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Variability in oil content and fatty acid composition in wild northern currants

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Abstract Seed oils of wild berries of alpine currant (AC) and northern redcurrant (NRC) were investigated. Oil contents of AC and NRC varied between 4.9 % and 9.7 % and between 12.8 % and 38.5 %, respectively. The proportions of α-linolenic, γ-linolenic and stearidonic acids were higher in NRC (means of 21.7, 12.3 and 5.4 %, respectively) than in AC (16.8, 8.6 and 3.0 %). The growth areas of NRC can be divided into two distinct groups according to the proportions of different polyunsaturated fatty acids. Oil content of NRC correlated negatively to the proportion of linoleic acid and positively to that of stearidonic acid. There were significant correlations between all polyunsaturated fatty acids in NRC but correlations were less obvious in AC. Interestingly, the correlation between linoleic and γ-linolenic acids was positive in NRC but negative in AC. α-Linolenic and stearidonic acids showed positive correlations in both species. In NRC the synthesis of triunsaturated fatty acids is directed either towards α-linolenic or γ-linolenic acids. Results showed great potential in NRC and AC for plant breeding due to the natural variation in the content and composition of the seed oils for functional nutrients.

Key words Northern currants · Seed oil · Fatty acid composition · Correlation · Natural variation

Introduction

There is an ever increasing demand for foods and food supplements with functional, health promoting properties. The oils containing, for example, γ-linolenic acid (18 : 3n-6) as a natural component could be described as functional in several senses. γ-Linolenic acid as a single fatty acid component or in combination with other essential fatty acids has been proposed and even proven to produce a wide range of beneficial health effects, e.g. relieving the symptoms of atopic eczema, lowering the plasma levels of cholesterol, inhibiting platelet aggregation, and balancing the immune defence of the body [1]. Pharmaceutical or dietary oils containing γ-linolenic acid are nowadays mainly produced from the seeds of evening primrose, borage and blackcurrant, as well as from certain fungi. As each oil is likely to have a particular spectrum of effects depending on the co-existing fatty acids and the precise structures of triacylglycerols [2], need for novel oil sources is justifiable.

Geographical location and the climatic conditions of the growth site are known to affect the oil contents and fatty acid compositions of the seeds of many plants [3, 4]. Particularly, a decrease in temperature has been shown to increase the degree of unsaturation and possibly to decrease the chain-length of the fatty acids and, hence, the fluidity of seed lipids, the mechanism of which is supposed to improve the cold-hardiness of a plant [5–9]. The relative amount of γ-linolenic acid in the seed oil of evening primrose (Oenethera biennis L.) has been reported to increase from south to north [10]. In addition, a lower γ-linolenic acid content is characteristic of Oenethera lamarckiana seeds developed at high temperatures (32 °C) compared with lower ones (≤ 25 °C) [11].

γ-Linolenic acid is formed by Δ6-desaturation of linoleic acid, and microsomal fractions from the developing seed embryo have been shown to contain an active Δ6-desaturase enzyme [12–14]. The Δ6-desaturase appears to utilize as substrate the linoleate bound to phosphatidylcholine. The Δ6-desaturase of borage seeds is position-specific, utilizing linoleate in position 2 of phosphatidylcholine only, a phenomenon reflected in the stereospecific structure of borage oil

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triacylglycerols [14]. In borage oil, γ-linolenic acid is found to be concentrated in the sn-2 position of the triacylglycerols, whereas in the seed oils of evening primrose and blackcurrant, γ-linolenic acid is concentrated in the sn-3 position which demonstrates differences in the biosynthesis routes of seed lipids in plants [13–16]. Another nutritionally interesting fatty acid component in the seed oils of currant berries, stearidonic acid (18 : 4n-3), is presumably formed from α-linolenic acid (18 : 3n-3) via Δ6-desaturation rather than from α-linolenic acid via Δ15-desaturation [17].

