (200:2, v/v, 10 mL), TAG 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 phospholipids with MTBE/methanol/ammonium acetate (5:8:2, v/v/v, 20 mL).

Fatty Acid Analysis. The total oil, TAG, and GPL fractions were transesterified by sodium methoxide catalysis (*35*). The fatty acid methyl esters (FAMEs) were analyzed with a Perkin-Elmer AutoSystem gas chromatograph equipped with programmed split/splitless injector and flame ionization detector, controlled by a Turbochrom Navigator 4 (Perkin-Elmer, San Jose, CA). Silica capillary GC column NB-351 (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 a split valve with the ratio 1:40 was opened after one minute. The temperature program was 120 °C held for 2 min, increased at a rate of 3 °C/min to 230 °C, and held for 20 min. The injector temperature was programmed from 170 °C to 250 °C at a rate of 200 °C/min. The detector temperature was 270 °C. FAMEs were identified by comparison with a standard mixture of known composition (68D, NuChek Prep, Elysian, MN) and the fatty acid composition was expressed as a weight percentage of the total fatty acids. The relative contents of fatty acids of the standard mixture agreed with the values stated by the manufacturer with deviations < 5%. No correction factors were used in the calculation of the weight percentages of fatty acids.

Statistical Analyses. The statistical analyses were carried out with the statistical program SPSS 7.5. Comparisons of the proportions of fatty acids in subsp. *sinensis* and subsp. *rhamnoides* were carried out with an Independent Sample T-test and a Mann−Whitney U-Test. The significance of the correlation between the proportions of fatty acids was tested with the Spearman Correlation Test.

RESULTS

Oil Content of Berries and Seeds. The yield and oil content of seeds and freeze-dried berries are summarized in Table 2. Seeds comprised 4−9% of the berries with no statistical difference between subsp. *sinensis* and subsp. *rhamnoides*. The oil content of berries varied over a wide range, especially in the soft parts. The average oil content in seeds was significantly higher ($p < 0.01$) in subsp. *rhamnoides* (11.3%) than in subsp.

Table 4. Fatty Acid Composition in Triacylglycerols in Seed Oil and Whole Berry Oil of Sea Buckthorn of Different Varieties or Origins (weight percentages)

seed oil

fatty acids	origin/variety/cultivar																				
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	R1	R2	R3	R4	R5	R6	R7	R8	R9
16:0	8.9	9.2	7.7	8.5	7.3	7.9	8.3	8.2	8.7	7.3	8.4	8.8	7.2	7.2	7.5	6.9	7.2	6.4	6.5	6.9	6.8
18:0	1.9	2.4	2.4	2.3	1.9	2.2	2.5	2.2	2.2	2.4	3.1	2.0	3.6	3.9	3.0	2.4	2.2	2.5	2.6	2.9	2.4
18:1n-9	20.3	21.0	20.5	26.5	12.6	16.5	19.4	22.7	19.1	15.1	22.1	15.9	17.8	20.1	17.8	17.6	18.3	17.0	13.9	18.5	14.8
18:1n-7	2.3	2.2	1.9	2.0	2.3	2.0	2.0	2.1	2.0	1.8	1.9	2.2	1.7	2.0	2.9	3.6	2.4	2.5	2.4	3.6	2.5
18:2n-6	37.4	42.8	42.9	39.8	38.3	41.2	40.4	41.8	38.7	39.8	40.8	43.2	38.9	40.6	37.7	36.5	38.3	42.8	39.7	36.6	36.0
18:3n-3	29.0	22.5	24.6	20.9	37.7	30.2	27.4	23.0	29.4	33.6	23.8	27.9	30.8	26.2	31.1	33.1	31.6	28.8	34.8	31.5	37.6
total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

