

Effect of Latitude and Weather Conditions on the Regioisomer Compositions of α - and γ -Linolenoyldilinoleoylglycerol in Currant Seed Oils

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The regioisomer compositions of triacylglycerols (TAG) 18:3(n-3)/18:2(n-6)/18:2(n-6) (Ala/L/L) and 18:3(n-6)/18:2(n-6)/18:2(n-6) (Gla/L/L) in seed oils of black and green currant (*Ribes nigrum* L.) and red and white currant (*Ribes rubrum* L.) varieties were determined by silver-ion high-performance liquid chromatography and mass spectrometry. The aim was to investigate whether latitude and weather conditions affect the regioisomer compositions of Ala/L/L and Gla/L/L and whether the regioisomerism differs between species and varieties. In *R. rubrum* the proportion of the symmetric regioisomer LAlaL among Ala/L/L was higher (14.1%) than in *R. nigrum* (12.1%) ($p < 0.001$). Generally in currants, the proportion of LAlaL was lower ($p < 0.001$) in northern Finland (12.1%) than in southern Finland (13.5%), where temperature and radiation sums were higher. In *R. rubrum* varieties grown in the south, the proportion of LGlaL among Gla/L/L was significantly higher in the years 2005 and 2007 (30.7–32.0%) than in 2006 (24.2–25.4%), when temperature and radiation sums were higher and the amount of precipitation was lower.

KEYWORDS: Currant; environmental factors; linolenic acid; regioisomer composition; *Ribes*; triacylglycerol

INTRODUCTION

Content and composition of seed oils of berries growing in southern and northern Finland are affected by the geography and local weather conditions (1). Influence of the conditions on lipid metabolism in other plants has also been discovered (2–4). Climate has also been shown to influence the regioisomer compositions of triacylglycerols (TAGs) in olives (5, 6).

Currant seed oils are rich in both α -linolenic acid 18:3(n-3) (Ala) and γ -linolenic acid 18:3(n-6) (Gla), which makes them special among plant oils. Black currant (*Ribes nigrum* L.) seed oil contains 11–25% of Gla and 10–19% of Ala depending on the genotype and origin (7–9). Other abundant fatty acids (FA) in black currant are 18:1(n-9) (7–13%) and 18:2(n-6) (42–53%) (7, 8). The FA proportions are in the same range also in northern red currant (*Ribes spicatum* L.) (10). However, lower proportions of Gla have been found in red currant (*Ribes rubrum* L.) (11). The most abundant TAG species in black currant and northern red currant seed oils are 54:8, 54:7, and 54:6 (acyl carbon number:number of double bonds between carbon atoms, ACN:DB) (12), and TAGs 18:3(n-3)/18:2(n-6)/18:2(n-6) (Ala/L/L) and 18:3(n-6)/18:2(n-6)/18:2(n-6) (Gla/L/L) are among the major FA combinations of TAGs in black currant seed oil (13, 14).

Mass spectrometry (MS) is a rapid and quantitative tool for the direct analysis of the positional distribution of FAs in

individual TAGs, without any enzymatic treatment of the sample. In electrospray ionization–tandem mass spectrometry (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 the primary (*sn*-1/3) glycerol positions (15, 16), and ESI-MS/MS of ammoniated precursor ions has been used for the regioisomerism analysis of various TAGs in fats and oils (17–19). Laakso and Voutilainen (13) were the pioneers in using the silver ion chromatographic (Ag-HPLC) method of Christie (20) in separating Ala- and Gla-containing TAGs in seed oils. In our previous study (21) we developed a Ag-HPLC method using nonchlorinated solvents and combined it with ESI-MS/MS to determine the regioisomer compositions of Ala/L/L and Gla/L/L in black currant seed oil. We found that the regioisomer compositions of Ala/L/L and Gla/L/L were different in black currant seed oil, which indicates that the enzymes involved in TAG synthesis of black currant seed discriminate between FAs Ala and Gla.

Information about the positional distribution of FA in TAGs is important from nutritional and technological points of view. This research also provides a biological approach to the regioisomer composition of currant seed oil TAGs by examining the influence of growth conditions on the TAGs. The aim was to investigate whether the cultivation site (southern vs northern Finland) and different weather conditions of the consecutive years have an effect on the regioisomer composition of TAGs Ala/L/L and Gla/L/L in currant seed oils and whether the regioisomer compositions are different between different currant species and varieties.

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MATERIALS AND METHODS

Abbreviations and Nomenclature. α -Linolenic acid [18:3(n-3)] is denoted Ala, γ -linolenic acid [18:3(n-6)] Gla, and linoleic acid 18:2(n-6) L. Linolenic acid is denoted Ln, when the double-bond positions are not considered. A/A/B denotes a TAG containing two different FAs A and B. 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 denoted also $[AA]^+$ and $[AB]^+$, where A and B denote different FAs.

Materials. The berries studied were varieties Ola, Melalahti, and Mortti of black currant, variety Vertti of green currant, variety Red Dutch of red currant, and variety White Dutch of white currant. Black currant and green currant were both of the species *R. nigrum* L. (Grossulariaceae), whereas red currant and white currant were both of the species *R. rubrum* L. Ripe berries were collected in 2005, 2006, and 2007 and stored frozen at -18°C until the seeds were isolated and analyzed. The cultivation sites were in southwestern Finland ($22^\circ 33' \text{E}$, $60^\circ 23' \text{N}$) and northern Finland ($26^\circ 01' \text{E}$, $66^\circ 35' \text{N}$). The corresponding altitudes were 10 and 100 m, respectively. The bushes were 3 years old in the first year of collection and of the same origin in both the north and south.

Reference TAGs *sn*-18:3(n-6)-18:2(n-6)-18:2(n-6) + *sn*-18:2(n-6)-18:2(n-6)-18:3(n-6), 18:2(n-6)-18:3(n-6)-18:2(n-6), *sn*-18:3(n-3)-18:2(n-6)-18:2(n-6) + *sn*-18:2(n-6)-18:2(n-6)-18:3(n-3), and 18:2(n-6)-18:3(n-3)-18:2(n-6) were purchased from Larodan Fine Chemicals (Malmö, Sweden). All solvents were of HPLC grade and used without further purification. Ammonium nitrate (p.a. grade) was from Merck (Darmstadt, Germany), silver nitrate (>99%) from Sigma-Aldrich GmbH (Steinheim, Germany), and ammonia-water (25%, analytical grade) from Mallinckrodt Baker B.V. (Deventer, The Netherlands).

