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Regioisomer Compositions of Vaccenic and Oleic Acid Containing Triacylglycerols in Sea Buckthorn (*Hippophaë rhamnoides*) Pulp Oils: Influence of Origin and Weather Conditions

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Triacylglycerols (TAGs) 16:1(n-7)/16:1(n-7)/18:1(n-7) (Po/Po/V) and 16:1(n-7)/16:1(n-7)/18:1(n-9) (Po/Po/O) in pulp/peel oils of various sea buckthorn (*Hippophaë rhamnoides*) subspecies and varieties were separated by reversed-phase high-performance liquid chromatography. The regioisomerism of the TAGs was determined by tandem mass spectrometry using ammonia supplemented in the nebulizer gas to produce ammonium adducts. The regioisomer compositions of Po/Po/V (8−24% of PoVPo) and Po/Po/O (43−61% of PoOPo) both differed from the random distribution of fatty acids (33.3% of ABA) in all 32 sea buckthorn samples investigated. The regioisomer compositions were different between cultivated ssp. *rhamnoides* varieties, wild ssp. *rhamnoides*, and wild ssp. *sinensis*. Differences were also found in the regioisomerism of both Po/Po/V and Po/Po/O between the two cultivated ssp. *rhamnoides* varieties, Tytti and Terhi. In addition, growth location and harvesting years showed clear impacts on the regioisomer compositions of Po/Po/V and Po/Po/O. Higher temperatures showed positive correlations with the proportion of PoOPo in ssp. *rhamnoides* and wild ssp. *sinensis* and with the proportion of *sn*-PoPoV + *sn*-VPoPo in wild ssp. *sinensis*. However, higher temperatures, higher temperature sums, and radiation sums increased the accumulation of PoVPo in wild ssp. *rhamnoides*.

KEYWORDS: Environmental factors; *Hippophaë rhamnoides*; oleic acid; regioisomer composition; sea buckthorn; triacylglycerol; vaccenic acid

INTRODUCTION

Both seeds and soft parts of sea buckthorn (*Hippophaë rhamnoides* L., Elaeagnaceae) berries are rich in oil, which is unique among edible berries. Berries of subspecies (ssp.) *rhamnoides*, *sinensis*, *salicifolia*, and *tibetana* are known to contain generally 1−4% of pulp oil from the fruit flesh and skin (*1*, *2*). Oils from the seeds and seedless parts of the berries have different fatty acid (FA) compositions, and the pulp oil is more saturated than the seed oil. The major ACN:DB (acyl carbon number:number of double bonds) species of triacylglycerol (TAG) in the pulp oil of ssp. *rhamnoides*, *mongolica*, and *sinensis* are 48:1, 48:2, 50:2, and 50:3, constituting together 49, 71, and 46% of the TAGs of the berries in the different subspecies, respectively (*3*). In these three subspecies, the proportion of TAG species 50:3 is 7−13%, of which 56−61% is TAG 16:1/16:1/18:1 in the ssp. *rhamnoides* and *sinensis* and 25% in the ssp. *mongolica* (*3*). Palmitoleic acid [16:1(n-7)], which is not common in the plant kingdom, is found to comprise 12−39% of the FAs of oil in the pulp/peel of ssp. *rhamnoides* and *sinensis*, but is practically absent in the seed oil (*2*). However, a Central Asian climatype (ssp. *turkistanica*) of sea buckthorn has been shown to contain 55% of palmitoleic acid in the mesocarp oil (*4*). The proportions of oleic acid [18:1(n-9)] and vaccenic acid [18:1(n-7)] in the pulp oil are 13−25 and 6−11%, respectively (*2*). Smaller proportions of oleic acids (3%) are also found in sea buckthorn berries grown in Canada (*5*). Other abundant FAs are palmitic acid (16:0) and linoleic acid [18:2(n-6)], comprising 19−47 and 2−27% of the pulp oils, respectively (*2*, *4*).

Mass spectrometry (MS) is a rapid and quantitative tool for a direct analysis of the positional distribution of FAs in individual TAGs. Duffin et al. (*6*) were the first to report the use of electrospray ionization−tandem mass spectrometry (ESI-MS/MS) and ESI-MS in the analysis of TAGs using ammonium adducts $[M + NH_4]^+$. In ESI-MS/MS of ammoniated TAGs $[M + NH_4]^+$, the loss of *sn*-2 FA has been shown to be energetically less favorable than the loss of FA from a primary (*sn*-1/3) glycerol position (*7*−*12*), and ESI-MS/MS of ammoniated precursor ions has been utilized to analyze various TAG regioisomers in fats and oils with (*13*−*15*) or without reference TAG regioisomers (*16*, *17*). Kalo et al. (*18*, *19*) have also used ESI-MS/MS of $[M + NH_4]^+$ ions in the identification of the short-chain TAG regioisomers after high-performance liquid chromatographic (HPLC) separation of the regioisomers. FA isomers of various double-bond positions and configurations cannot be differentiated in TAGs by

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MS only, and therefore chromatographic separation of TAGs containing different FA isomers, such as oleic and vaccenic acid, is essential.

Sea buckthorn berry oils are claimed to have several positive physiological effects including various antioxidant effects, beneficial effects on skin and mucosa, and effects on risk factors of cardiovascular diseases (*20*). Information about the positional distribution of FAs in TAGs may be significant from nutritional and technological points of view. In addition to such considerations, the present research also adopts a biological approach to the regioisomer composition of sea buckthorn pulp/peel oil TAGs by examining the influence of growth conditions as well as different subspecies and varieties on TAG regioisomerism. We have previously (*15*) studied differences in the FA positional distribution of TAGs 18:3(n-3)/18:2/18:2 (Ala/L/L) and 18:3(n-6)/18:2/18:2 (Gla/L/L) in seed oils of different currants, and the effects of environmental factors on the isomers. The regioisomer compositions of these two TAGs were different and showed that γ-linolenic acid had a stronger affinity to *sn*-2 position than α-linolenic acid. In addition, we found differences between currant varieties, and temperature as well as radiation was shown to affect the regioisomer composition. The aim of the present work was to study whether the regioisomer compositions of TAGs 16:1(n-7)/16:1(n-7)/18:1(n-7) (Po/Po/V) and 16:1(n-7)/16:1(n-7)/18:1(n-9) (Po/Po/O) in pulp oils of different sea buckthorn samples are different. In addition, the effects of different growth places and weather conditions as well as different subspecies and varieties on the regioisomer compositions of these TAGs were studied.

MATERIALS AND METHODS

Abbreviations and Nomenclature. Vaccenic acid [18:1(n-7)] is denoted V, oleic acid [18:1(n-9)] O, and palmitoleic acid [16:1(n-7)] Po. Octadecenoic acid (18:1) is denoted Od, when the double-bond position is not considered. A/A/B denotes a TAG containing two different FAs, A and B, in unknown *sn*-positions. Regioisomers are written as ABA (symmetric regioisomer) and *sn*-AAB + *sn*-BAA. No distinction is made between the *sn*-1 and *sn*-3 positions. Diacylglycerol (DAG) ions $[M + NH_4 - RCOONH_4]^+$ are also denoted $[AA]^+$ and $[AB]^+$, where A and B are different FAs.