Previous study has shown a rather narrow range (12–16 %) in the seed oil content of wild currants grown in Finland [18]. Seeds of alpine currant (AC) and northern redcurrant (NRC) were, however, several times bigger than the seeds of blackcurrant. In addition, the seed mass of AC and redcurrant berries was two times higher than that of blackcurrant, hence, AC and NRC yield almost twice the amount of oil on a fresh weight basis. γ-Linolenic and stearidonic acid contents of the wild NRC has been found to be high (16 mol % and 6 mol %, respectively) compared with the values reported earlier for cultivated redcurrant varieties [19, 20]. In this study we chose to apply a one-step extraction/methylation method to a detailed study of the seed oil contents and fatty acid compositions of NRC and AC. Special attention was paid to the geographical variation of the oil composition and to the correlations between the relative amounts of fatty acids providing information on the biosynthesis of the seed oils of currants.

Materials and methods

Berry samples. Berry samples of AC (Ribes alpinum L., 55 samples) and NRC (R. spicatum Robson ssp. Lapponicum Hylander, 49 samples) were collected in the coastal area and the archipelago of South-West Finland (from ca 60° to 61.5°N Lat.) and in northern Lapland (from ca 68.5° to 70°N Lat.), respectively. Seeds were isolated from the frozen, weighed berries, dried to a moisture content of 5–6 %, weighed and stored at the ambient temperature and humidity until analysed.

Extraction/methylation and analysis of fatty acids. The oil content and fatty acid composition of berry seeds was determined in duplicates by a rapid, one-step extraction/methylation method [21]. A sample of 50 mg of seeds was crushed and extracted/methylated in H_2SO_4/MeOH (1 : 99, v/v). Triheptadecanoylglycerol was used as the internal standard. The fatty acid methyl esters formed were dissolved in hexane and stored at –18 °C until analysed twice by gas chromatography on a capillary column (NB-351, 25 m × 0.32 mm i.d., 0.20 μm film thickness, Nordion, Finland). The oven temperature programme was 2 min at 120 °C, a 3 °C min – 1 increase to 230 °C and 10 min at 230 °C. The temperature of the flame ionization detector was 270 °C. Fatty acid methyl ester sample (0.5 μl) was introduced via a temperature programmable split/splitless injector (170 °C for 3 min, a 200 °C min – 1 increase to 250 °C, and 45 min at 250 °C). The injection of the sample was performed in splitless mode for the first minute, after which the split valve was opened (split ratio 40 : 1). Helium was used as the carrier gas (linear flow rate, 30 cm s – 1 at 120 °C). Peaks were identified by comparison to the retention of the fatty acid methyl esters standards. The oil content of the seeds was calculated by comparing the total area of the fatty acid methyl ester peaks to the area of the methyl heptadecanoate peak. The oil yield of the one-step method was compared with the yield of the Folch procedure in which $CHCl_3$/MeOH (2 : 1, v/v) is used as the extracting solvent [22, 23]. The NaOMe-catalysed transesterification was applied to the methylation of fatty acids after $CHCl_3$/MeOH extraction [24]. Berry seeds of a cultivated redcurrant variety (Rotes Wunder from Piikkiö, Finland) were used as reference material in the method comparisons. The extractions were done in triplicate by both methods.

Statistics. In order to examine the geographical variation in the seed oil content and fatty acid composition, the collecting sites of each species were divided into nine distinct growth area groups (Fig. 1). Exceptionally, the 'Others' group (9) of alpine currant consisted of one sample from Pori (ca 61.5°N Lat.) and two from Hanko (ca 60°N Lat.). Comparisons between the oil contents and fatty acid proportions of seeds in the eight groups of AC and nine groups of NRC were carried out using a one-way analysis of variance. The ninth group of alpine currant was excluded from the statistical analyses due to the geographical heterogeneity of the samples in the group. Specific differences were identified using the Newman-Keuls test. Correlation coefficients were calculated by the Pearson product-moment correlation test.