Environmental Variables. Meteorological data from the years 2005–2007 were obtained from the Finnish Meteorological Institute. The variables covered temperature, radiation, and precipitation (Tables 1 and 2; Figures 1 and 2). Temperature sum was calculated by summing the positive differences between daily mean temperatures and 5°C . The harvest dates varied between the years and between the species and varieties, and this was taken into account when the weather variables were calculated (Table 2). The soil of the growth sites was not analyzed, and hence the effects of nutrients and other soil-related factors on the results were not taken into consideration. Normal farming practices were followed in both the south and north.

Extraction of Currant Seed Oils. Frozen berries were thawed and pulped without breaking the seeds, and the seeds were separated and washed on a screen. The washed seeds were dried at 45°C and stored in a desiccator over silica gel at room temperature. Oil was extracted using a modified Folch extraction procedure (22, 23). A sample of dry seeds was crushed and homogenized in methanol and chloroform (1:2, v/v) (10 and 20 mL, respectively, for 1 g of seeds) 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% 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 evaporation of the solvent. The extracted oils were stored at -18°C until analyzed. Oil was extracted once from each currant seed sample.

Instrumentation. TAGs Ala/L/L and Gla/L/L were separated using Ag-HPLC. Ag-HPLC columns were prepared according to the procedure introduced by Christie (20, 24). The Ag-HPLC system consisted of two silver ion bonded EC 250/4.6 Nucleosil 100-5 SA columns (250 mm \times 4.6 mm i.d.; Macherey-Nagel GmbH & Co. KG, Düren, Germany) and Acquity Ultra Performance LC equipment (Waters Corp., Milford, MA). A binary solvent gradient (21) consisted of (A) acetone and (B) acetone/acetonitrile (4:1, v/v). The gradient program was as follows: initial A/B (100:0, v/v), held isocratic for 5 min; linear from 5 to 30 min to A/B (70:30); linear from 30 to 40 min to A/B (47:53); linear from 40 to 41 min to A/B (0:100); held isocratic for 8 min. During the analyses the flow rate was 0.8 mL/min, of which approximately 0.3 mL was introduced to MS by a T-piece. The columns were kept at constant room temperature, 21°C .

Table 1. Yearly Growing Seasons and Average Monthly Temperatures in Southern and Northern Finland

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
2005	south	April 26–Nov 15	204	1541	-5.6	4.1	9.3	13.7	18.6	16.0
2005	north	May 19–Oct 14	149	1108	-8.0	1.1	5.0	13.2	17.4	14.1
2006	south	April 24–Oct 27	187	1701	-6.4	3.4	10.2	14.7	18.3	18.4
2006	north	May 1–Sept 20	143	1155	-9.8	2.0	8.1	13.5	15.5	16.5
2007	south	April 11–Nov 1	205	1520	2.1	4.5	10.3	15.6	16.7	16.9
2007	north	May 14–Oct 4	144	958	-1.4	1.2	6.4	13.1	14.9	13.7

^aThe length of growing season in days. ^bTemperature sum in the growing season ($^\circ\text{C}$). ^cAverage monthly temperatures ($^\circ\text{C}$).

MS/MS analyses were performed with a Micromass Quattro Premier tandem quadrupole mass spectrometer (Waters Corp.) using positive ESI. The capillary was set to 5 kV, and the source and desolvation temperatures were set to 100 and 220°C , respectively. Nitrogen was used as desolvation and cone gas, and the flows were set to 550 and 80 L/h, respectively. Collision gas (argon) flow was set to 0.35 mL/min and collision energy to 27 eV. Ammonia/water (10%) was introduced at $2\ \mu\text{L}/\text{min}$ by syringe pump to the postcolumn flow prior to MS in order to produce ammonium adducts of TAGs $[M + NH_4]^+$. The daughter ion spectra (m/z 590–610) of the precursor ion $[M + NH_4]^+$ of TAG 54:7 (m/z 894.76) were collected in the actual analyses. The DAG ions examined were m/z 599.5 $[LL]^+$ and 597.5 $[LnL]^+$.

Determination of the Regioisomer Compositions. Analyses of reference TAGs LAlaL, *sn*-AlaLL + *sn*-LLAla, LGlaL, and *sn*-GlaLL + *sn*-LLGla by Ag-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 of 25:75, 50:50, and 75:25. Proportions of the intensities of the DAG ions $[AB]^+$ and $[AA]^+$ ($[AB]^+ / [AA]^+$) in ESI-MS/MS analyses were determined from each pure regioisomer and regioisomer mixture, and calibration curves were plotted for both Ala/L/L and Gla/L/L (21). The ratio of DAG ion intensities ($[AB]^+ / [AA]^+$) was plotted in the y -axis and the regioisomer proportion ABA ($100 \times \text{ABA} / (\text{ABA} + \textit{sn}\text{-AAB} + \textit{sn}\text{-BAA})$, %) in the x -axis. The relative proportions of regioisomers of TAGs Ala/L/L and Gla/L/L in currant seed oils were calculated on the basis of the corresponding ratios of the DAG ion intensities in the calibration curves. Analyses of each oil sample were also conducted in quadruplicate.

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. Statistical differences between the regioisomer compositions were studied using the one-way ANOVA or the Brown–Forsythe test depending on the homogeneity of variances. Tukey’s HSD and Tamhane’s tests were used as post hoc tests depending on the homogeneity of variances. Also, Fisher’s LSD test with Bonferroni corrections and the independent-sample t tests were used when considered to be appropriate. When data were not normally distributed, the Kruskal–Wallis test and the Mann–Whitney U test with Bonferroni corrections were used.

The following comparisons of the results were performed within a currant variety: harvest years were compared within a place of growth, and places were compared within each year. Combined 24 findings of each currant variety (results from 2 places in 3 years, 4 parallel analyses) were compared to study the differences between currant varieties. Combined 8 findings of each year (2 places in each year, 4 parallel analyses) were compared within each currant variety to examine the differences between different harvest years. Combined 12 findings of each place (3 years in 1 place, 4 parallel analyses) were compared within a currant variety to study the differences between the two cultivation sites.

