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Fatty Acid Content and Juice Characteristics in Black Currant (*Ribes nigrum* L.) Genotypes

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The fatty acid compositions of seeds from 29 black currant genotypes were determined using a rapid small-scale procedure. There was interest in α-linolenic, stearidonic, and, especially, γ-linolenic acid (GLA) contents, and most samples showed values between 11.1 and 18.7%, between 2.5 and 4.5%, and between 11.6 and 17.4%, respectively. However, six genotypes exhibited γ-linolenic contents >18%, and values >20% were recorded in four of these genotypes. The fatty acid contents of the six genotypes were also analyzed by using a conventional procedure, and only slight differences in fatty acid composition were found between the two methods. Although GLA content was not strongly correlated with juice parameters, some genotypes had both high GLA contents and desirable juice characteristics. The results obtained provide evidence that it is possible to select for GLA contents without negatively affecting juice quality, and both aspects can be combined in a single cultivar, thereby increasing the added value of the whole fruit.

KEYWORDS: **γ-Linolenic acid; *Ribes nigrum*; black currant seed; black currant juice; fatty acids**

INTRODUCTION

Black currants (*Ribes nigrum* L.) are cultivated for the production of their berries, which have been widely demonstrated to have considerable health benefits, mainly attributable to their relatively high amount of ascorbic acid (vitamin C) and, consequently, exceptional antioxidant activity compared with other fruits and vegetables (*1*). Black currant berries are mainly consumed in the form of juice, which has been reported to maintain berry antioxidant activity (*1*). Nowadays, health awareness acquired by the consumer has led to a demand for superior natural black currant juice color and reduced overall acidity as well as higher vitamin C content (*2*).

During black currant juice production, seeds are obtained in large quantities in the form of pomace remaining as a residue. The oil from black currant seeds contains some fatty acids of nutritional significance, namely, γ-linolenic [GLA, 18:3(*n*−6), ∼16% of the total fatty acids], α-linolenic [ALA, 18:3(*n*−3), ∼13%], and stearidonic acids [SA, 18:4(*n*−3), ∼3%]. In humans, ALA is the immediate precursor to SA, which after elongation and desaturation gives rise to eicosapentaenoic acid [EPA, 20:5(*n*−3)], the precursor of eicosanoids that show anti-inflammatory and antithrombotic activities (*3*).

GLA is a metabolite of linoleic acid [LA, 18:2(*n*−6)] and, in humans, is intermediate in the bioconversion of linoleic to arachidonic acid [AA, 20:4(*n*−6]. The transformation of LA to GLA involves a desaturation process by a Δ^6-desaturase enzyme that is believed to be the rate-determining step in the bioconversion of LA to AA (*4*). Δ^6-Desaturase enzymatic activity has been described to be impaired in certain pathologic conditions such as rheumatoid arthritis (*5*) or atopic eczema (*6*). In these cases, exogenous supplements of GLA have proved to be of great value because the rate-limiting step is bypassed. Hypertension (*7*), diabetes (*8*), and cancer (*9*) are other conditions shown to be attenuated by supplementation of the diet with GLA.

The positive effects on human health are the basis for the current interest in GLA and in those oils in which it occurs. GLA is found in varying amounts in the seeds of a wide range of plant species, but only two species, together with black currant, have commercial importance: evening primrose (*Oenothera biennis* L.) and borage (*Borago officinalis* L.) with GLA percentages of 7−10 and 17−25% of the total fatty acids, respectively (*10*).

Recently, several *Echium* species from Macronesia have been reported as the best sources of GLA so far found in nature, showing contents of up to 26.3%. Relatively high GLA amounts (up to 20.25%) have been described also in new plant species from Spain (*11*−*13*).

Black currant seed oil has been reported to contain 15−19% GLA (*10*). In a recent study of 10 cultivars, values of 12−16% were obtained (*14*), and in an earlier study, including 36 genotypes, GLA ranged from 11 to 19% for most genotypes, but 3 had especially high values between 22 and 24% (*15*). The present study aimed to determine the fatty acid composition, with special interest in GLA content, in black currant seeds from genotypes with high potential for fruit and juice production. At the same time, several parameters in the juice were measured.

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The aim was to highlight genotypes that were highly acceptable in both juice characteristics and fatty acid content, that is, those that show potential for greater added value. Possible linkages between juice quality parameters and GLA content were also analyzed.

MATERIALS AND METHODS

Material. Fruit samples from 29 different genotypes were picked by hand at commercial ripeness (85% black fruit) from 4-year-old black currant bushes grown under identical field conditions at the Scottish Crop Research Institute (SCRI), Dundee, U.K.

The fruits were immediately frozen and stored at −20 °C prior to juice and seed extractions. Subsequently, the fruit was liquidized, and the supernatant was decanted to separate the juice from the seeds. Juice samples from each genotype were diluted first and then subjected to pasteurization, which was achieved by heating at 75 °C in a water bath for 15 min. After that, the samples were rapidly cooled using a hot/cold water exchange.