Results and discussion

The seed oil content of a cultivated redcurrant (Rotes Wunder) determined by $CHCl_3$/MeOH (2 : 1, v/v) extraction was 12.5 % by weight (Table 1). When a sample of the same seed material was extracted by the one-step H_2SO_4/MeOH (1 : 99, v/v) method, a yield of 9.9 % was obtained. Thus, when compared with the yield of the $CHCl_3$/MeOH method, the oil yield of the rapid method was ca 79 %. However, repeatability of the two methods proved to be equal. When the fatty acid compositions of oils produced by the two different methods were compared, highly similar fatty acid compositions could be seen (Table 1). Similarly, in the study by Dahmer et al. [21] the oil yield from 50 mg soybean sample determined by the corresponding method was around 80 %, the yield decreasing with increasing sample size. The fatty acid composition of

Table 1 The extraction yield and fatty acid composition of redcurrant (Rotes Wunder) seed oil produced by $CHCl_3$–MeOH (2 : 1, v/v) extraction or by a one-step H_2SO_4–MeOH (1 : 99, v/v) method

Extraction method	$CHCl_3$–MeOH	H_2SO_4–MeOH
Oil yield	12.5 ± 0.9a	9.9 ± 0.9
Fatty acid		
16 : 0	4.6 ± 0.1a	4.8 ± 0.0
18 : 0	2.0 ± 0.0	3.0 ± 0.2
18 : 1n-9	17.1 ± 0.2	17.8 ± 1.0
18 : 1n-7	0.5 ± 0.1	0.6 ± 0.0
18 : 2n-6	41.3 ± 0.1	40.7 ± 0.4
18 : 3n-6	5.9 ± 0.1	5.6 ± 0.4
18 : 3n-3	25.1 ± 0.3	24.5 ± 0.4
18 : 4n-3	3.2 ± 0.0	3.0 ± 0.0
Others	0.3	0.0

