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Genotypic Variation in Fatty Acid Content of Blackcurrant Seeds

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The fatty acid composition and total fatty acid content of seeds from 36 blackcurrant genotypes developed at the Scottish Crop Research Institute were examined. A rapid small-scale procedure, involving homogenization of seeds in toluene followed by sodium methoxide transesterification and gas chromatography, was used. There was considerable variation between genotypes. The γ-linolenic acid content generally varied from 11 to 19% of the total fatty acids, but three genotypes had higher values of 22−24%, levels previously not reported for blackcurrant seed and similar to those for borage seed. Other nutritionally important fatty acids, stearidonic acid and α-linolenic acid, varied from 2 to 4% and 10−19%, respectively. The mean total fatty acid contents ranged from 14 to 23% of the seed, but repeatability was poor. The results are discussed. Blackcurrant seeds are mainly byproducts from juice production, and the study shows the potential for developing blackcurrant genotypes with optimal added value.

KEYWORDS: Alpha-linolenic acid; blackcurrant seed oil; fatty acids; gamma-linolenic acid; *Ribes nigrum*; stearidonic acid

INTRODUCTION

Gamma-linolenic acid [GLA, 18:3(*n*-6)] is the intermediate in the hepatic bioconversion of dietary linoleic acid [LA, 18:2(*n*-6)] to dihomogammalinolenic acid [DGLA, 20:3(*n*-6)] and then to arachidonic acid [AA, 20:4(*n*-6)]. DGLA and AA are precursors of eicosanoids that have a wide range of biological effects (*1*). The transformation of LA to GLA by the Δ6-desaturase enzyme is considered to be the rate-limiting step in the pathway (*2*). Supplementation with GLA has shown to be of value for a range of conditions. Its efficacy in alleviating conditions such as rheumatoid arthritis and atopic eczema appears to be due mainly to an efficient increase in antiinflammatory eicosanoids as a result of bypassing the rate-limiting step (*3*). Indeed, patients with atopic eczema appear to have impaired Δ6-desaturase activity. Other attributes of GLA include antitumor properties (*3*) and hypocholesterolemic activity (*4*).

There are few significant natural sources of GLA. The two major commercial sources are the seed oils of evening primrose (*Oenothera biennis* L.) and borage (*Borago officinalis* L.) with GLA contents of 7−10% and 17−25%, respectively (*5*). A third source is blackcurrant (*Ribes nigrum*) seeds (15−19% GLA), which are essentially byproducts from juice production. In contrast to the other two oils, blackcurrant oil contains significant amounts of other nutritionally important fatty acids, namely α-linolenic acid [ALA, 18:3(*n*-3), about 13%] and stearidonic acid [SA, 18:4(*n*-3), about 3%]. ALA is an essential fatty acid that is metabolized to eicosapentaenoic acid (EPA), a precursor of eicosanoids with antiinflammatory and antithrombotic activity. SA is formed from ALA by Δ6 desaturase and an increase in its consumption may be an efficient way to increase the longer chain *n*-3 acids (e.g., EPA) by overcoming the rate-limiting Δ6 desaturase step. *Echium plantagineum* is the only other commercial oil that contains GLA (about 10%), SA (about 15%), and ALA (about 30%).

The aim of the study was to determine the variation in fatty acid composition (GLA in particular, but also ALA and SA) and total fatty acid content in a range of blackcurrant genotypes, with a view to evaluating the potential for optimization of this parameter for commercialization.

MATERIALS AND METHODS

Material. Ripe fruit was collected from four-year-old blackcurrant plants of 36 different genotypes grown under similar field conditions at the Scottish Crop Research Institute (SCRI), Dundee, UK. The parentage of the material used is presented in Table 1. Genotypes were selected as a representative sample of the available germplasm in the SCRI *Ribes* breeding program, together with the Norwegian cultivar Hedda and SCRI-bred cultivar Ben Gairn. The fruit was liquidized, and the supernatant was decanted to leave the seeds.