RESULTS AND DISCUSSION

Chromatography. The separation of Ala/L/L and Gla/L/L was better in the current study with two columns than in our previous work with one column (21). The mass spectrum was selected as in the previous work from that area of the peak where

Table 2. Yearly Weather Variables of Growing Season until Harvest and Last Month before Harvest (30 Days) for Different Currant Varieties Grown in Southern and Northern Finland

Var. ^a	Y ^b	P ^c	HD ^d	H _g ^e _h	H _m ^f	T _g ^g	T _m ^h	AT _m ⁱ	ΔT _m ^j	MnT _m ^k	LT _m ^l	MxT _m ^m	HT _m ⁿ	R _g ^o	R _m ^p	P _g ^q	P _m ^r
Melal.	05	S	8/2	12	11	848	407	18.6	11.4	9.1	12.5	29.1	23.9	1937	641	259	188
Melal.	05	N	8/22	11	1	880	276	14.2	7.3	6.7	10.9	25.5	18.3	1634	391	251	113
Melal.	06	S	8/11	20	11	1058	392	18.1	12.3	6.8	11.6	29.3	23.9	2394	686	114	23
Melal.	06	N	8/9	7	1	798	296	14.9	9.7	6.1	10.2	26.1	19.9	1903	592	112	21
Melal.	07	S	8/2	10	1	901	347	16.6	7.8	5.8	12.8	26.7	20.6	2145	534	211	121
Melal.	07	N	8/23	4	2	867	316	15.5	7.4	8.5	12.2	26.8	19.5	1638	375	233	90
Mortti	05	S	8/15	13	1	995	354	16.8	8.5	8.8	12.7	26.0	21.2	2125	497	385	303
Mortti	05	N	8/31	11	1	958	275	14.2	6.9	6.8	11.1	25.5	18.2	1722	366	280	102
Mortti	06	S	8/18	21	12	1154	405	18.5	11.9	6.8	12.3	29.3	24.2	2478	587	135	44
Mortti	06	N	8/14	7	1	868	318	15.6	10.3	6.1	10.4	26.1	20.7	1986	596	112	2
Mortti	07	S	8/1	10	2	889	350	16.7	8.2	5.8	12.6	26.7	20.8	2126	541	211	121
Mortti	07	N	8/23	4	2	867	316	15.5	7.4	8.5	12.2	26.8	19.5	1638	375	233	90
Ola	05	S	8/19	13	1	1036	342	16.4	8.6	6.1	12.3	26.0	20.9	2197	496	385	257
Ola	05	N	8/31	11	1	958	275	14.2	6.9	6.8	11.1	25.5	18.2	1722	366	280	102
Ola	06	S	8/11	20	11	1058	392	18.1	12.3	6.8	11.6	29.3	23.9	2394	686	114	23
Ola	06	N	8/15	7	1	879	320	15.7	10.3	6.1	10.5	26.1	20.8	2005	590	112	2.2
Ola	07	S	8/2	10	1	901	347	16.6	7.8	5.8	12.8	26.7	20.6	2145	534	211	121
Ola	07	N	8/23	4	2	867	316	15.5	7.4	8.5	12.2	26.8	19.5	1638	375	233	90
Vertti	05	S	8/9	13	5	930	383	17.8	9.6	8.8	12.8	29.0	22.4	2041	572	355	273
Vertti	05	N	8/29	11	1	945	276	14.2	6.9	6.8	11.1	25.5	18.2	1698	383	276	107
Vertti	06	S	7/31	12	10	899	400	18.3	11.6	6.8	12.1	28.3	23.7	2171	743	108	26
Vertti	06	N	8/16	7	1	889	318	15.6	10.2	6.1	10.5	26.1	20.7	2017	581	112	2
Vertti	07	S	8/1	10	2	889	350	16.7	8.2	5.8	12.6	26.7	20.8	2126	541	211	121
Vertti	07	N	8/23	4	2	867	316	15.5	7.4	8.5	12.2	26.8	19.5	1638	375	233	90
W. D.	05	S	7/27	12	11	776	396	18.2	11.6	8.0	12.0	29.1	23.6	1822	622	235	173
W. D.	05	N	8/22	11	1	880	276	14.2	7.3	6.7	10.9	25.5	18.3	1634	391	251	113
W. D.	06	S	7/25	10	8	822	394	18.1	11.1	6.8	12.1	28.3	23.2	2036	703	95	32
W. D.	06	N	8/8	7	1	784	295	14.8	9.6	6.1	10.2	26.1	19.8	1882	595	112	21
W. D.	07	S	7/16	10	2	711	343	16.4	9.6	4.5	11.6	26.7	21.2	1844	562	133	80
W. D.	07	N	8/23	4	2	867	316	15.5	7.4	8.5	12.2	26.8	19.5	1638	375	233	90
R. D.	05	S	8/2	12	12	848	407	18.6	11.4	9.1	12.5	29.1	23.9	1937	641	259	188
R. D.	05	N	8/22	11	1	880	276	14.2	7.3	6.7	10.9	25.5	18.3	1634	391	251	113
R. D.	06	S	7/31	12	10	899	400	18.3	11.6	6.8	12.1	28.3	23.7	2171	743	108	26
R. D.	06	N	8/9	7	1	798	296	14.9	9.7	6.1	10.2	26.1	19.9	1903	592	112	21
R. D.	07	S	7/25	10	2	811	345	16.5	8.9	5.8	12.1	26.7	21.0	2016	520	154	99
R. D.	07	N	8/23	4	2	867	316	15.5	7.4	8.5	12.2	26.8	19.5	1638	375	233	90

^a Currant varieties. Abbreviations: Melal., Melalahti; W. D., White Dutch; R. D., Red Dutch. ^b Years 2005, 2006, and 2007. ^c Place of growth. Abbreviations: S, southern Finland; N, northern Finland. ^d Harvest date. ^e Number of hot days (temperature > 25 °C) from start of growing season until harvest. ^f Number of hot days in the last month before harvest. ^g Temperature sum from start of growing season until harvest (°C). ^h Temperature sum in the last month before harvest. ⁱ Average temperature in the last month before harvest (°C). ^j Mean difference between highest and lowest daily temperature during the last month before harvest. ^k Lowest temperature during the last month before harvest. ^l Average of lowest daily temperatures during the last month before harvest. ^m Highest temperature during the last month before harvest. ⁿ Average of highest daily temperatures during the last month before harvest. ^o Radiation sum from the start of the growing season until harvest (MJ/m²). ^p Radiation sum during the last month before harvest. ^q Precipitation from the start of growing season until harvest (mm). ^r Precipitation in the last month before harvest.