berry oil

fatty acids	origin/variety/cultivar																				
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	R1	R2	R3	R4	R5	R6	R7	R8	R9
16:0	30.1	28.9	15.9	16.4	23.0	26.8	28.5	21.2	23.5	20.4	24.6	25.5	26.2	11.0	23.7	26.6	21.0	18.5	22.8	25.9	24.7
16:1n-7	29.3	27.6	11.0	9.1	22.7	21.5	28.0	23.6	21.1	18.7	25.7	26.9	24.9	4.9	31.0	26.3	22.2	23.7	24.2	29.0	31.4
18:0	1.0	1.3	2.1	2.0	1.3	1.4	1.2	1.3	1.7	1.8	1.6	1.0	1.3	2.5	1.1	1.2	1.2	1.2	1.0	1.1	0.9
18:1n-9	14.3	14.3	19.3	23.6	12.5	16.4	15.8	18.1	24.5	17.5	18.0	18.6	18.9	19.0	18.6	18.2	18.2	18.5	22.0	14.3	13.3
18:1n-7	7.0	6.5	4.9	4.9	6.5	6.1	5.7	7.8	8.1	6.7	7.0	8.0	6.6	3.2	8.7	8.2	6.6	8.2	9.2	7.7	8.5
18:2n-6	12.3	15.0	33.1	29.4	19.2	17.4	12.1	16.9	13.4	20.2	14.9	12.2	14.1	33.9	10.5	12.3	18.7	19.0	13.2	14.0	12.9
18:3n-3	6.0	6.3	13.7	14.6	14.7	10.4	8.7	11.1	7.8	14.8	8.3	7.7	8.0	25.5	6.4	7.2	12.1	11.0	7.7	8.0	8.3
total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Comparison: subsp. *sinensis* vs subsp. *rhamnoides*

		seed oil						berry oil						
		16:0	18:0	18:1n-9	18:1n-7	18:2n-6	18:3n-3	16:0	16:1n-7	18:0	18:1n-9	18:1n-7	18:2n-6	18:3n-3
subsp. *sinensis*	mean	8.3[c]	2.3	19.3	2.1	40.6[a]	27.5	23.7	22.1	1.5	17.7	6.6	18.0	10.3
$n = 12$	std.	0.6	0.3	3.8	0.2	1.9	4.9	4.6	6.5	0.4	3.6	1.1	6.8	3.4
subsp. *rhamnoides*	mean	7.0	2.8[b]	17.3	2.6[a]	38.6	31.7[a]	22.3	24.2	1.3	17.9	7.4	16.5	10.5
$n = 9$	std.	0.4	0.6	1.9	0.6	2.2	3.3	5.0	7.9	0.5	2.6	1.8	7.1	5.9

[a] $p < 0.05$. [b] $p < 0.01$. [c] $p < 0.001$ between the two subspecies.

sinensis (7.3%), but at individual levels the seed oil contents were overlapping. The oil contents of berries (3.5%) of *subsp. rhamnoides* and their seedless parts (2.8%) were also higher than in subsp. *sinensis* (2.1% and 1.7%, respectively) ($p < 0.001$).

Fatty Acid Composition in Oil of Seeds, Pulp/Peel, and Berries. The fatty acid composition in the oil of seeds, pulp/peel, and whole berries is summarized in Table 3. The major fatty acids in descending order in seed oil were quite regularly linoleic, α-linolenic, oleic, palmitic (16:0), stearic (18:0), and vaccenic (18:1n-7) acids. In two samples of subsp. *sinensis* the proportion of oleic acid exceeded that of α-linolenic acid. A clear characteristic of the seed oil was the extremely low level of palmitoleic acid (<0.5%) which is not presented in the table. The fatty acid composition of sea buckthorn seeds seems to be well buffered. We found small variations in linoleic acid in seed oil from different berry origins (37−44 %), the average proportion in subsp. *sinensis* being slightly higher than in subsp. *rhamnoides* (40.9% vs 39.1%, $p < 0.05$). α-Linolenic acid showed greater variation, with a bigger average proportion in subsp. *rhamnoides* than in subsp. *sinensis* (30.6% vs 26.6%, $p < 0.05$). A higher proportion of palmitic acid (8.7% vs 7.4%, $p < 0.01$), and lower proportion of stearic (2.5% vs 3.0%, $p < 0.05$) and vaccenic (2.2% vs 2.8%, $p < 0.05$) acids were characteristic of the Chinese berries.