Materials. Sea buckthorn *H. rhamnoides* L. (Elaeagnaceae) berry samples were collected in different growth places in 2007 and 2008. Berries of two varieties of ssp. *rhamnoides*, Tytti and Terhi, were collected from three different locations: Sammalmäki, Turku (latitude 60° 23′ N, longitude 22° 09′ E, altitude 2 m) in southern Finland, Kittilä (latitude 68° 01′ N, longitude 24° 38′ E, altitude 212 m) in northern Finland, and Quebec (latitude 46° 47′ N, longitude 71° 23′ W, altitude 100 m) in Canada. The varieties of the berry samples C14, E10, and E20 in Kittilä were either Tytti or Terhi, but it was not possible to make an absolute identification. The wild ssp. *rhamnoides* berries were collected from Pyhämaa (latitude 60° 51 N, longitude 21° 11′ E, altitude 1 m) in southern Finland and from Taapajärvi (latitude 67° 07 N, longitude 24° 42′ E, altitude 216 m) and Kemi (see below for location details) in northern Finland. The wild sea buckthorn bushes in Taapajärvi had been transferred from Pyhämaa and were thus of the same origin, possibly even the same individuals. The wild berries in Kemi were picked from small islands close to each other: Pihlaja (latitude 65° 40′ N, longitude 24° 15′ E, altitude 0 m) in 2007, Maasarvi (latitude 65° 37′ N, longitude 24° 12′ E, altitude 0 m) in 2008, and Etukari (latitude 65° 40′ N, longitude 24° 12′ E, altitude 0 m) in 2008. The wild berries of the ssp. *sinensis* were collected in 2006−2008 from three altitudes (2000, 2500, and 3000 m) in the province of Sichuan (latitude 31° 01′ N, longitude 106° 54′ E) in China and from Sammalmäki in Finland. The wild ssp. *sinensis* bushes in Sammalmäki were grown from seeds originating from wild Chinese berries, but they were not of the same origin as the Sichuan berries.

The growth places and harvesting times of berries are listed in **Table 1**. Because of the exceptional weather conditions and cold summer, the berry samples in Taapajärvi and Kittilä had to be harvested slightly unripe. Also because of this, some berries grown in Taapajärvi and Kittilä were picked after the normal growing season. In Sichuan in 2006, only a small part of the wild bushes had berries, and in 2007, many of the wild bushes were cut down and the berries from different individuals were pooled.

Table 1. Growth Places and Harvest Dates of Different Sea Buckthorn Berry Samples

subspecies	variety/wild	growth place[a]	harvest date
rhamnoides	Tytti	S, Sammalmäki	Aug 30, 2007
			Aug 28, 2008
	Tytti	Quebec	Aug 28, 2007
			Sept 3, 2008
rhamnoides	Terhi	S, Sammalmäki	Aug 30, 2007
			Aug 28, 2008
	Terhi	Quebec	Aug 28, 2007
			Sept 3, 2008
rhamnoides	Tytti/Terhi C14, E10, E20	N, Kittilä	Sept 30, 2007
			Oct 22, 2008
rhamnoides	wild	S, Pyhämaa	Sept 12, 2007
			Sept 29, 2008
rhamnoides	wild	N, Taapajärvi	Sept 22, 2007
			Oct 4, 2008
rhamnoides	wild	N, Kemi	
		Pihlaja	Sept 11, 2007
		Maasarvi	Sept 16, 2008
		Etukari	Sept 11, 2008
sinensis	wild	Sichuan	
		2000 m	Oct 20, 2006
		2500 m	Oct 20, 2007
		3000 m	Oct 15, 2008
sinensis	wild	S, Sammalmäki	Aug 30, 2007
			Aug 7, 2008

[a] Abbreviations: S, southern Finland; N, northern Finland.

Reference TAGs *sn*-16:1(n-7)-16:1(n-7)-18:1(n-7) + *sn*-18:1(n-7)-16:1(n-7)-16:1(n-7), 16:1(n-7)-18:1(n-7)-16:1(n-7), *sn*-16:1(n-7)-16:1(n-7)-18:1(n-9) + *sn*-18:1(n-9)-16:1(n-7)-16:1(n-7), and 16:1(n-7)-18:1(n-9)-16:1(n-7) were purchased from Larodan Fine Chemicals (Malmö, Sweden). All of the solvents were of HPLC grade and used without further purification.

Environmental Variables. The meteorological data from the years 2007 and 2008 were obtained from the Finnish Meteorological Institute and from the National Climate Data and Information Archive of the Environment Canada. The data compiled by the weather stations closest to the harvesting places were used. The weather variables covered temperature, radiation, and precipitation (**Tables 2**−**4**). The temperature sum was calculated by summing the positive differences between the daily mean temperatures and 5 °C. The harvesting dates varied between the years and between the berry samples, and this was taken into account when the weather variables were calculated (**Table 4**). No daily weather data from Sichuan were available, but average weather variables were obtained from similar areas in western Sichuan covering the years 1963−1980. The weather variables were obtained from the altitudes of 2083, 2400, and 3000 m, which represent the altitudes of 2000, 2500, and 3000 m, respectively (**Table 5**). Normal farming practices were followed at all of the cultivation sites.

Extraction of Pulp Oils. The frozen berries were thawed, and seeds were removed from the berries mechanically. Oil was extracted using a modified Folch extraction procedure (*21*, *22*). A sample of sea buckthorn pulp was homogenized in methanol and chloroform (1:2, v/v) (10 and 20 mL, respectively, for 1 g of pulp) and filtered. The procedure was repeated, and the residue was washed with another portion of methanol/chloroform (1:2, v/v). The combined filtrates were washed with 0.88% of potassium chloride in water (one-fourth of the volume of the filtrate) and twice with methanol/water (1:1, v/v) (one-fourth of the volume of the filtrate) before the evaporation of the solvent. The extracted oils were stored at −18 °C until analyzed. Oil was extracted once from each berry sample.

Table 2. Yearly Growing Seasons and Average Monthly Temperatures in Different Growth Places in Finland and Canada

year	place	growing season	D_g[a]	ΣT_g[b]	T_{March}[c]	T_{April}[c]	T_{May}[c]	T_{June}[c]	T_{July}[c]	T_{Aug}[c]	T_{Sept}[c]	T_{Oct}[c]
2007	Sammalmäki	April 11−Nov 1	174	1520	2.1	4.5	10.3	15.6	16.7	16.9	11.2	7.3
2008	Sammalmäki	April 15−Oct 31	200	1407	−0.3	5.7	10.4	14.6	16.9	14.8	9.8	8.1
2007	Pyhämaa	April 24−Nov 2	162	1457	1.3	3.5	8.2	14.1	16.3	17.6	12.3	8.7
2008	Pyhämaa	April 22−Oct 27	189	1322	0.3	4.1	8.7	13.6	16.6	15.0	11.3	9.2
2007	Kemi	May 7−Oct 30	177	1038	−2.4	1.1	6.6	13.0	15.7	14.2	7.6	5.7
2008	Kemi	May 23−Sept 29	130	818	−6.2	0.5	6.3	11.9	14.8	11.8	7.2	4.0
2007	Kittilä	May 20−Oct 3	137	705	−3.1	−1.0	4.2	11.0	13.0	12.0	4.4	2.4
2008	Kittilä	May 31−Sept 5	98	474	−9.1	−2.8	3.3	9.5	12.4	9.0	4.3	−0.4
2007	Taapajärvi	May 14−Oct 4	144	958	−1.4	1.2	6.4	13.1	14.9	13.7	6.5	3.9
2008	Taapajärvi	May 23−Sept 24	125	747	−7.0	−0.2	5.6	11.5	14.2	10.9	6.1	2.0
2007	Quebec	April 20−Nov 1	195	1831	−5.3	3.3	12.0	16.8	19.0	17.3	14.1	9.5
2008	Quebec	April 17−Oct 16	183	1751	−6.7	4.7	10.7	16.9	19.5	18.0	13.9	6.7

[a] The length of growing season in days. [b] Temperature sum in growing season (°C). [c] Average monthly temperatures (°C).