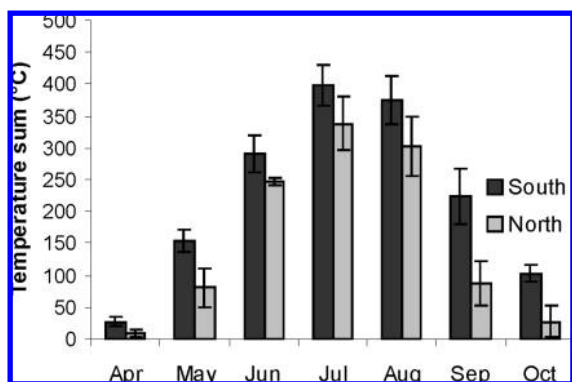


Figure 1. Average temperature sums from April to October in the years 2005–2007 in southern and northern Finland. Standard deviations are shown as error bars.

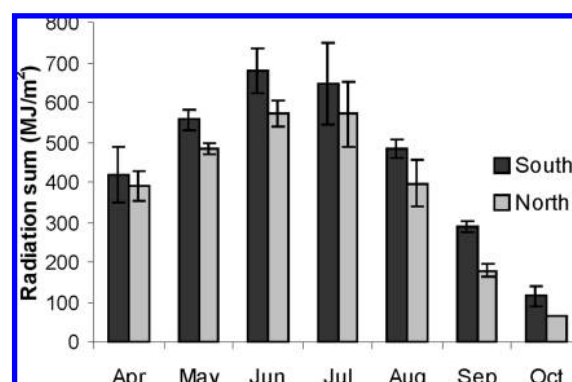


Figure 2. Average radiation sums from April to October in the years 2005–2007 in southern and northern Finland. Standard deviations are shown as error bars.

overlapping did not occur. The daughter ion chromatogram of m/z 894.76 (TAG 54:7) from black currant variety Ola is given as an example of the TAG separation (**Figure 3**).

Calibration Curves. The ratio of DAG ion intensities ($[AB]^+ / [AA]^+$) obtained from the reference TAG analyses was plotted in the y -axis and the corresponding regioisomer proportion

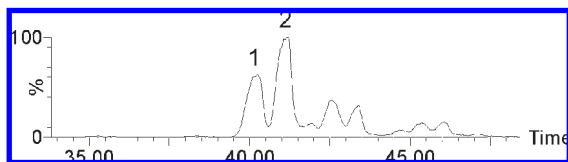


Figure 3. Ag-HPLC/ESI-MS/MS chromatogram of seed oil of Ola from 2005 grown in southern Finland. Ion chromatogram consists of the daughter ions of ammoniated TAGs with m/z 894.76. Peak 1 is Gla/L/L, and peak 2 is Ala/L/L. The peaks appearing later consist of the daughter ions of other TAG species, such as 18:3/18:3/18:1. See abbreviations in **Figure 4**.

ABA ($100 \times \text{ABA}/(\text{ABA} + sn\text{-AAB} + sn\text{-BAA})$, %) in the x -axis. Equations of the calibration curves were $y = 0.0057x + 0.8755$ for Gla/L/L and $y = 0.0104x + 1.5533$ for Ala/L/L. The R^2 values were 0.9868 and 0.9774, respectively. Gla was cleaved more efficiently than Ala from the TAG precursor ion $[\text{M} + \text{NH}_4]^+$, which can be seen from the different equations of the calibration curves. This phenomenon was found also in earlier studies (21, 25). To demonstrate this, the ESI-MS/MS spectra of pure regioisomers are shown in **Figure 4**.

Regioisomer Compositions of Ala/L/L and Gla/L/L in Currant Seed Oils. The 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 Gla/L/L and Ala/L/L in different currant samples are shown in **Tables 3** and **4**, respectively. The proportion of the TAG regioisomer LGlaL among the TAG Gla/L/L varied in different samples between 24.2 and 36.6% (**Table 3**). The narrow range of distribution of LGlaL shows the well-buffered system of the TAG synthesis in the currant seed and is close to the random distribution (33.3%) between the primary (sn -1 and sn -3) and secondary (sn -2) positions. The proportion of LAlaL in the TAG Ala/L/L was much lower than that of the corresponding regioisomer in the Gla/L/L, as shown in earlier investigations (21). Also, the Ala/L/L TAGs showed quite constant distribution between the two regioisomers LAlaL and sn -AlaLL + sn -LLAla. The proportion of regioisomer LAlaL ranged in all samples between 10.2 and 15.3%.

The results on the regioisomers of black currant seed oil TAGs in this study are in accordance with our previous findings obtained with different MS methods (21). Lawson and Hughes (26) determined enzymatically the stereospecific composition of black currant seed oil TAGs without separating different TAG species. Gla was found to be concentrated in the sn -3 position. Smaller proportions of Gla were found in the sn -2 position, and only a very small proportion of Gla was in the sn -1 position. Altogether, 36.8% of Gla was located in the sn -2 position and 63.2% in the sn -1/3 positions. Ala was found to be concentrated in primary positions (79.4%), predominantly in sn -1, and 20.6% of Ala was located in the sn -2 position. Linoleic acid was nearly evenly distributed in all sn -positions, but showed a small preference for the sn -2 position. These findings are in good agreement with the present study, although our results concern TAG Ln/L/L and the results of Lawson and Hughes concern all TAGs containing Ala and Gla. According to Kallio et al. (27), linolenic acid favors the sn -1/3 position in ACN:DB species 54:7 of black currant seed oil, which is consistent with our results. Kallio et al. (28) reported that in northern red currant *R. spicatum* L. the proportion of regioisomer LLnL was 16.3% of TAG Ln/L/L. These give also support to our results if noted that Kallio et al. were not able to distinguish Gla- and Ala-containing TAGs.