In the oil from berry pulp/peel, the dominating fatty acids were palmitoleic, palmitic, oleic, linoleic, vaccenic, and α-linolenic acids (Table 3). The highest deviations were recognized in the proportion of palmitoleic acid (12.1−39.0%). Comparing the two subspecies, subsp. *rhamnoides* contained a higher proportion of n-7 monounsaturated fatty acids (palmitoleic and vaccenic acids) in the berry pulp/peel ($p < 0.05$) (Table 3). The relative levels of α-linolenic and stearic acids in berry pulp/peel of subsp. *sinensis* were higher than those of subsp. *rhamnoides* ($p < 0.01$) (Table 3).

The fatty acid compositions of whole berries resembled those of the berry pulp/peel due to the dominance of pulp/peel in the berries.

TAG Fatty Acids in Seeds and Berries. The results of the composition analysis of fatty acids of the major lipid fraction TAG in seeds and whole berries are summarized in Table 4. The TAG fatty acid profiles were practically identical to the profiles of the total oil (Table 3). The same fatty acids dominated in the same order of magnitude.

In seed oil TAG, the same differences in the fatty acid composition were found between the two subspecies as with total seed oil (cf. Table 3). However, the differences observed in the TAG of whole berry oil were not statistically significant.

GPL Fatty Acids in Seeds and Berries. The results of GPL fatty acids composition are summarized in Table 5, showing the extreme differences between seeds and whole berries. Seed GPL linoleic and α-linolenic acids together comprised about 60% of the total fatty acids, the proportion of palmitoleic acid, again, being lower than 0.5%. Correspondingly, in whole berries the sum of linoleic and α-linolenic acids constituted about 40%, and palmitoleic acid comprised more than 15% of the GPL fatty acids.

In the seed oil GPL, the proportion of α-linolenic acid was higher in subsp. *rhamnoides* than in subsp. *sinensis* (18.7% vs 11.9%, $p < 0.01$) and vice versa for oleic acid (14.3% vs 20.0%, $p < 0.001$). A higher proportion of stearic acid was also found in subsp. *rhamnoides* than in subsp. *sinensis* (5.2% vs 3.8%, $p < 0.05$).

Table 5. Fatty Acid Composition in Glycerophospholipids in Seed Oil and Berry Oil of Sea Buckthorn of Different Varieties or Origins (weight percentages)

seed oil

fatty acid	origin/variety/cultivar																				
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	R1	R2	R3	R4	R5	R6	R7	R8	R9
16:0	14.6	13.4	11.8	12.2	12.4	14.3	13.9	13.7	15.3	12.3	12.5	14.1	12.1	11.4	13.1	13.7	7.2	6.5	12.9	13.0	13.7
18:0	3.3	3.4	3.6	3.5	3.5	3.8	4.3	3.5	3.9	4.6	4.9	3.6	6.3	7.0	5.5	4.8	2.2	2.6	5.9	5.8	6.6
18:1n-9	17.8	23.7	28.1	20.9	16.4	20.9	18.9	28.4	12.5	16.2	19.2	17.3	14.9	16.3	15.1	14.7	18.3	13.9	10.1	15.6	9.6
18:1n-7	5.5	4.7	4.5	5.2	5.9	4.5	5.0	4.2	4.9	5.1	4.3	5.6	4.2	4.4	6.1	6.2	2.4	2.4	6.1	5.4	5.2
18:2n-6	45.9	45.1	42.6	47.5	47.4	43.9	46.1	39.9	48.3	48.1	48.8	47.6	48.4	49.2	46.9	45.1	38.3	39.7	49.7	45.2	48.0
18:3n-3	12.9	9.8	9.6	10.8	14.3	12.6	11.8	10.3	15.2	13.9	10.3	11.9	14.2	11.7	13.4	15.5	31.6	34.8	15.4	15.0	16.9
total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