Table 3. Average of Highest Daily Temperatures in Different Months

year	place	March	April	May	June	July	Aug	Sept	Oct
2007	Quebec	−0.5	7.4	18.3	22.8	24.6	23.1	20.2	14.1
2008	Quebec	−1.4	9.2	16.0	21.3	24.3	22.8	19.2	11.5

Instrumentation. The TAGs Po/Po/V and Po/Po/O were separated using reversed phase (RP) HPLC. The HPLC system consisted of two Ascentis C18 columns (250 mm × 4.6 mm i.d., 5 μm particle size) (Supelco, Bellefonte, PA) and Acquity Ultra Performance LC equipment (Waters Corp., Milford, MA). A binary solvent gradient consisted of acetone (designated A) and acetonitrile (designated B). The gradient program was as follows: initial A/B (60:40, v/v), linear from 0 to 60 min to A/B (80:20). The flow rate was 1.0 mL/min, approximately 0.3 mL of which was introduced to the mass spectrometer via a tee. The columns were kept at constant room temperature, 21 °C.

MS/MS analyses were performed with a Micromass Quattro Premier tandem quadrupole mass spectrometer (Waters Corp.), using positive ESI. The capillary was set at 5 kV, and the source and the desolvation temperatures were set at 120 and 150 °C, respectively. Nitrogen was used as desolvation and cone gas, and the flows were set at 300 and 100 L/h, respectively. The collision gas (argon) flow was set at 0.30 mL/min and the collision energy at 27 eV. Ammonia gas (purity 5.0; Linde AG, Munich, Germany) was introduced to the nebulizer gas flow (nitrogen) to produce ammonium adducts of TAGs $[M + NH_4]^+$. The mass flow of the ammonia gas was optimized to generate a maximal intensity for $[M + NH_4]^+$ ions. The technique enabled convenient and continuous HPLC/ESI-MS/MS analyses, without introducing ammonia in water or ammonia salts in the postcolumn flow or mobile phases. The daughter ion spectra (m/z 500−600) of the precursor ion $[M + NH_4]^+$ of TAG 50:3 (m/z 846.76) were collected in the actual analyses. The DAG ions examined were m/z 547.5 for $[PoPo]^+$ and m/z 575.5 for $[PoO]^+$ and $[PoV]^+$.

Determination of Regioisomers of TAGs. Analyses of reference TAGs PoVPo, *sn*-PoPoV + *sn*-VPoPo, PoOPo, and *sn*-PoPoO + *sn*-OPoPo by RP-HPLC/ESI-MS/MS were conducted in quadruplicate as pure regioisomers and three regioisomer mixtures. The three mixtures were prepared at ABA/(*sn*-AAB + *sn*-BAA) ratios 25:75, 50:50, and 75:25. The ratio of DAG ion intensities ($[AB]^+/([AB]^+ + [AA]^+)$) in ESI-MS/MS analyses was determined for each pure regioisomer and regioisomer mixture, and calibration curves were plotted for both Po/Po/V and Po/Po/O. $[AB]^+/([AB]^+ + [AA]^+)$ was plotted in the y-axis and the regioisomer proportion ABA (100 × ABA/(ABA + *sn*-AAB + *sn*-BAA), %) in the x-axis. The ratio $[AB]^+/([AB]^+ + [AA]^+)$ is used because it clearly shows the proportion of the product DAG ions that are formed as a result of the cleavage of the fatty acid B from the *sn*-2 position. The relative proportions of regioisomers of the TAGs Po/Po/V and Po/Po/O in sea buckthorn pulp oils were calculated on the basis of the corresponding ratios of the DAG ion intensities in the calibration curves as was first done by Jakab et al. (*23*). Analyses from each oil sample were also conducted in quadruplicate. All reference TAGs were injected simultaneously into the column to create similar chromatograms from the reference TAGs and the sample oils. The DAG ion intensities were taken from the averaged mass spectra, which were selected across the peak in the total ion chromatogram (**Figure 1**). The mass spectrum (**Figure 2**) was obtained from the segment of the peak where there was no overlapping of Po/Po/V and Po/Po/O. Mass spectra were extracted from the peaks in a similar manner from both the reference TAG chromatograms and the sample oil chromatograms to avoid the possible fluctuations in product ion ratios across the peak, which was shown to happen in HPLC/APCI-MS analyses (*24*).

Statistical Methods. SPSS 14.0 for Windows (Chicago, IL) was used for data analysis. Normal distribution of the data was tested with the Shapiro−Wilk test, and the homogeneity of variances was tested with the Levene test. The statistical differences between the regioisomer compositions were studied using the one-way analysis of variance (ANOVA) or the Brown−Forsythe test, depending on the homogeneity of variances. Tukey's honestly significant difference (HSD) test and Tamhane's tests were used as post hoc tests depending on the homogeneity of variances. Also, Fisher's least significant difference (LSD) test with Bonferroni corrections and the independent-sample t tests were used when considered to be appropriate. When the data were not normally distributed, the Kruskal−Wallis test was employed. The one-sample t test was used when the regioisomer compositions of Po/Po/V and Po/Po/O were compared to the random distribution of FAs (33.3% of PoOdPo).

RESULTS AND DISCUSSION

Chromatography. Daughter ion chromatograms of m/z 846.76 (TAG 50:3) from the varieties Tytti and Terhi and wild Finnish and wild Chinese berries are given as examples of the TAG separation and TAG proportions (**Figure 1**). To our knowledge, this is the first time that excellent HPLC separation of vaccenic and oleic acid containing TAGs is reported. Nikolova-Damyanova and Christie (*25*) used RP-HPLC with mobile phase containing silver ions, but they found no conditions under which vaccenic and oleic acids were separated. Instead, 18:1(n-11) and oleic acid were readily resolved as phenacyl and phenethyl esters on an octadecyl column with methanol/water mixtures as the mobile phase without using silver ions. In an earlier study, Borch (*26*) was not able to separate oleic acid and vaccenic acid as their phenacyl esters on an octadecyl column using an ACN/water gradient. The separation of TAGs by RP-HPLC is mainly affected by the length of the FA chains and the degree of unsaturation (*27*). The separation of TAGs according to the positions of double bonds is also possible with RP-HPLC. Phillips et al. (*28*) were able to separate triolein and tripetroselinin, which might be easier than separation of TAGs having only one FA that differs in double-bond position, as is the case in this study. Later, Laakso (*29*) managed to separate α- and γ-linolenic acid containing TAGs by using two octadecyl columns in series and a gradient consisting of acetone and dichloromethane/dichloroethane (4:1). Aitzetmüller and Grönheim (*30*) were also able to separate α- and γ-linolenic acid containing TAGs by one octadecyl column and mobile phase ACN/2-propanol/hexane. The elution order of TAGs is reversed in silver ion HPLC