Procedures. *Rapid Method for Extracting Oil and Forming Fatty Acid Methyl Esters*. A rapid small-scale method involving simultaneous oil extraction and transesterification to fatty acid methyl esters (FAMEs) was utilized. Around 100 mg of sample was transferred to a test tube, and 2.0 mL of C21:0 methyl ester internal standard solution (25 mg/20 mL of toluene) was added. After homogenizing the sample for 4 min using an Ultra-Turrax blender and decanting the liquid into a clean test tube, 2.0 mL of 0.5 N sodium methoxide solution was added, and

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Table 1. Parentage of Blackcurrant Genotypes

selection number	female parent	male parent
S36-4-8	243/7	Sunderbyn II
9139-1	Ben Avon	Tifon
8966-9	S13-2-9	S18-24-60
S36-1-2	Ben Alder	C7-4-24
9146-1	Ben Alder	Andega
Hedda	Ojebyn	Melalahti
S36-1-100	Ben Alder	Ben Loyal
912-1	S18-1-8	B1834-120
S12-3-43	C2-1-62	open pollinated
918-1	S26-6-91	open pollinated
9141-1	Ben Alder	S18-15-1
S36-2-39	C7-4-24	Imandra
9118-1	Ben Avon	open pollinated
9137-1	Ben Avon	Ben Hope
9126-1	Ben Alder	Andega
Ben Gairn	Ben Alder	Golubka
S18-1-8	Ben More	C2-1-62
9135-1	S26-6-91	open pollinated
9119-1	S18-4-40	open pollinated
917-1	S18-25-62	Ben Connan
S18-4-40	C2-4-51	RiIII-10
9133-1	Ben Tirran	Tifon
S36-3-11	Ben Gairn	C7-5-24
S18-4-37	C2-4-51	RiIII-10
C12-22-31/33	Baldwin	Sunderbyn II
S34-1-4	AB3/7	C6-12-36
914-1	Ben Tirran	B1610-68
S26-5-1	C2-4-51	Ben Alder
S36-4-55	P10-9-20	74020-19
9120-1	S18-1-18	S18-24-54
9142-1	S18-25-62	B1834-120
9138-1	S26-9-67	open pollinated
913-1	Ben Avon	Tifon
9111-1	Ben Alder	B1610-68
S26-10-70	complex parentage	Ben Lomond
9114-1	S18-22-24	open pollinated

the mixture was heated at 50 °C on a heating block for 10 min. Subsequently, 100 μL of glacial acetic acid, 5 mL of saturated sodium chloride, and 3 mL of isohexane containing butylated hydroxytoluene (BHT; 50 ppm) were added. After shaking the tube and centrifuging the contents, the upper isohexane layer was removed and put through an anhydrous sodium sulfate column. The extraction was repeated once more with 3 mL of isohexane with BHT, and finally the column was washed through with 1 mL of isohexane with BHT. The sample was then ready for analysis by gas chromatography (GC).

Large-Scale Extraction of Oil Followed by Formation of Fatty Acid Methyl Esters. An aliquot of approximately 10 g of seeds was weighed and ground in an electric coffee mill for 30 min, and the oil was Soxhlet extracted with 250 mL of isohexane for 1.5 h. After re-grinding the residue, the extraction was repeated with the same solvent for a further 1.5 h. The bulk of the solvent was removed by rotary evaporation, and the remainder was removed under nitrogen on a heating block at 100 °C for 30 min until constant weight was achieved.

FAMEs were prepared by dissolving an aliquot (30−50 mg) of the oil in 1 mL of dichloromethane and adding 2 mL of 0.5 M sodium methoxide in methanol. The mixture was treated as described above .

Gas Chromatography of Fatty Acid Methyl Esters. GC of FAMEs was performed using a Hewlett-Packard model 5890 series II gas chromatograph equipped with split/splitless injector, autosampler, and flame ionization detector (FID). A capillary column of fused silica coated with CP-Wax 52CB (0.25 mm i.d. × 25 m in length, 0.2 μm film thickness; Chrompack UK Ltd., London) was used, and hydrogen was the carrier gas at an initial flow rate of 1 mL/min. After holding the temperature at 170 °C for 3 min, the column was temperature-programmed at 4 °C/min to 220 °C, then was held at this point for a further 15 min. In all analyses, the detector was set at 300 °C, the injector was set at 230 °C, and a split ratio of 50:1 was used. An HP 3365 Chemstation (Hewlett-Packard Ltd., Stockport, UK) was used for data acquisition.