berry oil

fatty acids	origin/variety/cultivar																				
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	R1	R2	R3	R4	R5	R6	R7	R8	R9
16:0	17.2	17.5	15.0	15.1	16.7	16.9	17.2	14.5	15.9	15.3	15.0	14.6	16.5	17.1	16.7	18.5	16.1	15.9	17.0	18.4	17.1
16:1n-7	18.2	16.8	9.7	7.5	14.9	13.9	20.7	13.3	15.8	13.9	18.3	18.5	21.2	23.2	21.0	16.8	13.8	16.2	15.9	19.1	20.0
18:0	1.3	1.5	2.0	2.2	2.0	1.8	1.9	1.7	1.7	2.1	1.8	1.2	1.7	1.5	2.0	2.2	2.3	2.2	2.0	2.5	2.9
18:1n-9	14.1	15.4	16.6	17.8	11.9	17.4	16.3	24.5	15.3	16.4	16.6	17.4	17.5	18.9	18.5	17.7	15.6	15.4	17.5	14.7	12.9
18:1n-7	9.5	9.7	8.3	7.7	9.5	8.6	9.0	10.2	10.0	10.1	10.0	11.3	8.5	9.9	9.3	8.1	7.6	8.8	9.2	7.9	8.4
18:2n-6	24.0	24.5	31.9	33.6	30.5	27.2	19.7	21.6	24.9	25.0	22.4	22.8	21.2	18.1	20.9	23.7	31.2	26.7	23.7	26.7	25.9
18:3n-3	15.7	14.6	16.5	16.1	14.5	14.3	15.3	14.2	16.5	17.1	16.0	14.2	13.4	11.3	11.6	13.0	13.4	14.9	14.7	10.7	12.8
total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

Comparison: subsp. *sinensis* vs subsp. *rhamnoides*

		seed oil						berry oil						
		16:0	18:0	18:1n-9	18:1n-7	18:2n-6	18:3n-3	16:0	16:1n-7	18:0	18:1n-9	18:1n-7	18:2n-6	18:3n-3
subsp. *sinensis*	mean	1.34	3.8	20.0[c]	4.9	45.9	11.9	15.9	15.1	1.8	16.6	9.5[a]	25.7	15.4[c]
$n = 12$	std.	1.1	0.5	4.8	0.5	2.7	1.9	1.1	3.8	0.3	3.0	1.0	4.3	1.0
subsp. *rhamnoides*	mean	11.5	5.2[a]	14.3	4.7	45.6	18.7[b]	17.0[a]	18.6[a]	2.2[a]	16.5	8.6	24.2	12.9
$n = 9$	std.	2.7	1.7	2.8	1.5	4.1	8.4	0.9	3.1	0.4	2.0	0.8	3.9	1.4

[a] $p < 0.05$. [b] $p < 0.01$. [c] $p < 0.001$ between the two subspecies.

Table 6. Comparison between the Fatty Acid Composition in Triacylglycerols (TAG) and Glycerophospholipids (GPL) in Seed Oil and Berry Oil

seed oil

		fatty acids					
		16:0	18:0	18:1n-9	18:1n-7	18:2n-6	18:3n-3
TAG	mean	7.7	2.5	18.5	2.3	39.7	29.3[b]
$n = 21$	std.	0.8	0.5	3.2	0.5	2.2	4.7
GPL	mean	12.6[b]	4.4[b]	17.6	4.8[b]	45.8[b]	14.8
$n = 21$	std.	2.1	1.3	4.9	1.0	3.3	6.5

berry oil

		fatty acids						
		16:0	16:1n-7	18:0	18:1n-9	18:1n-7	18:2n-6	18:3n-3
TAG	mean	23.1[b]	23.0[a]	1.4	17.8	7.0	17.4	10.4
n = 21	std.	4.7	7.0	0.4	3.1	1.5	6.8	4.5
GPL	mean	16.4	16.6	1.9[b]	16.6	9.1[b]	25.1[b]	14.3[a]
n = 21	std.	1.2	3.8	0.4	2.5	1.0	4.1	1.8

[a] $p < 0.01$. [b] $p < 0.001$ between TAG and GPL.

In berry oil GPL, the proportions of palmitic (15.9% vs 17.0%), palmitoleic (15.1% vs 18.6%), and stearic (1.8% vs 2.2%) acids were lower in subsp. *sinensis* ($p < 0.05$). α-Linolenic (15.4% vs 12.9%, $p < 0.001$) and vaccenic (9.5% vs 8.6%) acids were more abundant in subsp. *sinensis.*

Comparison of Fatty Acid Composition of TAG and GPL. As shown in Table 6, the linoleic acid level was higher in seed oil GPL than in the corresponding TAG fraction (45.8% vs 39.7%, $p < 0.001$) in all 21 samples analyzed. The difference was greatest in the proportions of α-linolenic acid (14.8% vs 29.3%, $p < 0.001$), but in the opposite direction. The levels of palmitic (12.6% vs 7.7%, $p < 0.001$), stearic (4.4% vs 2.5%, $p < 0.001$), and vaccenic (4.8% vs 2.3%, $p < 0.001$) acids were all significantly higher in seed oil GPL than in the corresponding TAG.