Table 4. Yearly Weather Variables of Growing Season until Harvest and Last Month before Harvest (30 Days) for Different Growth Places in Finland and Canada[a]

place, year	H_g[b]	H_m[c]	T_g[d]	T_m[e]	AT_m[f]	ΔT_m[g]	min T_m[h]	LT_m[i]	max T_m[j]	HT_m[k]	R_g[l]	R_m[m]	P_g[n]	P_m[o]
Sammalmäki, 2007	16	6	1243	366	17.2	9.9	3.0	12.3	27.9	22.2	2745	493	261	49
Sammalmäki, Aug 7, 2008	8	5	926	350	16.7	10.4	3.7	11.1	28.2	21.6	2341	592	146	43
Sammalmäki, Aug 28, 2008	8	1	1147	313	15.4	7.7	3.7	11.6	27.1	19.3	2595	387	200	79
Pyhämaa, 2007	1	0	1212	300	15.0	4.2	5.9	12.9	22.2	17.1	2553	393	299	104
Pyhämaa, 2008	0	0	1240	188	11.3	3.4	6.1	9.8	17.4	13.2	2749	248	241	39
Kemi, 2007	2	0	950	174	10.8	8.3	−2.4	6.5	20.5	14.8	2266	337	270	57
Kemi, Sept 11, 2008	1	0	786	161	10.3	7.9	−2.6	6.2	19.7	14.1	1861	321	219	67
Kemi, Sept 16, 2008	1	0	792	120	9.0	8.0	−2.6	4.7	17.8	12.6	1883	265	219	47
Kittilä, 2007	1	0	702	20	4.4	9.3	−9.0	−0.2	12.5	9.1	1856	191	231	50
Kittilä, 2008[p]	0	0	519	2	1.0	5.8	−10.7	−1.9	12.6	3.9	1599	100	296	51
Taapajärvi, 2007	4	0	924	57	6.9	6.6	−1.1	3.8	18.3	10.4	1906	248	294	62
Taapajärvi, 2008[p]	1	0	748	36	5.3	6.0	−4.3	2.6	13.1	8.6	1731	179	325	31
Quebec, 2007	39	11	1381	376	17.5	11.8	6.2	11.6	31.8	23.4	2597	537	516	114
Quebec, 2008	20	9	1343	397	18.2	10.9	7.5	12.8	29.0	23.6	2712	532	616	98

[a] For the types and harvest dates of the berries, refer to **Table 1**. [b] Number of hot days (temperature ≥ 25 °C) from start of growing season until harvest. [c] Number of hot days in the last month before harvest. [d] Temperature sum from start of growing season until harvest (°C). [e] Temperature sum in the last month before harvest. [f] Average temperature in the last month before harvest (°C). [g] Mean difference between highest and lowest daily temperature during the last month before harvest. [h] Lowest temperature during the last month before harvest. [i] Average of lowest daily temperatures during the last month before harvest. [j] Highest temperature during the last month before harvest. [k] Average of highest daily temperatures during the last month before harvest. [l] Radiation sum from the start of the growing season until harvest (MJ/m^2). [m] Radiation sum during the last month before harvest. [n] Precipitation from the start of growing season until harvest (mm). [o] Precipitation in the last month before harvest. [p] In Kittilä 2008 and Taapajärvi 2008, the growing season ended before the berries were harvested.

Table 5. Average Monthly Weather Variables in Sichuan, China

parameter	altitude (m)	March	April	May	June	July	Aug	Sept	Oct	annual
T[a]	2000	9.3	13.0	15.3	16.8	19.5	18.9	15.8	11.8	11.4
	2500	6.6	10.8	13.5	14.9	17.5	17.3	13.8	9.3	9.0
	3000	4.6	8.6	12.3	13.7	15.1	14.6	12.4	7.9	7.4
P[b]	2000	14	55	107	167	148	120	105	66	818
	2500	31	64	130	143	131	95	134	76	833
	3000	7	22.	63	130	129	110	99	33	602
sun[c]	2000	215	205	169	108	164	173	118	105	1811
	2500	153	154	154	125	155	155	121	131	1744
	3000	182	187	200	166	170	178	165	180	2149
humidity[d]	2000	58	64	71	78	78	76	81	81	72
	2500	56	60	70	76	77	75	78	74	65
	3000	41	47	56	69	74	73	74	65	55

[a] Average temperature (°C). [b] Precipitation (mm). [c] Sunshine (h). [d] Relative humidity (%).

compared with RP-HPLC, and we found RP-HPLC to be better than Ag-HPLC (data not shown) in the separation of oleic and vaccenic acid containing TAGs.

Proportions of Triacylglycerols. There was a difference between different sea buckthorn pulp/peel oil samples in the proportions of Po/Po/V and Po/Po/O (**Figure 1**; **Table 6**). In the variety Tytti, Po/Po/V was more abundant than in the variety Terhi. This was seen in berries grown both in Finland and in Quebec (**Figure 1A**, **B**; **Table 6A**). We hypothesized that the varieties Terhi and Tytti grown in Kittilä could be distinguished on the basis of the profile of these two TAGs, but no conclusive identification could be made (**Table 6A**). The TAG profile in the wild ssp. *rhamnoides* berries grown in Pyhämaa was quite similar to the TAG profile in the berries grown in Kemi (**Figure 1D**; **Table 6B**), whereas the wild ssp. *rhamnoides* grown in Taapajärvi differed from these two in having lower proportions of Po/Po/O (**Figure 1C**). This is interesting because the wild sea buckthorn bushes in Taapajärvi had been transferred from Pyhämaa. It seems that the latitudes with different weather conditions affect the TAG profiles of these two TAG in sea buckthorn pulp oil. The phenomenon may also be explained by the half-ripe berries in Taapajärvi. In the wild ssp. *sinensis* berries grown in Finland and China, the proportion of

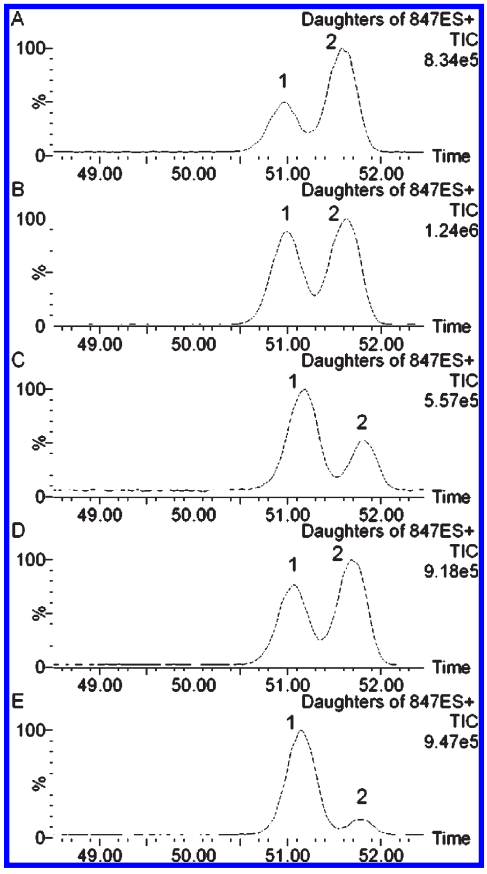

Figure 1. RP-HPLC/ESI-MS/MS chromatogram of pulp oil of ssp. *rhamnoides* varieties Terhi (**A**) and Tytti (**B**) in Sammalmäki, wild ssp. *rhamnoides* berries in Taapajärvi (**C**) and Kemi (**D**), and wild ssp. *sinensis* berries in Sichuan (**E**) at 2500 m. All of the samples are from the year 2007. Ion chromatograms consist of the daughter ions of ammoniated TAGs with *m*/*z* 846.76. Peak 1 is Po/Po/V, and peak 2 is Po/Po/O. For abbreviations, refer to **Figure 2**.