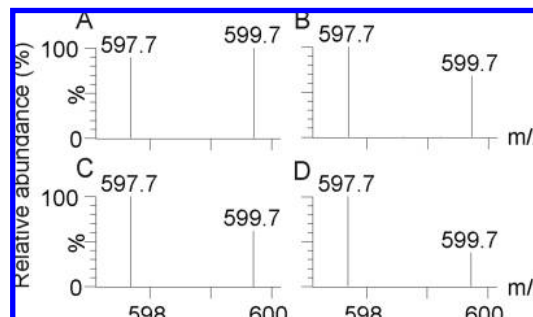


Figure 4. ESI-MS/MS spectra of (A) sn -GlaLL + sn -LLGla, (B) LGlaL, (C) sn -AlaLL + sn -LLAla, and (D) LAlaL showing the diacylglycerol daughter ions. The diacylglycerol ions corresponding to m/z 597.7 and 599.7 are $[\text{LnL}]^+$ and $[\text{LL}]^+$, respectively. Abbreviations: Ala, α -linolenic acid; Gla, γ -linolenic acid; L, linoleic acid; Ln, linolenic acid.

Comparison of Different Currant Varieties. All 24 analyses (results from 3 years in 2 places of growth, 4 parallel analyses) performed for each currant variety were combined and compared with other varieties to determine whether the regioisomer compositions of TAGs in different currant varieties differ from each other. Statistically significant differences in the regioisomer compositions of Gla/L/L were found between Vertti and Melalahti ($p = 0.018$) and between Vertti and Red Dutch ($p = 0.039$), the proportion of LGlaL being highest in Vertti ($32.6 \pm 3.4\%$) in the averaged results (**Table 3**).

Differences in the regioisomer composition of Ala/L/L were found between Melalahti and White Dutch ($p = 0.001$), Melalahti and Red Dutch ($p = 0.023$), Mortti and White Dutch ($p = 0.002$), Mortti and Red Dutch ($p = 0.035$), and Ola and White Dutch ($p = 0.024$) (**Table 4**). An almost statistically significant difference was found between Vertti and White Dutch ($p = 0.070$). The combined results of *R. nigrum* ($12.1 \pm 2.4\%$) were significantly lower than the combined results of *R. rubrum* ($14.1 \pm 2.2\%$) ($p < 0.001$). Thus, it can be stated that in *R. rubrum* varieties the proportion of regioisomer LAlaL is more abundant than in *R. nigrum* varieties, although statistically significant differences were found neither between Ola and Red Dutch nor between Vertti and *R. rubrum* varieties.

Kallio et al. (28) have reported differences in the regioisomer compositions of seed oil TAGs between different *Ribes* species, namely, wild northern red currant *R. spicatum* and wild alpine currant *R. alpinum*. Differences in the regioisomer compositions of TAGs have been discovered also in the seed oils of various seabuckthorn (*Hippophaë rhamnoides*) subspecies (29). However, in these two studies the different currant species and sea buckthorn subspecies were growing in different geographical locations, which may have affected the oil composition.

Comparison of Harvest Years and Growth Places. The findings from each harvest year were compared separately in southern (S2005–S2007) and northern Finland (N2005–N2007) within each currant variety. Differences were found neither in Ala/L/L nor Gla/L/L in Ola and Vertti. The proportion of LAlaL in Melalahti in the year 2007 was lower when compared with 2005 ($p = 0.030$) and 2006 ($p = 0.044$) in southern Finland (**Table 4**), whereas in Mortti, difference was noticed in Gla/L/L when the years 2005 and 2007 in northern Finland were compared ($p = 0.027$) (**Table 3**).

In White Dutch and Red Dutch, differences were found in Gla/L/L. The results of White Dutch from southern Finland were different in 2006 and 2007 ($p = 0.016$), LGlaL being less abundant in 2006 (**Table 3**). An almost statistically significant difference was noted between 2005 and 2006 ($p = 0.063$). Differences were also found in Red Dutch grown in southern

Table 3. Regioisomer Compositions of 18:3(n-6)/18:2(n-6)/18:2(n-6) (Gla/L/L) Given As a Percentual Proportion of LGlaL (100 × LGlaL/(LGlaL + sn-GlaLL + sn-LLGla), %) in Different Currant Varieties in Southern and Northern Finland and in Different Harvest Years 2005–2007 Determined by Ag-HPLC/ESI-MS/MS

result ^a	Melalahti	Mortti	Ola	Vertti	White Dutch	Red Dutch
S2005 ^{b,c}	32.3 ± 1.7 *	31.9 ± 3.4	30.1 ± 1.9	32.1 ± 2.6	31.1 ± 2.3	30.7 ± 2.2 a
S2006 ^{b,c}	30.3 ± 0.9	31.1 ± 4.2	32.5 ± 3.1	34.9 ± 3.7	24.2 ± 2.9 a*	25.4 ± 2.0 b
S2007 ^{b,c}	28.5 ± 1.0	33.0 ± 1.6	34.1 ± 1.1	31.5 ± 2.6	32.0 ± 1.4 b	31.6 ± 2.6 a
N2005 ^{b,c}	26.7 ± 1.3 *	27.7 ± 2.0 a	29.4 ± 1.4	29.6 ± 1.1	33.6 ± 5.0	30.7 ± 2.5
N2006 ^{b,c}	29.6 ± 3.5	31.1 ± 2.5	32.9 ± 3.8	32.2 ± 3.5	33.2 ± 2.6 *	28.7 ± 3.8
N2007 ^{b,c}	29.2 ± 4.1	34.0 ± 1.2 b	32.1 ± 3.1	35.4 ± 3.9	36.6 ± 1.5	31.0 ± 3.7
Avg ^d	29.4 ± 2.8 c	31.5 ± 3.1	31.8 ± 2.8	32.6 ± 3.4 d	31.8 ± 4.7	29.7 ± 3.3 c
Avg S ^e	30.4 ± 2.0	32.0 ± 3.1	32.2 ± 2.6	32.8 ± 3.1	29.1 ± 4.2 †	29.3 ± 3.5
Avg N ^e	28.5 ± 3.2	31.0 ± 3.2	31.4 ± 3.1	32.4 ± 3.7	34.5 ± 3.5 †	30.1 ± 3.2
Avg 2005 ^f	29.5 ± 3.3	29.8 ± 3.4 e	29.8 ± 1.6 e	30.8 ± 2.3	32.4 ± 3.9	30.7 ± 2.2 e
Avg 2006 ^f	29.9 ± 2.4	31.1 ± 3.2	32.7 ± 3.3	33.6 ± 3.6	28.7 ± 5.5 e	27.0 ± 3.3 f
Avg 2007 ^f	28.8 ± 2.8	33.5 ± 1.4 f	33.1 ± 2.4 f	33.4 ± 3.7	34.3 ± 2.8 f	31.3 ± 3.0 e