In the case of berry oil, the levels of linoleic (25.1% vs 17.4%, $p < 0.001$), α-linolenic (14.3% vs 10.4%, $p < 0.01$), vaccenic (9.1% vs 7.0%, $p < 0.001$), and stearic (1.9% vs1.4%, $p < 0.001$) acids were significantly higher in GPL than in TAG, and *vice versa* in palmitic (16.4% vs 23.1%, $p < 0.001$) and pamitoleic (16.6% vs 23.0%, $p < 0.01$) acids.

Correlation between Proportions of Different Fatty Acids. The correlation between the proportions of different fatty acids in seed oil, seed oil TAG, and seed oil GPL is shown in Figure 1. In the nonfractionated seed oil, the proportion of α-linolenic acid was negatively correlated with oleic acid (Figure 1A) and linoleic acid (Figure 1B). In the purified seed oil TAG, the proportion of α-linolenic acid was analogously negatively correlated with the oleic (Figure 1A) and linoleic acids (Figure 1B). A negative correlation between the proportions of α-linolenic and oleic acids was also found in seed oil GPL (Figure 1C).

The proportions of palmitic, oleic, vaccenic, and α-linolenic acids in seed oil GPL correlated positively with the proportions of these fatty acids in seed oil TAG.

The correlation between some of the major fatty acids in whole berries is shown in Figure 2. In berry oil and berry oil TAG the proportion of palmitoleic acid, a fatty acid mainly from the soft parts of the berries, correlated positively with the proportion of palmitic acid (Figure 2A) and negatively with the sum of linoleic and α-linolenic acids (Figure 2B). The same trend was seen in GPL (Figure 2C). Positive correlation was observed between the proportions of linoleic and α-linolenic acids in berry oil and berry oil TAG (Figure 2D).

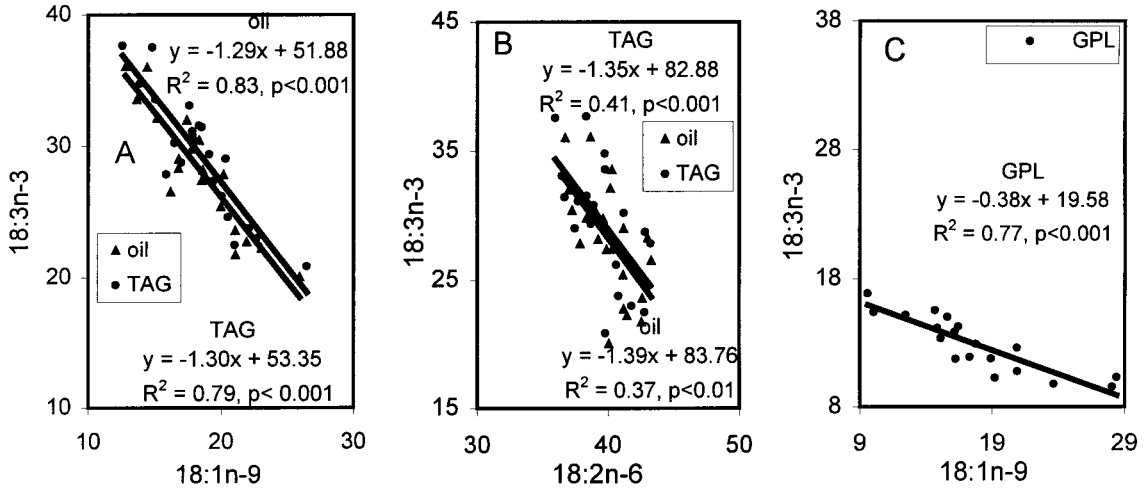

Figure 1. Correlation between proportions of fatty acids in seed oil, seed oil triaclyglycerols (TAG), and seed oil glycerolphospholipids (GPL) of sea buckthorn berries.

The proportions of palmitic, palmitoleic, oleic, and linoleic acids in berry GPL increased significantly with the increasing proportions of the corresponding fatty acids in TAG.