Po/Po/O was much lower than the proportion of Po/Po/V (**Figure 1E**; **Table 6C**). An exception is observed in the berries grown at an altitude of 2000 m in Sichuan, where the proportions of these two TAGs varied between the years (**Table 6C**). The

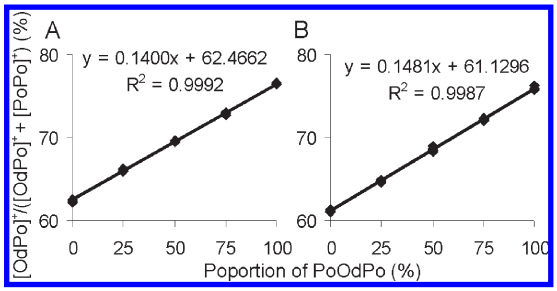

Figure 2. Calibration curves of 16:1(n-7)/16:1(n-7)/18:1(n-7) (Po/Po/V) (**A**) and 16:1(n-7)/16:1(n-7)/18:1(n-9) (Po/Po/O) (**B**) obtained by RP-HPLC/ESI-MS/MS. Equations with standard errors, multiple R and R^2 are $y = (0.1400 \pm 0.0010)x + 62.4662 \pm 0.0585$, $R = 0.9996$, $R^2 = 0.9992$ (**A**), and $y = (0.1481 \pm 0.0012)x + 61.1296 \pm 0.0760$, $R = 0.9994$, and $R^2 = 0.9987$ (**B**). The ratio of diacylglycerol ion intensities $[OdPo]^+/([OdPo]^+ + [PoPo]^+)$ (%) is determined from the mixtures of PoOdPo and *sn*-PoPoOd + *sn*-OdPoPo. Regioisomer PoOdPo content (%) is calculated as follows: 100 × PoOdPo/(PoOdPo + *sn*-PoPoOd + *sn*-OdPoPo). Four parallel analyses were conducted from each regioisomer mixture. Abbreviations: Po, palmitoleic acid [16:1(n-7)]; V, vaccenic acid [18:1(n-7)]; O, oleic acid [18:1(n-9)]; Od, octadecenoic acid (18:1, double-bond position is not considered).

variation may derive from different weather conditions or differences among the bushes within the same population.

Calibration Curves. The calibration curve of Po/Po/V was similar to that of Po/Po/O (**Figure 2**). However, small differences can be found in the calibration curves and spectra, which result from the preferential cleavage of oleic acid compared to vaccenic acid. To demonstrate this, the ESI-MS/MS spectra of pure regioisomers are provided in **Figure 3**.

Regioisomer Compositions of Po/Po/V and Po/Po/O in Sea Buckthorn Pulp Oils. Regioisomer compositions of the samples were determined on the basis of the obtained ratio of DAG ion intensities and corresponding calibration curves. The regioisomer compositions of Po/Po/V and Po/Po/O in different sea buckthorn berry samples are shown in **Tables 7** and **8**, respectively. The proportion of the TAG regioisomer PoVPo among the TAG Po/Po/V varied in different samples between 7.9 and 24.1% (**Table 7**). The quite narrow range of distribution of PoVPo shows the well-controlled system of the TAG synthesis in the soft parts of sea buckthorn. The proportion of PoOPo in the TAG Po/Po/O was much higher than that of the corresponding regioisomer PoVPo. Furthermore, the Po/Po/O TAGs showed quite constant distribution between the two regioisomers PoOPo and *sn*-PoPoO + *sn*-OPoPo. PoOPo ranged in all samples between 43.3 and 61.2% (**Table 8**). The regioisomer composition of both Po/Po/V and Po/Po/O differed from the random distribution of FAs (33.3% of PoOdPo) ($p < 0.001$). In some berry samples, particularly in the wild ssp. *sinensis* berries, the amount of Po/Po/O was very small, which produced high standard deviations for the regioisomers. The current study and previous investigations (*3*, *4*) show that both the regioisomer proportions of Po/Po/Od in general as well as those of Po/Po/V and Po/Po/O vary greatly between different sea buckthorn samples. It has been demonstrated that sea buckthorn of the Caucasian climatype (ssp. *caucasica*) has different pathways of TAG biosynthesis than the Siberian (ssp. *mongolica*), Central Asian (ssp. *turkistanica*), and Baltic climatypes (ssp. *rhamnoides*) (*4*, *31*).

In a previous study of Yang and Kallio (*3*), the regioisomer composition of Po/Po/Od was found to be 48.6% of PoOdPo in wild ssp. *sinensis* and 29.4% in wild ssp. *rhamnoides* when normalized to 100%. The method did not distinguish between TAGs PoVPo and PoOPo. It is also noteworthy that the results reported by Yang and Kallio (*3*) have considerable standard deviations, probably because of the diverse harvesting places of the berries. According to the current results, Po/Po/V is more abundant than Po/Po/O in ssp. *sinensis* except in the samples of Sichuan at 2000 m (**Figure 1E**; **Table 6C**), indicating that the proportion of PoOdPo in ssp. *sinensis* would be closer to PoVPo (8−22%). This differs from the result (48.6%) of Yang and Kallio (*3*). However, it can be assumed that the different results of these two studies may partially be explained by different berry samples. As seen in the present study, oleic acid seems to have a preference for the *sn*-2 position, whereas vaccenic acid has a preference for *sn*-1/3. As the proportions of Po/Po/O and Po/Po/V were roughly 50:50 in wild ssp. *rhamnoides* (**Table 6B**), it is evident that the proportion of regioisomer PoOdPo is near the random distribution (33%) in ssp. *rhamnoides*. This is in accordance with the previous results (29.4% of PoOdPo) (*3*). Ozerinina et al. (*4*) found that in Siberian (ssp. *mongolica*), Central Asian (ssp. *turkistanica*), Baltic (ssp. *rhamnoides*), and Caucasian (ssp. *caucasica*) climatype sea buckthorns, octadecenoic acid had higher affinity to the *sn*-2 position than palmitoleic acid. However, when the TAG species were calculated on the basis of FA composition of TAGs and TAG hydrolysis products, the proportion of PoOdPo was slightly less than that of *sn*-PoPoOd+*sn*-OdPoPo (30−47 vs 53−70%, respectively), except in the Caucasian climatype (58 vs 42%) (*4*). In the present study, it was shown that oleic acid had overall higher affinity for *sn*-2 position than vaccenic acid or palmitoleic acid, when TAGs Po/Po/V and Po/Po/O are considered. In addition, vaccenic acid had lower affinity for *sn*-2 position than palmitoleic acid.