^a Values are expressed as molar percentages ± standard deviation. Samples are analyzed in quadruplicate. Abbreviations: S, southern Finland; N, northern Finland; Avg, average. Clarifications: Avg is total average of all samples S2005–N2007; Avg S and Avg N are averages of the results of southern Finland and northern Finland, respectively; Avg 2005, Avg 2006, and Avg 2007 are averages of the results of the different harvest years. ^b Harvest years compared within the same place of growth, S or N. Results in a column marked with different letters (a, b) are significantly different $p < 0.05$. ^c Southern and northern Finland compared within the same harvest year. Results in a column marked with an asterisk (*) are significantly different $p < 0.05$. ^d Results in a row marked with different letters (c, d) are significantly different $p < 0.05$. ^e Results in a column marked with a dagger (†) are significantly different $p < 0.05$. ^f Results in a column marked with different letters (e, f) are significantly different $p < 0.05$.

Table 4. Regioisomer Compositions of 18:3(n-3)/18:2(n-6)/18:2(n-6) (Ala/L/L) Given As a Percentual Proportion of LAlaL (100 × LAlaL/(LAlaL + sn-AlaLL + sn-LLAla), %) in Different Currant Varieties in Southern and Northern Finland and in Different Harvest Years 2005–2007 Determined by Ag-HPLC/ESI-MS/MS

result ^a	Melalahti	Mortti	Ola	Vertti	White Dutch	Red Dutch
S2005 ^{b,c}	14.4 ± 1.5 a*	14.1 ± 2.2	12.6 ± 1.8	13.6 ± 2.5	14.4 ± 2.0	15.1 ± 3.8
S2006 ^{b,c}	14.2 ± 1.9 a	11.2 ± 2.1	14.2 ± 2.1	13.0 ± 1.2	15.1 ± 2.2	14.2 ± 2.0
S2007 ^{b,c}	10.2 ± 2.1 b	10.3 ± 1.2	13.7 ± 1.9	13.1 ± 1.4	15.0 ± 1.8	15.3 ± 0.8
N2005 ^{b,c}	10.2 ± 0.9 *	11.6 ± 2.1	10.4 ± 2.9	12.7 ± 2.7	13.0 ± 2.1	12.7 ± 2.5
N2006 ^{b,c}	10.3 ± 2.1	12.6 ± 3.9	12.6 ± 3.2	10.9 ± 2.1	14.7 ± 1.8	11.4 ± 2.4
N2007 ^{b,c}	11.2 ± 3.1	11.3 ± 0.5	10.9 ± 1.5	12.4 ± 1.5	14.5 ± 1.9	14.3 ± 1.4
Avg ^d	11.7 ± 2.6 c	11.8 ± 2.4 c	12.4 ± 2.5 ce	12.6 ± 2.0	14.5 ± 1.9 d	13.8 ± 2.5 de
Avg S ^e	12.9 ± 2.6 †	11.9 ± 2.4	13.5 ± 1.9 †	13.2 ± 1.6	14.8 ± 1.9	14.8 ± 2.3 †
Avg N ^e	10.6 ± 2.1 †	11.8 ± 2.4	11.3 ± 2.6 †	12.0 ± 2.1	14.1 ± 1.9	12.8 ± 2.3 †
Avg 2005	12.3 ± 2.5	12.9 ± 2.4	11.5 ± 2.5	13.2 ± 2.5	13.7 ± 2.1	13.9 ± 3.2
Avg 2006	12.2 ± 2.8	11.9 ± 3.0	13.4 ± 2.7	12.0 ± 1.9	14.9 ± 1.9	12.8 ± 2.5
Avg 2007	10.7 ± 2.5	10.8 ± 1.0	12.3 ± 2.2	12.7 ± 1.4	14.8 ± 1.8	14.8 ± 1.2

^a Values are expressed as molar percentages ± standard deviation. Samples are analyzed in quadruplicate. Abbreviations: S, southern Finland; N, northern Finland; Avg, average. Clarifications: Avg is total average of all samples S2005–N2007; Avg S and Avg N are averages of the results of southern Finland and northern Finland, respectively; Avg 2005, Avg 2006, and Avg 2007 are averages of the results of the different harvest years. ^b Harvest years compared within the same place of growth, S or N. Results in a column marked with different letters (a, b) are significantly different $p < 0.05$. ^c Southern and northern Finland compared within the same harvest year. Results in a column marked with an asterisk (*) are significantly different $p < 0.05$. ^d Results in a row marked with different letters (c, d, e) are significantly different $p < 0.05$. ^e Results in a column marked with a dagger (†) are significantly different $p < 0.05$.

Finland (**Table 3**). The differences between the year 2006 and the years 2005 and 2007 were significant ($p = 0.018$ and 0.007 , respectively). Consequently, it can be concluded that 2006 differs from 2005 and 2007 in *R. rubrum* grown in southern Finland with respect to the regioisomer composition of Gla/L/L. No clear tendency in the average monthly temperatures from May to July between the years was seen that could have explained the possible differences in the regioisomer compositions, but the mean temperatures of March and April were lower in 2006 (**Table 1**). For White Dutch the temperature sum in the growing season until harvest in southern Finland was 46 and 111 °C higher in 2006 (822 °C) compared with 2005 and 2007,

respectively (**Table 2**). However, the temperature sum in the last month (30 days) before harvest and the average temperature of the last month were somewhat the same in 2005 (396 and 18.2 °C, respectively) as in 2006 (394 and 18.1 °C, respectively) (**Table 2**). In 2007 the temperature sum and mean temperature of the last month were lower (343 and 16.4 °C, respectively). The amount of radiation during the growing period until harvest was 214 and 192 MJ/m² higher in 2006 (2036 MJ/m²) compared with 2005 and 2007, respectively (**Table 2**). The radiation in the last month before harvest was also higher in 2006 (**Table 2**). The precipitation during the period of growth until harvest was 140 and 38 mm lower in 2006 (95 mm) than in 2005 and 2007,