[a] Results are weight percentage of fresh weight ± SD

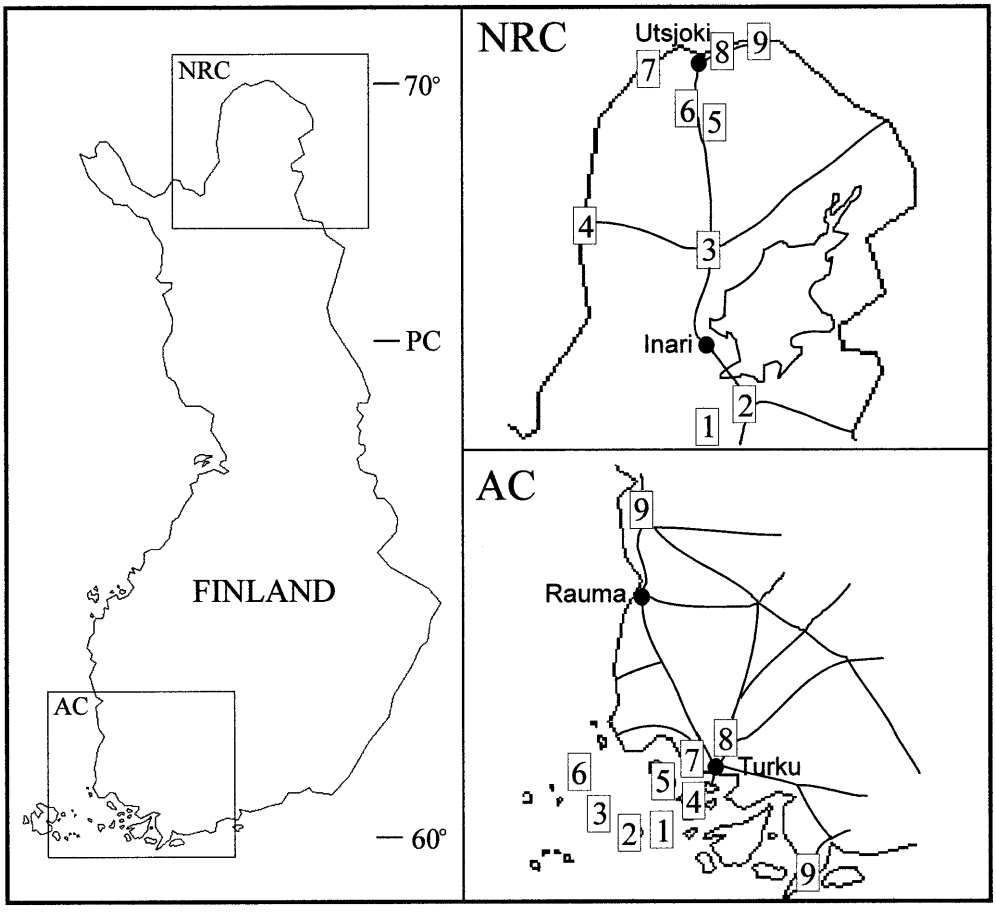

Fig. 1 Collecting areas of alpine currant (*AC*) and northern redcurrant (*NRC*) samples in Finland. AC: Nauvo (*1*), Korppoo (*2*), Houtskär (*3*), Parainen (*4*), Rymättylä (*5*), Brändö (*6*), Naantali (*7*), Lieto (*8*), Others including one sample from Pori and two from Hanko (*9*). NRC: Tolonen (*1*), Ivalo (*2*), Kaamanen (*3*), Roviniemi (*4*), Tsieskuljoki (*5*), Tsarsjoki (*6*), Kostejoki (*7*), Vetsijoki (*8*), Nuorgam (*9*)

the soybean oil produced was unaffected by the low oil yield. Despite a lower oil yield, the rapid, one-step method is thus applicable to the screening of the fatty acid compositions of large numbers of currant seed samples. The seed oil contents of AC and NRC reported here were corrected by dividing the yield of the rapid method by a correction factor of 0.79.

The oil contents of alpine currant seed samples varied between 4.9 % and 9.7 % by weight (Table 2). There was no statistical association between the growth area and seed oil content. The high seed oil content (12.3 %) determined earlier for a single AC sample collected in the archipelago of South-West Finland [18] could have been an occasional finding or a consequence of, for