Subspecies *rhamnoides* Varieties Tytti and Terhi. The proportion of regioisomer *sn*-ABA was more abundant in Terhi than in Tytti with regard to both Po/Po/V (**Table 7A**) and Po/Po/O (**Table 8A**) when all of the results were combined (total av). Tytti and Terhi were also different regarding Po/Po/O when compared with results from Sammalmäki and Quebec (**Table 8A**). The results within both varieties were similar between different years and growth places. Only the regioisomer composition of Po/Po/O shows variation between the years 2007 and 2008 in the variety Terhi grown in Quebec (**Table 8A**).

Wild Subspecies *rhamnoides* Berries. Wild ssp. *rhamnoides* berries from different growth places had different regioisomer compositions in Po/Po/V, the proportion of PoVPo being lowest in berries from Taapajärvi and highest in berries from Kemi (three different places) (**Table 7B**). Similarly, with regard to Po/Po/O, berries grown in Taapajärvi had a significantly lower proportion of PoOPo than berries from Pyhämaa and Kemi (**Table 8B**). This may be due to the unripeness of the berries from Taapajärvi. The proportion of PoVPo was significantly higher in berries from Etukari harvested in 2008 than in berries from the other two places in Kemi (**Table 7B**). In berries from Pyhämaa, PoOPo was more abundant in 2007 compared to 2008 (**Table 8B**).

Wild Subspecies *sinensis* Berries. The results of wild ssp. *sinensis* berries varied widely between years and growth places in both Po/Po/V and Po/Po/O. Berries grown at 2500 m in Sichuan differed from the other wild Sichuan berry samples with regard to the regioisomer composition of Po/Po/O (**Table 8C**). The wild ssp. *sinensis* berries grown in Finland did not differ from the corresponding berry samples from Sichuan. The ssp. *sinensis* berry samples collected in consecutive years from Finland were significantly different with regard to both Po/Po/V and Po/Po/O (**Tables 7C** and **8C**). In Sichuan, berries picked in 2006 from the altitudes of 2500 and 3000 m had significantly lower proportions of PoVPo than berries from the years 2007 and 2008 (**Table 7C**). With regard to berries grown at 2000 m, differences were found between 2007 and 2008 in Po/Po/O (**Table 8C**).

Comparison between Different Berry Groups. The berries were divided into three groups: cultivated ssp. *rhamnoides* varieties Tytti and Terhi, wild ssp. *rhamnoides*, and wild ssp. *sinensis*. When these groups were compared, differences were found between the cultivated variety group and wild berry groups regarding Po/Po/V (**Table 9**), whereas the only difference was found in Po/Po/O between wild ssp. *rhamnoides* and wild ssp. *sinensis* berries (**Table 9**).

Table 6. Proportions of TAGs Po/Po/V and Po/Po/O Given as Percentages (Po/Po/V + Po/Po/O = 100%) in Pulp Oils of Subspecies *rhamnoides* Varieties Tytti and Terhi (**A**), Wild Subspecies *rhamnoides* (**B**), and Wild Subspecies *sinensis* (**C**) Determined by HPLC-ESI-MS/MS[a]

A	growth place and year[b]	Po/Po/V	Po/Po/O
Tytti	Sammalmäki, 2007	46.3 ± 1.2	53.7 ± 1.2
	2008	47.0 ± 1.0	53.0 ± 1.0
	av	46.7 ± 1.1	53.4 ± 1.1
	Quebec, 2007	43.8 ± 1.7	56.2 ± 1.7
	2008	44.6 ± 0.9	55.4 ± 0.9
	av	44.2 ± 1.3	55.8 ± 1.3
	total av	45.4 ± 1.7	54.6 ± 1.7
Terhi	Sammalmäki, 2007	33.2 ± 0.8	66.8 ± 0.8
	2008	32.5 ± 0.8	67.5 ± 0.8
	av	32.8 ± 0.8	67.2 ± 0.8
	Quebec, 2007	32.9 ± 1.9	67.1 ± 1.9
	2008	32.2 ± 0.9	67.8 ± 0.9
	av	32.6 ± 1.4	67.4 ± 1.4
	total av	32.7 ± 1.1	67.3 ± 1.1
C14	Kittilä, 2007[c]	43.3 ± 0.5	56.6 ± 0.5
	2008[c]	28.6 ± 1.5	71.4 ± 1.5
	av	36.0 ± 8.0	64.0 ± 8.0
E10	Kittilä, 2007[c]	37.6 ± 0.3	62.4 ± 0.3
	2008[c]	39.9 ± 2.8	60.1 ± 2.8
	av	38.7 ± 2.2	61.3 ± 2.2
E20	Kittilä 2007[c]	49.2 ± 0.9	50.8 ± 0.9
	2008[c]	26.5 ± 1.0	73.5 ± 1.0
	av	37.8 ± 12.2	62.2 ± 12.2
B	**wild ssp. *rhamnoides* growth place and year**	**Po/Po/V**	**Po/Po/O**
	Pyhämaa, 2007	40.3 ± 0.9	59.7 ± 0.9
	2008	47.5 ± 2.1	52.5 ± 2.1
	av	43.9 ± 4.1	56.1 ± 4.1
	Taapajärvi, 2007[c]	70.6 ± 1.5	29.4 ± 1.5
	2008[c]	62.4 ± 2.0	37.6 ± 2.0
	av	66.5 ± 4.7	33.5 ± 4.7
	Kemi, 2007	45.2 ± 4.0	54.8 ± 4.0
	2008[d]	45.5 ± 0.7	54.5 ± 0.7
	2008[e]	46.9 ± 0.9	53.1 ± 0.9
	av	45.9 ± 2.3	54.1 ± 2.3
C	**wild ssp. *sinensis* growth place and year**	**Po/Po/V**	**Po/Po/O**
	Sammalmäki, 2007	92.7 ± 0.4	7.3 ± 0.4
	2008	91.7 ± 1.2	8.3 ± 1.2
	av	92.2 ± 1.0	7.8 ± 1.0
	Sichuan, 2000 m, 2006	47.5 ± 1.6	52.5 ± 1.6
	2007	71.9 ± 0.8	28.1 ± 0.8
	2008	37.5 ± 1.2	62.5 ± 1.2
	av	52.3 ± 15.1	47.7 ± 15.1
	Sichuan, 2500 m, 2006	96.4 ± 0.8	3.6 ± 0.8
	2007	89.2 ± 0.9	10.8 ± 0.9
	2008	89.5 ± 0.6	10.5 ± 0.6
	av	91.7 ± 3.6	8.3 ± 3.6
	Sichuan, 3000 m, 2006	97.4 ± 0.6	2.6 ± 0.6
	2007	89.8 ± 0.6	10.2 ± 0.6
	2008	95.8 ± 0.3	4.2 ± 0.3
	av	94.3 ± 3.5	5.7 ± 3.5

[a] Calculation of proportions is based on the daughter ion chromatograms (**Figure 1**). For abbreviations, refer to **Figure 2**. Values are expressed as molar percentages ± standard deviation. Samples are analyzed in quadruplicate. [b] Av, averaged value from different years within growth place; total av, averaged value from different years and different growth places. [c] Berries were harvested as slightly unripe. [d] Growth place Etukari. [e] Growth place Maasarvi.