respectively (Table 2). Also, the amount of precipitation in the last month before harvest was lower in 2006. Similar tendencies can be seen in the weather variables of Red Dutch (Table 2). It can be speculated that the average temperatures in March and April, the temperature sum, the radiation sum, and the amount of precipitation in the growing season affect the regioisomer composition of Gla/L/L in *R. rubrum* varieties so that lower average temperatures in March and April, increase in the amount of radiation and temperature, and decrease in the precipitation promote the formation of *sn*-GlaLL + *sn*-LLGla. No clear influence of temperatures in the last month before harvest was noted. In contrast to *R. rubrum* from southern Finland, no differences between the years exist in the results of *R. rubrum* grown in the north, although radiation and precipitation variables differed similarly between the years in northern Finland as in southern Finland. Temperature sum in the period of growth until harvest in northern Finland was lower in 2006 compared with 2005 and 2007, which is opposite to the values in southern Finland. This may have partly equalized the regioisomer compositions in the north. It is also noteworthy that a similar effect of weather on the regioisomer compositions of Gla/L/L in *R. nigrum* varieties was not noted, although the weather variables between the years in southern Finland differed similarly as in *R. rubrum*.

Cultivation sites were compared in each year within a currant variety. The regioisomer composition of Gla/L/L in White Dutch grown in southern Finland (S2006) was different from that in northern Finland (N2006) in 2006 ($p = 0.005$) (Table 3). In Melalahti, the regioisomer compositions were different between southern and northern Finland in 2005 in both Gla/L/L and Ala/L/L ($p = 0.037$ and $p = 0.030$, respectively) (Tables 3 and 4).

Comparison of Growth Places. The influence of cultivation site was studied by combining the 12 findings from the three harvest years of each currant variety grown either in southern or northern Finland (results from the 3 years in one place, 4 parallel analyses). The regioisomer composition of Gla/L/L was statistically significantly different between southern (Avg S) and northern Finland (Avg N) only in White Dutch ($p = 0.002$) (Table 3). No evident, common trend in the results of the six berry species and varieties was found. Distribution of FAs in TAG Ala/L/L was significantly different between southern and northern Finland in Melalahti ($p = 0.023$), Ola ($p = 0.026$), and Red Dutch ($p = 0.042$), the regioisomer LAlaL being more abundant in southern Finland (Table 4). The same tendency was seen also in other currants, although the differences were not statistically significant. Generally in currants, when all 72 findings from one cultivation site (6 varieties, 3 years, 4 parallel analyses) were combined, the proportion of regioisomer LAlaL was significantly higher in southern ($13.5 \pm 2.3\%$) than in northern Finland ($12.1 \pm 2.4\%$) ($p < 0.001$). It can be thus concluded that the proportion of regioisomer LAlaL in currant seed oil TAGs is more abundant in southern Finland than in northern Finland. Decrease in monthly temperatures (Table 1), temperature sums (Figure 1), and radiation sums (Figure 2) in northern Finland may promote the formation of regioisomer *sn*-AlaLL + *sn*-LLAla in currant seeds.

Comparison of Harvest Years. When the 8 findings of one harvest year in both cultivation sites were combined within one currant variety (results from one year in 2 places, 4 parallel analyses) and compared with the other years, differences in the regioisomer compositions were found in Gla/L/L (Table 3) but not in Ala/L/L (Table 4). In Mortti ($p = 0.043$) and Ola ($p = 0.039$), the proportion of the regioisomer LGlaL was significantly higher in 2007 (Avg 2007) compared with 2005

(Avg 2005). In White Dutch, again, the proportion of the isomers differed between the harvest years 2006 (Avg 2006) and 2007 ($p = 0.036$). With regard to Red Dutch the proportion of LGlaL was lower in 2006 compared with 2005 ($p = 0.046$) and 2007 ($p = 0.018$).

To our knowledge, this study is the first to indicate that weather variables have statistically significant effects on the regioisomer compositions of TAGs in berry seed oils. Interestingly, a lower amount of radiation and lower temperatures seem to promote the formation of regioisomer *sn*-LnLL + *sn*-LLLn among Ala/L/L in currant seeds, whereas the influence on Gla/L/L in *R. rubrum* seeds seems to be opposite. The present study also shows that the regioisomer compositions of Gla/L/L and Ala/L/L are different and that the regioisomer compositions of these TAGs in currant seed oils are quite consistent regardless of species, variety, harvest year, and cultivation site. In the future it would be worthwhile to examine the regioisomer compositions of other TAGs in currant seed oils. Also, the use of more extreme environmental conditions for cultivation of currants could further clarify the results of this study.

ABBREVIATIONS USED

ACN, acyl carbon number; Ag, silver ion; Ala, α -linolenic acid; DAG, diacylglycerol; DB, double bond; ESI, electrospray ionization; FA, fatty acid; Gla, γ -linolenic acid; HPLC, high-performance liquid chromatography; L, linoleic acid; Ln, linolenic acid; MS, mass spectrometry; MS/MS, tandem mass spectrometry; RP, reversed-phase; TAG, triacylglycerol.

SAFETY

Acetonitrile used in this study as a mobile phase in HPLC has harmful properties and can be metabolized to produce hydrogen cyanide.