Procedure. *Rapid Small-Scale Method for Extracting Oil and Forming Fatty Acid Methyl Esters (FAMEs).* This method involves simultaneous oil extraction and transesterification to form FAME derivatives. Approximately 100 mg of seeds was weighed in a test tube, and 2.0 mL of C21:0 methyl ester internal standard solution (1.25 mg/mL) was added. The sample was homogenized for 4 min using an Ultra-Turrax blender and the liquid decanted into a clean test tube. Subsequently, 2.0 mL of 0.5 N sodium methoxide solution was added to each sample, which was, subsequently, heated at 50 °C on a heating block for 10 min. After that, 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 the mixture had been shaken and centrifuged (5 min, 300 rpm), the upper isohexane layer containing the FAMEs was removed and put through an anhydrous sodium sulfate column. The extraction was repeated again with 3 mL of isohexane with BHT, and finally the column was washed through with 1 mL of isohexane of BHT. The sample was then ready for gas chromatographic (GC) analysis.

Regular Procedure for Extracting Oil Followed by Formation of FAMEs. Around 10 g of seeds, without any kind of prior treatment, was weighed and ground in an electric coffee mill for 30 min, and the oil was extracted with 250 mL of isohexane for 1.5 h using a Soxhlet apparatus. The residue was reground and the extraction repeated once more using the same solvent for another 1.5 h. The bulk of the solvent was removed by rotary evaporation and the remainder under nitrogen on a heating block at 100 °C for 30 min until constant weight was reached.

FAMEs were prepared by dissolving an aliquot (30−50 mg) of the oil in 1 mL of dichloromethane and adding 2.0 mL of 0.5 M sodium methoxide in methanol. The mixture was treated and the FAMEs were extracted as described above.

Analysis of FAMEs by GC. A Hewlett-Packard model 5890 series II gas chromatograph equipped with a split/splitless injector, an autosampler, and a flame ionization detector (FID) was used. Chromatographic separations of FAMEs were performed on a CP-Wax 52CB capillary column (0.25 mm i.d. × 25 m in length, 0.2 μm film thickness; Chrompack U.K. Ltd., London, U.K.), using hydrogen as the carrier gas at a flow rate of 1 mL/min. Initially the column was maintained at 170 °C for 3 min, and then the temperature was increased at 4 °C/min to 220 °C and held at this value for 15 min. The detector and injector temperatures were 300 and 230 °C, respectively, and a split ratio of 50:1 was used. An HP 3365 Chemstation (Hewlett-Packard Ltd., Stockport, U.K.) was utilized for data acquisition. Fatty acid composition was expressed as weight percents of total fatty acids. The total oil content was considered to be approximately equal to total fatty acid content, but with the addition of small amounts of nonsaponifiable matter.

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

$$\%\ \mathrm{FAME} = \Sigma \frac{(A_{XA} \times \mathrm{CF}_X) \times W_{IS}}{A_{IS} \times W_A} \times 100$$

where A_{XA} = area counts of individual FAME, A_{IS} = area count of C21:0 methyl ester internal standard, W_{IS} = mass of internal standard added to the sample, W_A = mass of seeds used, and CF_X = theoretical correction factor relative to C21:0 methyl ester (IS) (*16*).

The percent FAME was essentially equal to the percent triacylglycerols in the seed.

Total oil contents (wt % wt^{-1}) were measured gravimetrically for oils extracted according to the regular procedure.

Juice Analyses. Parameters of juice quality were determined as follows. Total anthocyanin content was measured by spectrophotometric analysis. Juice samples were diluted to 5% v/v in pH 1.0 buffer (0.2 M KCl/HCl), and absorbances were recorded at 515 nm using a Pye Unicam SP6-300 spectrophotometer.

To determine titratable acidity (expressed as percent w/v citric acid monohydrate), neat juice was diluted with water to give a "standardized juice" with a specific gravity of 1.0545. The standardized juice (1 mL) was diluted with distilled water (50 mL) and titrated with 0.1 N sodium hydroxide until a pH of 8.15 was achieved. Percent acidity is equal to $100 \times TFN \times \mathrm{SG}/V$ [where T is the volume (mL) 0.1 N sodium hydroxide used, F is a factor equal to 0.070 for citric acid monohydrate, N is the exact normality of 0.1 N sodium hydroxide used, determined by titration against 1 M hydrochloric acid, SG is the specific gravity of the standardized juice, and V is the volume (mL) of standardized juice used] or $7.3815 \times TN$.