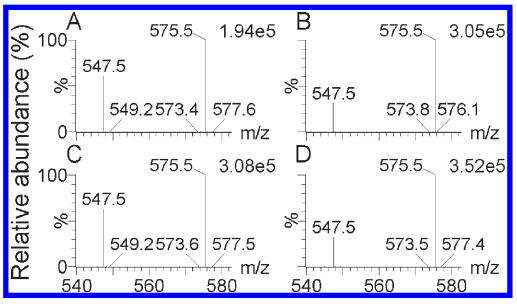

Figure 3. HPLC-ESI-MS/MS spectra of *sn*-PoPoV + *sn*-VPoPo (**A**), PoVPo (**B**), *sn*-PoPoO + *sn*-OPoPo (**C**), and PoOPo (**D**) showing the diacylglycerol daughter ions. The diacylglycerol ions corresponding to *m*/*z* 547.5 and 575.5 are $[PoPo]^+$ and $[PoOd]^+$, respectively. For abbreviations, refer to **Figure 2**.

Effect of Latitude on the Regioisomer Compositions. The results of the varieties Tytti and Terhi were combined within each growth place: Sammalmäki (latitude 60° N), Quebec (latitude 46° N), and Kittilä (latitude 68° N). In Tytti and Terhi grown in Kittilä, the proportion of regioisomer PoOPo was significantly lower among Po/Po/O compared to results from Sammalmäki and Quebec (**Table 10**), which indicates that northern locations increase the synthesis of regioisomer *sn*-PoPoO + *sn*-OPoPo. The wild ssp. *rhamnoides* berries from Taapajärvi in northern Finland and Pyhämaa in southern Finland had the same origin, which also enabled the comparison of different growth places. In wild ssp. *rhamnoides* berries, as in the varieties Tytti and Terhi, northern growth place increases the formation of *sn*-PoPoO + *sn*-OPoPo (**Table 8B**). The formation of PoVPo was also higher in Pyhämaa than in Taapajärvi (**Table 7B**). These observations suggest that northern growth places promote the synthesis of regioisomer *sn*-PoPoOd + *sn*-OdPoPo in ssp. *rhamnoides* berries. This may be due to the overall lower temperatures, lower temperature sums, and lower radiation sums in the north (**Tables 2** and **4**). The berries grown in northern Finland in Kittilä and Taapajärvi had to be harvested when they were still slightly unripe, which also could affect the results. However, the lower temperatures and radiation sums cause unripeness of the berries, and thus these factors cannot be separated from each other.

It has been recognized that within a single origin, the FA composition in seeds of ssp. *sinensis* remains fairly constant during the harvesting period, whereas the FA profile of the whole berries is subject to greater variation (*32*). Furthermore, the time of berry harvesting in a single year has been shown to have more

Table 7. Regioisomer Compositions of Po/Po/V Given as Percentages of PoVPo (100 × PoVPo/(PoVPo + *sn*-PoPoV + *sn*-VPoPo), %) in Pulp Oils of Subspecies *rhamnoides* Varieties Tytti and Terhi (**A**), Wild Subspecies *rhamnoides* (**B**), and Wild Subspecies *sinensis* (**C**) Determined by HPLC-ESI-MS/MS[a]

A	growth place and year[b]	Tytti	Terhi	C14[c]	E10[c]	E20[c]
	Sammalmäki, 2007	18.3 ± 0.8	21.6 ± 1.4			
	2008	20.8 ± 1.6	20.7 ± 1.6			
	av	19.5 ± 1.8	21.2 ± 1.4			
	Quebec, 2007	18.4 ± 1.8	20.3 ± 2.0			
	2008	18.8 ± 1.3	20.3 ± 2.6			
	av	18.6 ± 1.5	20.3 ± 2.1			
	total av	19.1 ± 1.7 a	20.8 ± 1.8 b			
	Kittilä, 2007			17.8 ± 2.1	20.5 ± 3.1	19.3 ± 0.7
	2008			18.0 ± 2.0	17.7 ± 5.0	24.1 ± 2.8
	av			17.9 ± 1.9	19.1 ± 4.1	21.7 ± 3.2

B	wild ssp. *rhamnoides* harvest year	growth places: Pyhämaa	Taapajärvi[c]	Kemi
	2007	15.2 ± 2.8	10.6 ± 2.2	18.4 ± 1.1*
	2008	17.3 ± 2.7	12.7 ± 1.1	21.5 ± 1.4[d]†
	2008			18.9 ± 1.0[e]*
	av	16.3 ± 2.8 a	11.6 ± 2.0 b	19.6 ± 1.8 c

C	wild ssp. *sinensis* harvest year	growth places: Sammalmäki	Sichuan, 2000 m	Sichuan, 2500 m	Sichuan, 3000 m
	2006		15.9 ± 2.8	9.2 ± 1.9 *	11.5 ± 0.5 *
	2007	7.9 ± 2.0 *	12.6 ± 4.4	19.2 ± 1.8 †	19.7 ± 3.0 †
	2008	15.1 ± 1.2 †	16.8 ± 2.6	22.1 ± 1.9 †	16.1 ± 2.1 †
	av	11.5 ± 4.1	15.1 ± 3.6	16.8 ± 6.0	15.8 ± 4.0

[a] For abbreviations, refer to **Figure 2**. Values are expressed as molar percentages ± standard deviation. Samples are analyzed in quadruplicate. Results in a row marked with different letters (a, b, c) are significantly different ($p < 0.05$). Results in a column marked with different symbols (*, †) are significantly different ($p < 0.05$). [b] Abbreviations: av, averaged value from different years within growth place; total av, averaged value from different years and different growth places. [c] Berries were harvested as slightly unripe. [d] Growth place Etukari. [e] Growth place Maasarvi.

Table 8. Regioisomer Compositions of Po/Po/O Given as Percentages of PoOPo (100 × PoOPo/(PoOPo + *sn*-PoPoO + *sn*-OPoPo), %) in Pulp Oils of Subspecies *rhamnoides* Varieties Tytti and Terhi (**A**), Wild Subspecies *rhamnoides* (**B**), and Wild Subspecies *sinensis* (**C**) Determined by HPLC-ESI-MS/MS[a]

A	growth place and year[b]	Tytti	Terhi	C14[c]	E10[c]	E20[c]
	Sammalmäki, 2007	55.1 ± 0.7	57.8 ± 1.2			
	2008	53.7 ± 0.8	57.2 ± 0.7			
	av	54.4 ± 1.0 a	57.5 ± 1.0 b			
	Quebec, 2007	54.9 ± 2.8	59.3 ± 1.1 *			
	2008	54.6 ± 1.4	57.1 ± 1.5 †			
	av	54.8 ± 2.0 a	58.2 ± 1.7 b			
	total av	54.6 ± 1.6 a	57.9 ± 1.4 b			
	Kittilä, 2007			48.1 ± 2.9	52.8 ± 2.6	53.0 ± 2.3
	2008			49.4 ± 2.4	49.6 ± 3.7	48.7 ± 0.9
	av			48.8 ± 2.5	51.2 ± 3.4	50.9 ± 2.8