LITERATURE CITED

- (1) Johansson, A. K.; Kuusisto, P. H.; Laakso, P. H.; Derome, K. K.; Sepponen, P. J.; Katajisto, J. K.; Kallio, H. P. Geographical variations in seed oils from *Rubus chamaemorus* and *Empetrum nigrum*. *Phytochemistry* **1997**, *44*, 1421–1427.
- (2) Harwood, J. L. Environmental factors which can alter lipid metabolism. *Prog. Lipid Res.* **1994**, *33*, 193–202.
- (3) Cherry, J. H.; Bishop, L.; Hasegawa, P. M.; Leffler, H. R. Differences in the fatty acid composition of soybean seed produced in northern and southern areas of the U.S.A. *Phytochemistry* **1985**, *24*, 237–241.
- (4) Trémolières, A.; Dubacq, J. P.; Drapier, D. Unsaturated fatty acids in maturing seeds of sunflower and rape; regulation by temperature and light intensity. *Phytochemistry* **1982**, *21*, 41–45.
- (5) Damiani, P.; Cossignani, L.; Simonetti, M. S.; Campisi, B.; Favretto, L.; Favretto, L. G. Stereospecific analysis of the triacylglycerol fraction and linear discriminant analysis in a climatic differentiation of Umbrian extra-virgin olive oils. *J. Chromatogr., A* **1997**, *758*, 109–116.
- (6) Vichi, S.; Pizzale, L.; Conte, L. S. Stereospecific distribution of fatty acids in triacylglycerols of olive oils. *Eur. J. Lipid Sci. Technol.* **2007**, *109*, 72–78.
- (7) Johansson, A.; Laakso, P.; Kallio, H. Characterization of seed oils of wild, edible Finnish berries. *Z. Lebensm. Unters. Forsch. A* **1997**, *204*, 300–307.
- (8) Ruiz del Castillo, M. L.; Dobson, G.; Brennan, R.; Gordon, S. Genotypic variation in fatty acid content of blackcurrant seeds. *J. Agric. Food Chem.* **2002**, *50*, 332–335.
- (9) Ruiz del Castillo, M. L.; Dobson, G.; Brennan, R.; Gordon, S. Fatty acid content and juice characteristics in black currant (*Ribes nigrum* L.) genotypes. *J. Agric. Food Chem.* **2004**, *52*, 948–952.

- (10) Johansson, A.; Laine, T.; Linna, M.-M.; Kallio, H. Variability in oil content and fatty acid composition in wild northern currants. *Eur. Food Res. Technol.* **2000**, *211*, 277–283.
- (11) Goffman, F. D.; Galletti, S. Gamma-linolenic acid and tocopherol contents in the seed oil of 47 accessions from several *Ribes* species. *J. Agric. Food Chem.* **2001**, *49*, 349–354.
- (12) Johansson, A.; Laakso, P.; Kallio, H. Molecular weight distribution of the triacylglycerols of berry seed oils analyzed by negative-ion chemical ionization mass spectrometry. *Z. Lebensm. Unters. Forsch. A* **1997**, *204*, 308–315.
- (13) Laakso, P.; Voutilainen, P. Analysis of triacylglycerols by silver-ion high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. *Lipids* **1996**, *31*, 1311–1322.
- (14) Laakso, P. Characterization of α - and γ -linolenic acid oils by reversed-phase high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. *J. Am. Oil Chem. Soc.* **1997**, *74*, 1291–1300.
- (15) Hvattum, E. Analysis of triacylglycerols with non-aqueous reversed-phase liquid chromatography and positive ion electrospray tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 187–190.
- (16) Marzilli, L. A.; Fay, L. B.; Dionisi, F.; Vouros, P. Structural characterization of triacylglycerols using electrospray ionization–MSⁿ ion-trap MS. *J. Am. Oil Chem. Soc.* **2003**, *80*, 195–202.
- (17) Leskinen, H.; Suomela, J.-P.; Kallio, H. Quantification of triacylglycerol regioisomers in oils and fat using different mass spectrometric and liquid chromatographic methods. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 2361–2373.
- (18) Kalo, P.; Kempainen, A.; Ollilainen, V.; Kuksis, A. Regiospecific determination of short-chain triacylglycerols in butterfat by normal-phase HPLC with on-line electrospray–tandem mass spectrometry. *Lipids* **2004**, *39*, 915–928.
- (19) Malone, M.; Evans, J. J. Determining the relative amounts of positional isomers in complex mixtures of triglycerides using reversed-phase high-performance liquid chromatography–tandem mass spectrometry. *Lipids* **2004**, *39*, 273–284.
- (20) Christie, W. W. A stable silver-loaded column for the separation of lipids by high performance liquid chromatography. *J. High Resolut. Chromatogr. Chromatogr. Commun.* **1987**, *10*, 148–150.
- (21) Leskinen, H.; Suomela, J.-P.; Pinta, J.; Kallio, H. Regioisomeric structure determination of α - and γ -linolenoyldilinoleoylglycerol in blackcurrant seed oil by silver ion high-performance liquid chromatography and mass spectrometry. *Anal. Chem.* **2008**, *80*, 5788–5793.
- (22) Folch, J.; Lees, M.; Stanley, S. G. H. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497–509.
- (23) Ways, P.; Hanahan, D. J. Characterization and quantification of red cell lipids in normal man. *J. Lipid Res.* **1964**, *5*, 318–328.
- (24) Christie, W. W. Separation of molecular species of triacylglycerols by high-performance liquid chromatography with a silver ion column. *J. Chromatogr.* **1988**, *454*, 273–284.
- (25) Manninen, P.; Laakso, P. Capillary supercritical fluid chromatography–atmospheric pressure chemical ionization mass spectrometry of γ - and α -linolenic acid containing triacylglycerols in berry oils. *Lipids* **1997**, *32*, 825–831.
- (26) Lawson, L. D.; Hughes, B. G. Triacylglycerol structure of plant and fungal oils containing γ -linolenic acid. *Lipids* **1988**, *23*, 313–317.
- (27) Kallio, H.; Currie, G.; Gibson, R.; Kallio, S. Mass spectrometry of food lipids: negative ion chemical ionization/collision induced dissociation analysis of oils containing γ -linolenic acid as an example. *Ann. Chim.* **1997**, *87*, 187–198.
- (28) Kallio, H.; Tuomasjukka, S.; Johansson, A.; Tahvonen, R.; Nieminen, N.; Sjövall, O.; Kurvinen, J.-P.; Kivini, H. Analysis of regioisomers of triacylglycerols of northern currant seed oil by tandem mass spectrometry. *Eur. J. Lipid Sci. Technol.* **2005**, *107*, 101–106.
- (29) Yang, B.; Kallio, H. Analysis of triacylglycerols of seeds and berries of sea buckthorn (*Hippophaë rhamnoides*) of different origins by mass spectrometry and tandem mass spectrometry. *Lipids* **2006**, *41*, 381–392.

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