Calculations. Fatty acid compositions, as weight percents of total fatty acids, were calculated using area counts from the gas chromatogram of the FAME.

Total fatty acid contents, as weight percents of seeds, were measured using the following equation:

$$\%\ \mathrm{FAME} = \frac{\Sigma(A_{\mathrm{XA}} \times \mathrm{CF_X})W_{\mathrm{IS}}}{A_{\mathrm{IS}} \times W_{\mathrm{A}}} \times 100$$

where A_{XA} = area counts of individual FAME, A_{IS} = area count of C21:0 methyl ester internal standard, W_{IS} = mass internal standard added to sample, W_{A} = mass of seeds used, and $\mathrm{CF_X}$ = theoretical correction factor relative to C21:0 methyl ester (IS) (*6*).

The % FAME was essentially equal to the % triacylglycerol in the seed.

Total oil content, as a weight percent of seeds, was measured gravimetrically for large-scale oil extractions.

RESULTS AND DISCUSSION

For each sample, the fatty acid composition and total fatty acid content were determined by the rapid method for at least three replicates. The fatty acid profiles of all genotypes were typical of blackcurrant oil. LA (42.3−53.3% of total fatty acids) was the major component, followed by GLA (11.6−24.6%) and ALA (10.0−19.2%), then oleic acid (6.6−11.9%), palmitic acid (5.6−7.3%), SA (2.4−4.3%), and stearic acid (1.1−1.8%). 18:1(*n*-7) and 20:1(*n*-9) were present at around 0.8%, and there was about 0.3% of 20:2 (*n*-6). 14:0, 16:1(*n*-7), 20:0, 20:1(*n*-11), and 22:0 were all about 0.2% or less.

The percentage fatty acid compositions varied little between replicates (Table 2), and agreed well with the values determined for FAMEs prepared from oil extracted by the large-scale method. For example, the mean GLA, ALA, and SA values for genotype 9114-1 were 24.6, 10.4, and 3.3%, and 24.3, 10.9, and 3.4% by the two methods, respectively. For Hedda, the values were 13.8, 17.3, and 3.8%, and 13.8, 17.2, and 3.8%, respectively. These data validated the use of the rapid small-scale method. As an aside, because of the good repeatability and because the method used only a few seeds, it was implied that there was only minor seed-to-seed variation in fatty acid composition.

There was considerable variation in fatty acid composition between cultivars. We focused this study on only GLA, ALA, and SA contents because of their nutritional interest. With regard to ALA and SA (Table 2), their percentages of total fatty acids ranged from 10.0% in S26/10/70 to 19.2% in S36/4/8 and from 2.4% in S12/3/43 and 9118-1 to 4.3% in S36/3/11, respectively. It has been reported previously (*7*) that the oils from blackcurrant seeds contain 12−14% ALA and 3−4% SA.

The GLA content (Table 2) ranged from 11.6% (S36/4/8) to 18.9% (913-1 and 9138-1) for the majority of genotypes. Nevertheless, three of the 36 genotypes (9111-1, S26/10/70, and 9114-1) exhibited higher than 20% GLA, with values of 22.0, 24.3, and 24.6%, respectively. In previous studies (*7*−*9*) GLA levels up to 19% have been described, but our study is the most extensive so far. The highest levels we found in three of our genotypes were similar to those reported in borage oil (*5*), and are one of the richest sources of GLA to date.