example, different temperature conditions during the seed development. Depending on the plant species, a decrease in growth temperature either increases or decreases the seed oil content of a plant [9, 25, 26]. The oil content of evening primrose, for example, decreases significantly at high temperatures compared with lower ones [11].

The oil contents of NRC seed samples (mean 19.9 %) were clearly higher than those of AC and that of cultivated red currant (Tables 1, 2). As in AC, the seed oil content was unaffacted by the growth area.

Table 2 Seed oil contents of alpine currant and northern redcurrant berry samples collected at different growth areas (see Fig. 1)

Alpine currant	N[a]	Oil content[b]	Range[c]
(1) Nauvo	4	7.7	5.6– 8.7
(2) Korppoo	6	7.5	5.1– 9.7
(3) Houtskär	7	7.0	4.9– 9.1
(4) Parainen	12	7.2	4.9– 9.2
(5) Rymättylä	7	6.6	5.2– 8.4
(6) Brändö	5	7.2	6.1– 8.0
(7) Naantali	8	7.2	5.7– 9.2
(8) Lieto	3	6.8	6.2– 7.5
(9) Others	3	6.7	5.7– 8.2
Redcurrant	**N**	**Oil content**	**Range**
(1) Tolonen	4	18.4	16.6–19.9
(2) Ivalo	10	20.8	17.8–23.9
(3) Kaamanen	7	21.3	16.2–27.2
(4) Roviniemi	7	20.0	12.8–23.2
(5) Tsieskuljoki	3	17.8	15.8–21.3
(6) Tsarsjoki	9	22.2	15.8–38.5
(7) Kostejoki	2	16.8	16.5–17.1
(8) Vetsijoki	4	21.0	15.7–27.7
(9) Nuorgam	3	20.9	15.8–24.7