DISCUSSION

In the samples investigated, the seed content in berries was similar in both subspecies. The oil content in seeds, seedless parts, and whole berries was higher in subsp. *rhamnoides* than in subsp. *sinensis*. Freeze-drying of berries of subsp. *sinensis* generally gave better yields from their soft parts, however, with a lower oil content than subsp. *rhamnoides*. These results are consistent with the well-known higher contents of sugars and fruit acids in the juice of the Chinese berries (*14, 17, 36, 37*).

As structural components of cell membranes and precursors of other n-6 and n-3 fatty acids and eicosanoids, linoleic and α-linolenic acids play an important role in the physiology of man. Even though the contents of these essential fatty acids seem to be always high in seeds of berries of any sea buckthorn sources, a slight difference was found between the berries of the two subspecies. The seeds of subsp. *sinensis* seem to be a better source of linoleic acid, and seeds of subsp. *rhamnoides* seem to be a better source of α-linolenic acid.

Palmitoleic acid has a fluidity similar to that of polyunsaturated fatty acids but low susceptibility to lipid peroxidation. An increase in the level of dietary palmitoleic acid has been suggested to improve the metabolism of vascular smooth muscle cells (*38*). Hypocholesterolemic and hypoglyceridemic activity comparable to that of linoleic and α-linolenic acids has also been reported of palmitoleic acid (*39*). Increased levels of the fatty acid have been found in tissues under special conditions such as deficiency of essential fatty acid and fatty liver (*25, 40, 41*). All this information provides an interesting background for additional clinical experiments.

Oil from the soft parts of sea buckthorn berries is know to contain a high level of palmitoleic acid (*15, 16, 20, 22, 23*). Its proportion varies significantly among berries of different origins (*14, 19, 42*−*46*). Vereshchagin et al. (*43*) investigated the regio-isomers and fatty acid composition of TAG in pulp/peel of wild berries of three geographic (Central Asian, Baltic, and Caucasian) forms, from Tadzhikistan, the Baltic seashore, and Georgia, respectively. The Central Asian and the Baltic forms contained higher proportions of palmitoleic acid in the TAG of berry mesocarp (55% and 42%, respectively) than the Caucasian form (16%). The fatty acid composition of mesocarp oil of a Siberian geographic form reported by Ozerinina and coworkers (*19*) was similiar to that of the Central Asian and Baltic forms (*43*). The geographic forms of sea buckthorn may be divided into different groups according to the different mechanisms of TAG synthesis in berry mesocarp: the Siberian, Central Asian, and Baltic forms in one group, and the Caucasian form in the other (*42, 43*). The authors did not define the classification status of these geographic forms.

According to Rousi (*1*), the Central Asian, Baltic, Caucasian, and Siberian forms probably belong to subsp. *turkestanica*, subsp. *rhamnoides*, subsp. *caucasica*, and subsp. *mongolica*, respectively. Chen et al. (*14*) reported 16% and 25% palmitoleic acid in the berry pulp oil of wild subsp. *turkestanica* collected from two different locations (Hetian and Hashi), respectively, in Xinjiang, P. R. China. Wang (*17*) investigated the fatty acid composition of berry pulp/peel oil of three subspecies of sea buckthorn. The reported level of palmitoleic acid in berry pulp/peel oil was 31% in subsp. *sinensis*, 43% in subsp. *mongolica*, and 37% in subsp. *turkestanica*. The results of the present study also showed extreme variations of palmitoleic acid, from 12% to 39% in the oil from the berry pulp/peel, and 9% to 31% in the oil of whole berries even with this small number of samples. In addition to the variation among the different subspecies and geographic forms, these results also indicate the potential of breeding and industrial application when seeking an extreme source of palmitoleic acid from the plant kingdom.

The practically identical fatty acid composition of the nonfractionated oil and the TAG was due to the quantitative dominance of the TAG fraction in the oil. According to earlier research (*37*), 85−90% of the seed oil and whole berry oil was TAG, and 10−15% was phospolipids. Other lipid material such as sterols, tocopherols and carotenoids comprised 1−3% of the oil.