B	wild ssp. *rhamnoides* harvest year	growth places: Pyhämaa	Taapajärvi[c]	Kemi
	2007	60.0 ± 1.7 *	50.2 ± 6.5	56.7 ± 2.0
	2008	54.6 ± 3.5 †	52.7 ± 3.4	56.7 ± 0.9[d]
	2008			58.0 ± 0.6[e]
	av	57.3 ± 3.8 a	51.4 ± 5.0 b	57.1 ± 1.4 a

C	wild ssp. *sinensis* harvest year	growth places: Sammalmäki	Sichuan, 2000 m	Sichuan, 2500 m	Sichuan, 3000 m
	2006		56.2 ± 1.4	50.0 ± 4.0	61.2 ± 6.5
	2007	55.1 ± 7.5 *	48.8 ± 3.9 *	44.2 ± 4.6	58.0 ± 3.0
	2008	43.3 ± 5.9 †	59.1 ± 1.9 †	45.3 ± 3.9	51.7 ± 8.6
	av	49.2 ± 8.9 ab	54.7 ± 5.1 a	46.5 ± 4.6 b	57.0 ± 7.1 a

[a] For abbreviations, refer to **Figure 2**. Values are expressed as molar percentages ± standard deviation. Samples are analyzed in quadruplicate. Results in a row marked with different letters (a, b) are significantly different ($p < 0.05$). Results in a column marked with different symbols (*, †) are significantly different ($p < 0.05$). [b] Abbreviations: av, averaged value from different years within growth place; total av, averaged value from different years and different growth places. [c] Berries were harvested as slightly unripe. [d] Growth place Etukari. [e] Growth place Maasarvi.

pronounced effects on the content and composition of TAGs in ssp. *sinensis* berries than the harvesting year (*32*). This may also be the case in FA positional distribution. In developing sea buckthorn berries of Siberian climatype the proportions of PoOdPo among Po/Po/Od in mesocarp were 25% 90 days after pollination (DAP) and 46% 107 DAP (*33*). In addition, the maturation stage of sea buckthorn berries has been shown to affect the incorporation of particular FAs into specific *sn*-positions in seed oil TAGs (*34*). However, in these two studies (*33*, *34*) the TAG species were calculated on the basis of FA composition of TAGs and hydrolysis products of all TAGs.

Effect of Annual Weather Variables on the Regioisomer Compositions. There was variation in the regioisomer composition of Po/Po/O between the years 2007 and 2008 in the variety Terhi grown in Quebec (**Table 8A**). In 2007, the daily maximum temperatures in Quebec were higher (**Table 3**) and the daily minimum temperatures were lower than in 2008 (**Table 4**). Also, the number of hot days was higher in 2007 than in 2008 (**Table 4**). Because of the lower minimum temperatures, the average monthly temperatures were lower in 2007 as well (**Table 2**), whereas the temperature sums in the growth season until harvest

Table 9. Average Regioisomer Compositions of Po/Po/V and Po/Po/O for Three Different Sea Buckthorn Berry Groups Given in Proportions of PoVPo and PoOPo[a]

triacylglycerol	Tytti and Terhi	wild ssp. *rhamnoides*	wild ssp. *sinensis*
PoVPo	19.8 ± 0.4 a	16.4 ± 0.7 b	15.1 ± 0.7 b
PoOPo	53.7 ± 0.5 ab	55.5 ± 0.8 a	52.1 ± 1.1 b

[a] For abbreviations, refer to **Figure 2**. Values are expressed as molar percentages ± standard deviation. Results in a row marked with different letters (a, b) are significantly different ($p < 0.05$)

(**Table 4**) were almost identical for both years. Perhaps higher maximum temperatures could promote the formation of regioisomer PoOPo in Terhi grown in Quebec. By comparison, in Sammalmäki, the temperatures in 2007 were also higher than in 2008, but no statistically significant differences can be found in these Terhi samples. However, the temperatures in Quebec 2007 were higher than in Sammalmäki 2007, so their effect may be also greater. In the wild ssp. *rhamnoides* berries grown in Pyhämaa, the effect of temperatures on the FA positional distribution in Po/Po/O is also visible (**Table 8B**). The proportion of PoOPo was higher

Table 10. Average Regioisomer Compositions of Po/Po/V and Po/Po/O for Combined Results of Varieties Tytti and Terhi in Three Different Growth Places, Sammalmäki, Quebec, and Kittilä, Given in Proportions of PoVPo and PoOPo[a]

triacylglycerol	Sammalmäki	Quebec	Kittilä
PoVPo	20.4 ± 1.8	19.5 ± 2.0	19.6 ± 3.5
PoOPo	56.0 ± 1.9 a	56.5 ± 2.5 a	50.3 ± 3.0 b

[a] For abbreviations, see **Figure 2**. Values are expressed as molar percentages ± standard deviation. Results in a row marked with different letters (a, b) are significantly different ($p < 0.05$)

in 2007 when temperatures were higher, at least in the last months before harvest. In the wild ssp. *sinensis* berries grown in Sammalmäki, the regioisomer composition was notably different between the years 2007 and 2008 in Po/Po/O (**Table 8C**). Perhaps this is also explained by the differences in temperatures between these years. The effect was more pronounced in ssp. *sinensis* than in ssp. *rhamnoides*. An opposite phenomenon was found in Po/Po/V of ssp. *sinensis* in Sammalmäki, because in 2007 the proportion of PoVPo was lower than in 2008 (**Table 7C**). Such differences in the regioisomer compositions were seen neither in Terhi grown in Sammalmäki nor in the variety Tytti. Similarly, this effect of temperatures did not occur in the berries grown in northern Finland, maybe because of the lower overall temperatures. Finally, no clear tendency can be found in the radiation or precipitation variables that could explain the differences in the regioisomer compositions.

Effect of Altitude on the Regioisomer Compositions. It is difficult to draw conclusions on the environmental effects on the wild ssp. *sinensis* grown in Sichuan, because no daily weather data were available. However, the regioisomer compositions of berries grown at the altitude of 2500 m are significantly different from those of berries grown at 2000 and 3000 m. One explanation could be the lower average amount of sunshine and higher amount of precipitation at 2000 m compared to the other altitudes (**Table 5**).

A complete HPLC separation of TAGs containing vaccenic acid and oleic acid is reported in this study. In addition, a technique to produce ammonium adducts by ammonia as nebulizer gas is presented. The results show that the regioisomerisms of Po/Po/V and Po/Po/O differ from each other in sea buckthorn pulp/peel oils, which indicates partial discrimination of vaccenic acid and oleic acid in the biosynthesis of storage TAGs in sea buckthorn fruit flesh. The regioisomer compositions of Po/Po/V and Po/Po/O are quite consistent regardless of subspecies, variety, harvest year, or cultivation site, although some differences exist between different sea buckthorn samples. Differences in the FA positional distribution of Po/Po/V as well as Po/Po/O were found between cultivated ssp. *rhamnoides*, wild ssp. *rhamnoides*, and wild ssp. *sinensis*. The results of this research show that higher temperatures may promote the formation of PoOPo in ssp. *rhamnoides* and ssp. *sinensis* and, on the other hand, the formation of *sn*-PoPoV + *sn*-VPoPo in ssp. *sinensis*. In contrast, higher temperatures, temperature sums, and radiation sums induced the synthesis of PoVPo in wild ssp. *rhamnoides*, which was seen when different latitudes were compared.

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