[a] Number of berry samples in the group
[b] Mean of the group (weight percentage of fresh weight)
[c] Range of oil contents in the group

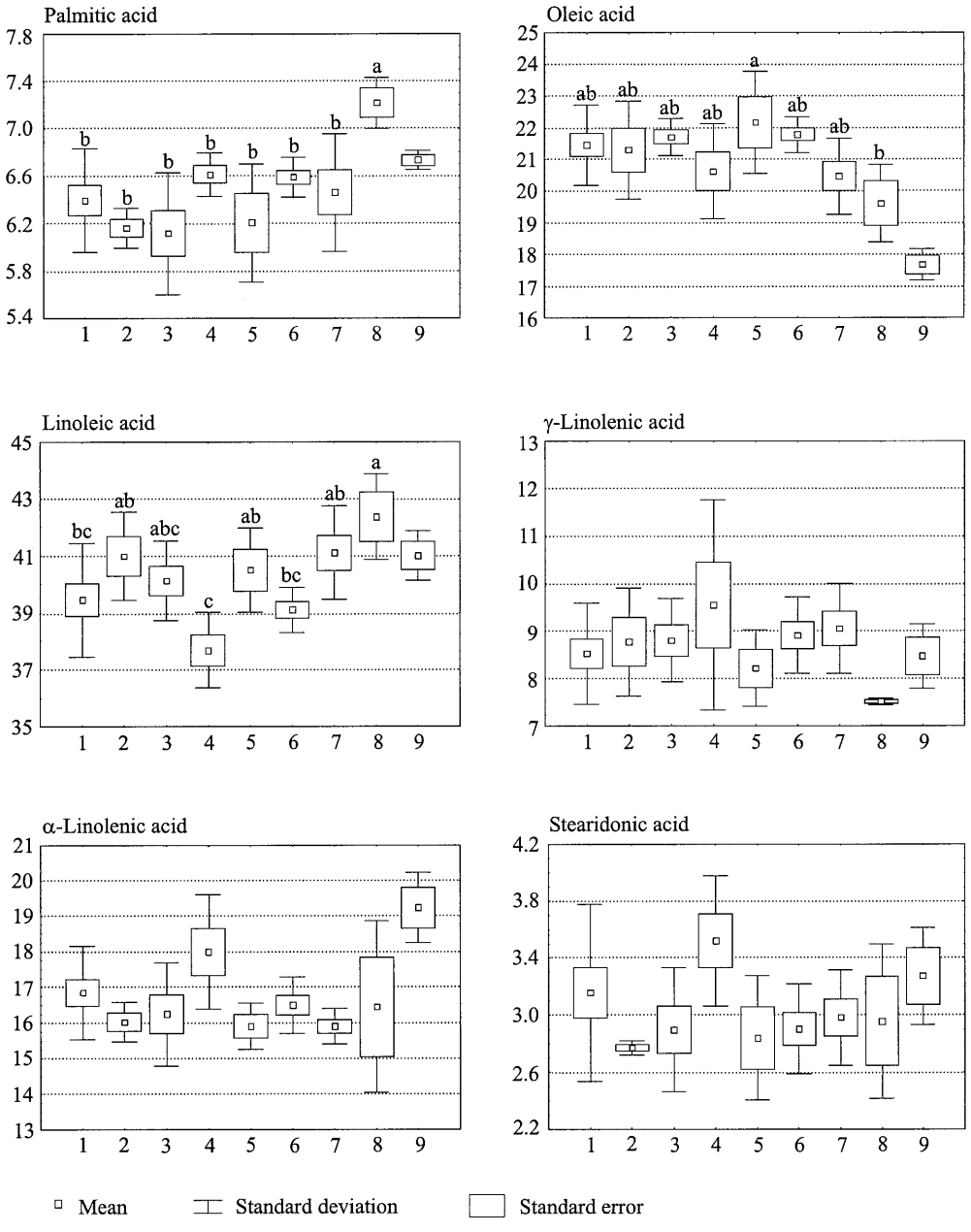

Fig. 2 Variability in the fatty acid composition of AC samples. Nauvo (*1*), Korppoo (*2*), Houtskär (*3*), Parainen (*4*), Rymättylä (*5*), Brändö (*6*), Naantali (*7*), Lieto (*8*), Others (*9*) (see Fig. 1). Sample groups with different letters differ significantly at $P \leq 0.05$

However, seed oil content of the individual redcurrant berry samples varied considerably. There was, for example, one seed sample from Tsarsjoki in which the oil content was as high as 38.5 %.

The seed masses of AC and NRC berries, calculated as weight percentage of fresh weight, varied from 3.3 % to 16.2 % (mean 8.7 %) and from 3.3 % to 8.2 % (mean 5.6 %), respectively. AC berries would therefore yield on the average 6 g and NRC 11 g oil from 1 kg fresh berries. For the sake of comparison, Rotes Wunder would yield ca 4 g oil/kg.

The most abundant fatty acids in both AC and NRC were linoleic (18:2n-6), α-linolenic (18:3n-3), oleic (18:1n-9), α-linolenic (18:3n-6), palmitic (16:0) and stearidonic (18:4n-3) acids, comprising 96–98 % of the total fatty acids (Figs. 2, 3). Some 14:0, 18:0, 20:0, 22:0, 24:0, 16:1n-7, 18:1n-7 and 20:1n-9 fatty acids were also detected. The proportions of γ-linolenic, α-linolenic and stearidonic acids were markedly higher in NRC (means of 12.3, 21.7 and 5.4 %, respectively) than in AC (means of 8.6, 16.8 and 3.0 %, respectively). Instead, the proportion of oleic acid was higher in AC than in NRC (20.7 % and 15.3 %, respectively). When the fatty acid compositions of wild currants were compared with that of Rotes Wunder, some clear differences regarding the polyunsaturated fatty acids were observed (Table 1, Figs. 2, 3). The relative amounts of γ-linolenic and stearidonic acids were much higher and that of α-linolenic acid clearly lower in wild currants than in Rotes Wunder