The correlation shown in Figures 1 and 2 is in line with the common biosynthesis pathways of these fatty acids. In berry oil and berry oil TAG and GPL, palmi-

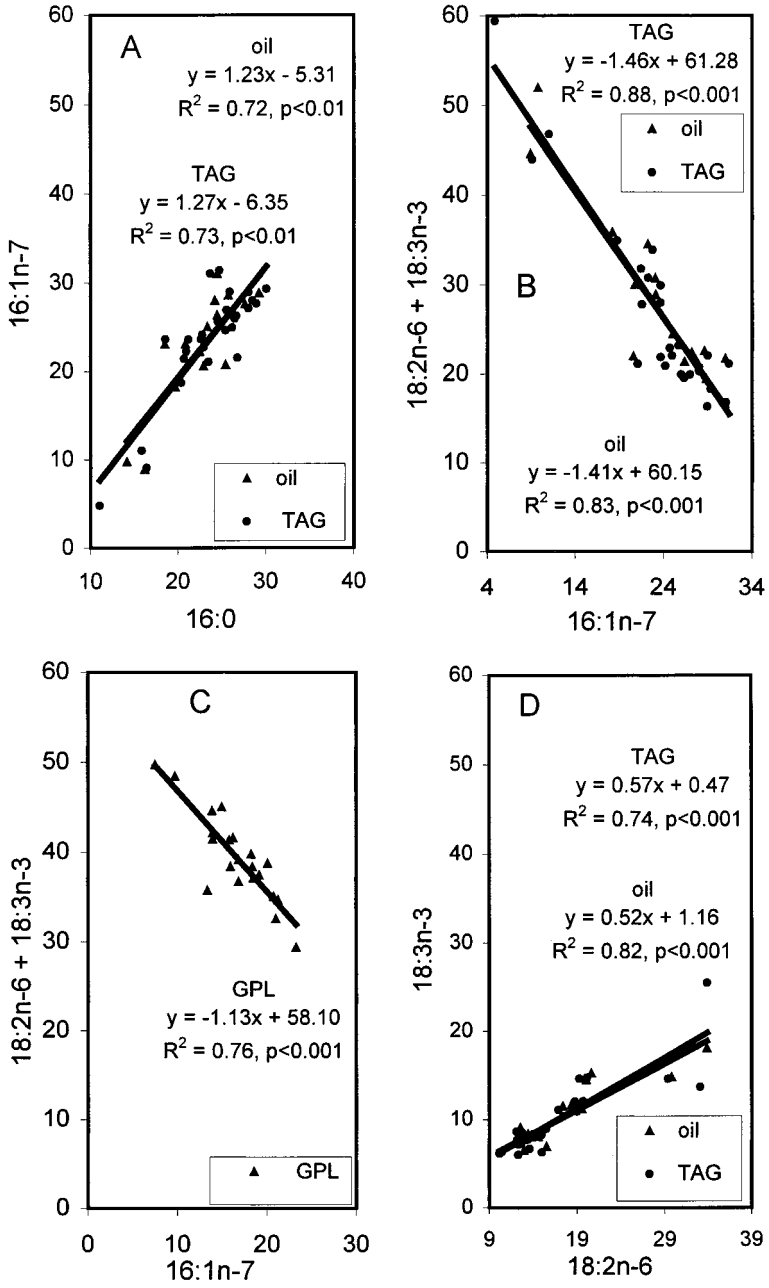

Figure 2. Correlation between proportions of fatty acids in berry oil, berry triacylglycerols (TAG), and glycerophospholipids (GPL) of sea buckthorn berries.

toleic acid correlated positively with palmitic acid, reflecting the elevation in the biosynthesis of the fatty acid with the increasing availability of its precursor. The same was true with stearic and linoleic, and stearic and α-linolenic acids. The commonly existing negative correlation between 16-carbon and 18-carbon fatty acids may be explained by the competitive mechanism of the two main pathways. The present results also suggest that, despite the interest in both palmitoleic and the essential fatty acids, the highest levels of both are not to be found in the same berries. The same is true between linoleic and α-linolenic acids.

Information provided by the present study is useful when organizing the breeding programs of sea buckthorn. However, in addition to the difference in genetic background among the different subspecies, climate and soil conditions and cultivating activities may also have influenced the oil content and fatty acid compositions. Further studies with berries from different natural populations should provide more precise information on compositional variation among different subspecies of sea buckthorn.

ABBREVIATIONS USED

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

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