The full fatty acid profiles of the three high-GLA genotypes are presented in Table 3. It can be seen that these genotypes contain the lowest levels of ALA of all samples (Table 2). Conversely, some of the high ALA samples (e.g., S36/4/8 and S36/1/2) have the lowest GLA contents. This phenomenon could indicate competitive Δ6 and Δ15 desaturation activities on LA, the common precursor. However, an absolute inverse trend was

Table 2. GLA, ALA, SA, (weight % of total fatty acids) and Total Fatty Acid Contents (weight % of seeds), in Order of Increasing GLA Content, in Seeds of Blackcurrant Genotypes

genotype	GLA content	ALA content	SA content	total content
S36-4-8	11.6 (0.2)[a]	19.2 (0.4)	3.9 (0.1)	21.8 (4.1)
9139-1	12.6 (0.7)	14.4 (0.3)	2.7 (0.1)	18.0 (1.4)
8966-9	12.9 (0.3)	16.0 (0.1)	2.9 (0.1)	22.2 (6.8)
S36-1-2	13.6 (0.2)	18.9 (0.1)	4.1 (0.1)	18.1 (3.8)
9146-1	13.7 (0.1)	14.1 (0.6)	2.9 (NS)	17.8 (2.1)
Hedda	13.8 (0.1)	17.3 (NS)[b]	3.8 (NS)	19.7 (3.3)
S36-1-100	14.2 (0.1)	14.6 (0.2)	3.0 (0.1)	18.0 (5.2)
912-1	14.2 (0.2)	15.4 (1.2)	3.4 (NS)	18.6 (1.4)
S12-3-43	14.3 (0.2)	12.4 (0.1)	2.4 (NS)	19.7 (3.9)
918-1	14.5 (0.5)	15.0 (0.9)	3.4 (0.1)	17.5 (1.7)
9141-1	14.5 (0.3)	12.6 (0.2)	2.8 (0.1)	16.0 (2.7)
S36-2-39	14.7 (0.1)	12.8 (0.2)	2.9 (0.1)	18.2 (3.8)
9118-1	14.9 (0.1)	12.3 (0.1)	2.4 (0.1)	17.0 (2.0)
9137-1	14.9 (0.9)	13.3 (0.1)	3.1 (0.2)	14.7 (2.0)
9126-1	15.0 (0.2)	14.6 (0.3)	3.3 (0.1)	16.4 (1.9)
Ben Gairn	15.8 (0.3)	15.0 (0.1)	3.5 (0.1)	18.1 (2.3)
S18-1-8	16.0 (0.1)	13.6 (0.3)	3.2 (0.1)	20.8 (4.0)
9135-1	16.2 (0.2)	14.0 (0.2)	3.2 (0.1)	13.7 (1.9)
9119-1	16.2 (0.1)	11.9 (0.1)	3.0 (NS)	17.7 (1.4)
917-1	16.5 (0.1)	13.5 (0.1)	3.4 (0.1)	15.0 (0.2)
S18-4-40	16.5 (0.3)	13.6 (0.1)	3.5 (0.1)	18.6 (2.6)
9133-1	16.8 (0.1)	13.6 (0.3)	3.5 (0.1)	18.1 (6.4)
S36-3-11	16.8 (0.2)	15.9 (0.2)	4.3 (0.1)	18.6 (3.4)
S18-4-37	17.1 (0.1)	15.3 (1.3)	3.9 (0.1)	22.7 (7.3)
C12-22-31/33	17.1 (0.3)	15.7 (0.2)	4.2 (0.1)	17.5 (3.7)
S34-1-4	17.3 (0.2)	14.0 (0.2)	3.6 (0.1)	16.4 (1.9)
914-1	17.5 (0.5)	14.6 (0.4)	3.5 (0.1)	17.9 (1.1)
S26-5-1	17.7 (0.5)	12.7 (0.4)	3.1 (0.1)	18.7 (3.6)
S36-4-55	18.2 (0.1)	13.5 (0.2)	3.5 (0.1)	17.3 (5.6)
9120-1	18.4 (0.2)	13.9 (0.3)	3.8 (0.1)	15.7 (3.1)
9142-1	18.6 (0.8)	12.3 (0.2)	3.3 (0.1)	17.6 (3.2)
9138-1	18.9 (0.1)	14.6 (0.4)	4.2 (0.1)	16.0 (2.4)
913-1	18.9 (0.4)	12.6 (0.1)	3.5 (0.1)	15.2 (2.4)
9111-1	22.0 (0.5)	11.5 (0.1)	3.4 (NS)	16.1 (1.6)
S26-10-70	24.3 (0.6)	10.0 (0.2)	3.2 (0.1)	16.9 (4.2)
9114-1	24.6 (0.5)	10.4 (0.2)	3.3 (NS)	16.2 (2.4)

[a] Standard deviation values are given in parentheses. [b] Not significant.