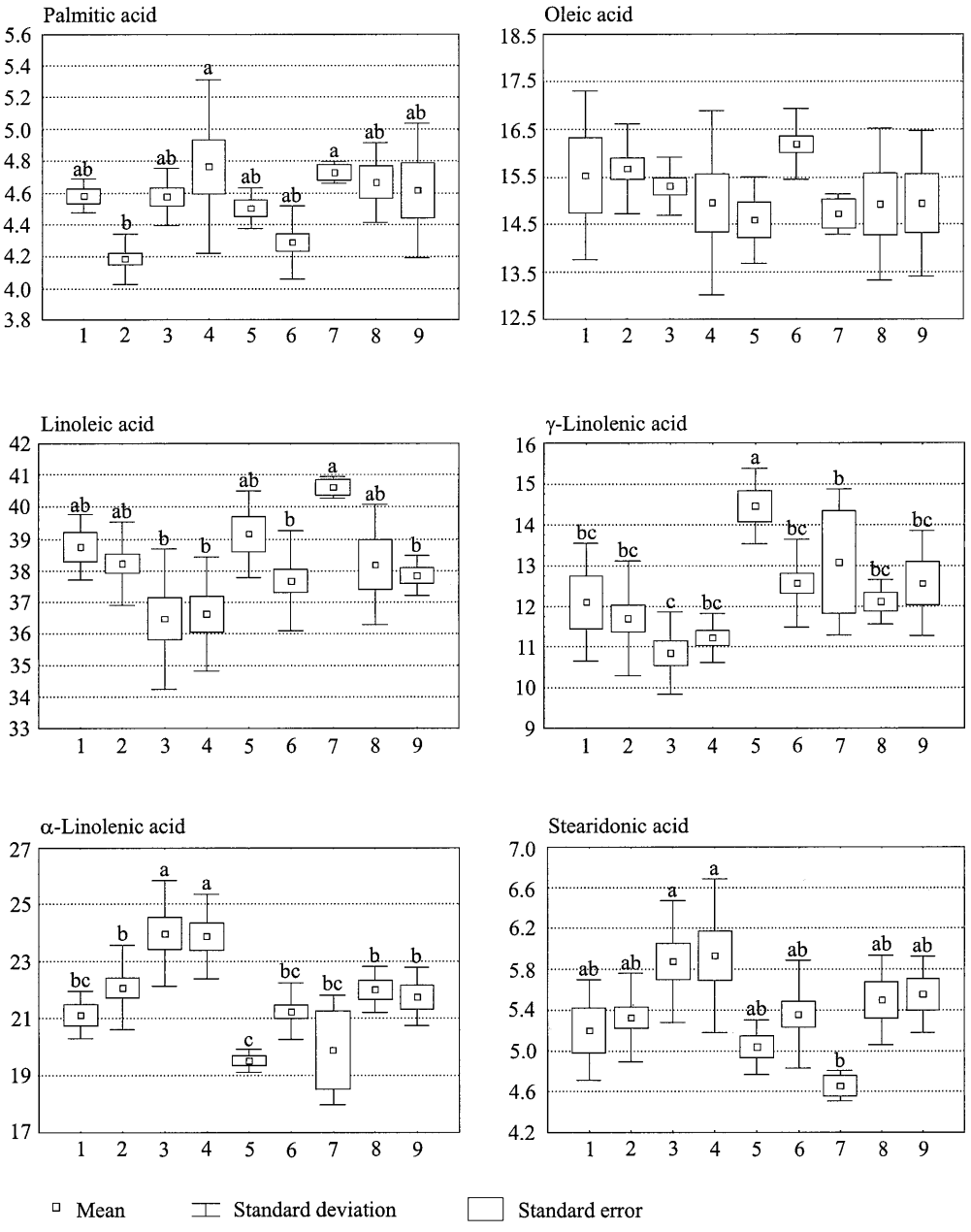

Fig. 3 Variability in the fatty acid composition of NRC samples. Tolonen (*1*), Ivalo (*2*), Kaamanen (*3*), Roviniemi (*4*), Tsieskuljoki (*5*), Tsarsjoki (*6*), Kostejoki (*7*), Vetsijoki (*8*), Nuorgam (*9*) (see Fig. 1). Sample groups with different letters differ significantly at $P \leq 0.05$

seed oil. This together with clearly higher oil contents show a greater value of wild currants with regard to the nutritionally important γ-linolenic and stearidonic acids.

The seed oil proportions of different fatty acids varied remarkably in both currant species. Due to high within-group deviations, no statistical between-group differences in the proportions of polyunsaturated fatty acids of AC could be observed (Fig. 2). The fatty acid composition of NRC was more growth area-specific, i.e., there were statistically significant geographical differences regarding almost all fatty acids present (Fig. 3). The γ-linolenic acid content of NRC from Tsieskuljoki (site 5), for example, was exceptionally high (14.5 %). Most interestingly, the growth areas of NRC could be divided into two distinct groups according to the proportions of polyunsaturated fatty acids. In the more northern group (areas 5–9), the content of γ-linolenic acid was high (mean of five groups, 13.0 %) whereas that of α-linolenic acid was low (20.9 %) compared with the more southern group (areas 1–4, mean of four groups, 11.5 % and 22.8 %, respectively). In addition, the proportion of linoleic acid closely parallelled that of γ-linolenic acid, and the proportion of stearidonic acid that of α-linolenic acid. Lotti et al. [10] have found that the relative amount of γ-linolenic acid in evening primrose increases from south to north in locations between 38° and 56°N Lat. A tendency to increasing content of linoleic acid and decreasing content of α-linolenic acid

Table 3 Correlation coefficients between the polyunsaturated fatty acids of alpine currant and northern redcurrant seed oil

	Alpine currant		
	Linoleic acid	γ-Linolenic acid	α-Linolenic acid
γ-Linolenic acid	−0.76*	–	0.16
α-Linolenic acid	−0.27	0.16	–
Stearidonic acid	−0.72*	0.65	0.73*
	Northern redcurrant		
	Linoleic acid	γ-Linolenic acid	α-Linolenic acid
γ-Linolenic acid	0.72*	–	−0.92**
α-Linolenic acid	−0.89**	−0.92**	–
Stearidonic acid	−0.98**	−0.74*	0.93**

* Significant at P %<0.05; ** significant at $P<0.001$

of seed oil towards the more northern growth sites within Finland has been observed for cloudberry (Rubus chamaemorus) and crowberry (Empetrum nigrum) [6].

The seed oil content of NRC correlated negatively to the proportion of linoleic acid and positively to that of stearidonic acid ($P<0.05$). No correlation between the oil content and the proportions of individual fatty acids was observed in AC. Oil content and linoleic acid proportion have also been shown to correlate negatively in the seeds of lupine (Lubinus albus) [27]. In lupine, however, the seed oil content correlated negatively to α-linolenic acid too, but positively to oleic acid [27].

When the correlation coefficients between the polyunsaturated fatty acids of currant seeds were examined, some clear differences between the two wild currant species could be doserved. There were significant correlations between all polyunsaturated fatty acids in NRC, whereas weak correlations were found in the pairs linoleic/γ-linolenic, linoleic/stearidonic and α-linolenic/stearidonic acids in AC only (Table 3). Interestingly, the correlation between linoleic and γ-linolenic acids (i.e. substrate and product of Δ6-desaturase) was positive (r=0.72) in redcurrant but negative ($r=-0.76$) in AC. The corresponding pair of the n-3 fatty acid family, i.e. α-linolenic and stearidonic acids, showed positive correlations in both currant species. The production of triunsaturated fatty acids in NRC is directed either towards α-linolenic acid or γ-linolenic acid with a correlation coefficient of $r=-0.92$. There was no correlation between the triunsaturated fatty acids in AC. In spite of the generally assumed same biosynthesis route via Δ6-desaturase, γ-linolenic and stearidonic acids correlated negatively in NRC. The trend was positive in AC although not statistically significant. This means that it would be highly unlikely to find a natural NRC variety with high contents of both γ-linolenic and stearidonic acids. Big differences between the correlation matrices of the currants could be explained by such factors as availability of substrates, alternative biosynthesis routes, activation/inhibition of enzymes etc.

The present study shows that a rapid, one-step extraction/methylation method utilizing H_2SO_4–MeOH is applicable to the determination of fatty acid composition and variation in oil content of large numbers of currant seed samples. It would, therefore, be a suitable method of analysis in plant breeding experiments where a large number of small samples is to be analysed. The study also shows that due to the naturally high seed masses of wild alpine and redcurrant berries, the oil yield from the berries is reasonably high. These currant species would, therefore, be of great value when novel, productive oil seed crops are to be breeded. There was variation in the seed oil content and in the proportions of nutritionally important polyunsaturated fatty acids in both currant species and, thus, potential for plant breeding. Whether the variance is due to differences in genotype or to the different growth conditions, i.e. latitude, altitude, vicinity of the sea etc., will be tested by cultivating the same varieties in controlled conditions. According to the correlation coefficients obtained, selection for improved fatty acid composition should not have a negative effect on seed oil yield from currant berries.

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