Table 3. Fatty Acid Composition of the Seeds (weight % of total fatty acids) of the Three Blackcurrant Genotypes with Higher than 20% GLA Content

	genotype		
fatty acid	9111-1	S26/10/70	9114-1
16:0	6.0 (0.1)[a]	6.3 (0.1)	6.2 (0.2)
16:1 (n-7)	0.1 (NS)[b]	0.1 (NS)	0.1 (NS)
18:0	1.6 (NS)	1.4 (NS)	1.3 (NS)
18:1(n-9)	9.6 (0.1)	8.9 (0.1)	9.3 (0.1)
18:1 (n-7)	0.7 (NS)	0.7 (NS)	0.8 (NS)
18:2	43.5 (0.3)	43.2 (0.3)	42.7 (0.3)
γ-18:3	22.0 (0.5)	24.3 (0.6)	24.6 (0.5)
α-18:3	11.5 (0.1)	10.0 (0.3)	10.4 (0.2)
18:4	3.4 (NS)	3.2 (0.1)	3.3 (NS)
20:0	0.2 (NS)	0.2 (NS)	0.1 (NS)
20:1 (n-11)	0.1 (NS)	0.1 (NS)	0.1 (NS)
20:1 (n-9)	0.9 (NS)	1.4 (NS)	0.8 (NS)
20:2 (n-6)	0.4 (NS)	0.4 (NS)	0.4 (NS)

[a] Standard deviation values are given in parentheses. [b] Not significant.

not apparent across the full range of genotypes (Table 2). It is interesting that there does not appear to be a correlation of GLA content with parentage (Table 1).

There was considerable variation in total fatty acid content (as a percentage of the total seed weight) between the genotypes, ranging from mean values of 13.7% (9135-1) to 22.7% (S18/4/37) (Table 2). The mean values for the 3 genotypes of high GLA content were low (16−17%). For most samples, there was considerable variation between replicates. This can be explained by either incomplete extraction/transmethylation during the analytical procedure or by considerable seed to seed variation in oil content, which became apparent due to the small sample size required for the analysis. The latter explanation is possibly more likely as we observed considerably less variability using the same methodology with borage and evening primrose seeds (unpublished). On the other hand, differences in moisture content or in surface properties among the different types of seeds, neither of which were investigated, could affect the extraction/transmethylation efficiency. The total oil content (approximately equal to total fatty acid content, but with the addition of small amounts of nonsaponifiable matter) was determined for two samples for comparison. One sample (Hedda) gave the same result (19.8%) as with the rapid method, but the other sample (9114-1) gave a significantly higher value (19.2% compared with a mean value of 16.2% for the rapid method). The value for the total oil content is likely to be more representative of the bulk because of the much larger sample used.

In conclusion, a pilot study has shown the potential for breeding and selection of blackcurrant genotypes with high GLA content. The genotypes with high GLA content had the lowest ALA content. In terms of the nutritional profile of the oil, from one standpoint, this would appear to be unimportant, as high ALA oils for *n*-3 supplementation are available from other sources (e.g., linseed) and, in any case, the two types of fatty acids are usually targeted at different conditions. On the other hand, blackcurrant seed oil has been shown to be effective in treatment of rheumatoid arthritis patients (*10*), possibly due to a combination of the different antiinflammatory effects of the eicosanoids derived from DGLA (derived from GLA) and EPA (derived from ALA).

The scope for increasing the SA content of blackcurrant oil is limited, and other sources, e.g., *Echium plantagineum*, have higher levels (*5, 11*). Interestingly, in a recent study on the seed oils of several *Echium* species (*11*), species with high GLA, ALA, and SA levels were detected. For example, *E. callithyrsum* contained 26.3, 24.9, and 9.4%, respectively.

The aim is now to extend the study to potentially commercial genotypes that will be optimized for both juice quality and fatty acid profile of the seed oil, enabling the development of cultivars with a greater added value.

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