Ascorbic acid concentrations (milligrams per 100 mL of juice) were measured by LC-MS. Neat juice was diluted with 25 mM aqueous ammonium acetate, containing 4 mM dithiothreitol, at pH 4.5, to give a 5% (v/v) solution. A strong anion exchange column (Spheroclone SAX; 4.0 × 250 mm, 5 μm) was used isocratically with a mobile phase of 0.1% (v/v) aqueous trifluoroacetic acid at a flow rate of 0.25 mL min^{-1}. After UV detection (220 nm), the eluent was directly fed to the APCI interface of a Finnigan MAT SSQ 710C single-quadrupole mass spectrometer (Thermo Quest, Hemel Hempstead, U.K.) operating in positive single ion monitoring mode at m/z 177 ($[M + 1]^+$ ion), with a scan time of 1 s. Ascorbic acid [0.5 mM in 0.2% (v/v) aqueous trifluoroacetic acid, containing 4 mM dithiothreitol] was used as calibrant.

Percent soluble solids was determined by measuring the refractive index of neat juice using a digital refractometer (Atago PR-100). The refractive index was converted automatically to Brix percent, which is equivalent to percent soluble solids.

RESULTS AND DISCUSSION

Total fatty acid contents of the seeds varied from 7.6 to 19.3%, but replication was poor (RSD values between 0.8 and 25.9%). Considerable variation in total fatty acid content between replicates was also observed in the previous study (*15*). It was suggested that the reason for this might be either incomplete extraction/transmethylation during the analytical procedure or considerable seed to seed variation in oil content, which becomes more apparent due to the small sample size required for the analysis.

The parentage and GLA, ALA, and SA contents for all genotypes are displayed in **Tables 1** and **2**, respectively. RSD values (calculated from three replicates for each sample) for fatty acid composition were in all cases <5.3%. The fatty acid profiles of the 29 genotypes were in good agreement with those described for black currant seed oil in the literature (*4*, *15*). LA was the major component, at a level of ∼50% of the total fatty acids. We centered this study on ALA, SA, and, particularly, GLA contents because of their major nutritional importance. The ALA, SA, and GLA contents ranged from 11.1 to 18.7%, from 2.5 to 4.5%, and from 11.6 to 22.7%, respectively, which were similar to the values in the previous study on 36 different genotypes (*15*). As far as GLA content is concerned (**Table 2**), 4 genotypes showed >20%, which is unusual for black currants, but such levels were detected in 3 of the 36 genotypes examined

Table 1. Parentage of Black Currant Genotypes

genotype	female parent	male parent
S18-1-8	Ben More	C2-1-62
S36-1-66	Ben Alder	Imandra
S36-1-98	Ben Alder	Ben Loyal
S36-1-100	C7-4-24	Ben Alder
S36-3-34	Ben Gairn	Imandra
S36-5-115-7	Polar	OP
9111-14	Ben Alder	B1610-68
9123-3	Ben Avon	Ben Connan
9133-4	Ben Tirran	Tifon
9134-7	Ben Avon	B1610-68
9139-8	Ben Avon	Tifon
9141-6	Ben Alder	C2-1-62 × C7-T1-51
9143-1	Ben More × C2-1-62	OP
9148-9	Ben Dorain	AB3/7 × Ben Alder
9152-3	Ben More × C2-1-62	OP
9161-9	**Ben Alder**	**B1610-68**
9165-6	**C2-1-62 × Ben More**	**Ben Connan**
9165-7	C2-1-62 × Ben More	Ben Connan
9170-1	**C2-1-62 × Ben More**	**Ben Connan**
9170-11	**C2-1-62 × Ben More**	**Ben Connan**
9179-13	Ben More × C2-1-62	C2-1-62 × Rilll-10
9197-2	Ben Tirran	Ben Loyal
9198-1	C2-1-62 × C7-T1-51	OP
9198-2	C2-1-62 × C7-T1-51	OP
9199-4	Ben More × C2-1-62	B1834-120
91108-1	Ben Avon	C2-4-53 × Rilll-10
91110-1	**C2-1-62 × Rilll-10**	**B1834-120**
91129-1	**Ben Dorain**	**B1834-120**
91153-1	Ben Avon	Ben Connan

Table 2. GLA, ALA, and SA Contents (Weight Percent of Total Fatty Acids) in Seeds and Levels of Various Juice Parameters in 29 Black Currant Genotypes

genotype	GLA (wt %)	ALA (wt %)	SA (wt %)	ascorbic acid (mg/100 mL)	A_{515nm}	total acidity (%)	soluble solids (%)
S18-1-8	16.2	12.7	3.1	170	1.12	4.2	15.4
S36-1-66	15.1	15.4	3.7	215	1.14	4.1	14.9
S36-1-98	11.6	15.2	2.9	206	0.86	3.2	16.6
S36-1-100	13.2	18.7	4.1	255	0.49	3.7	14.9
S36-3-34	13.9	13.3	2.8	257	0.71	4.1	16.0
S36-5-115-7	16.3	14.4	3.6	403	0.94	3.7	16.6
9111-14	15.7	13.8	3.2	86	0.94	3.5	15.3
9123-3	14.2	14.8	3.1	75	0.42	4.7	13.4
9133-4	16.3	16.7	4.5	139	0.93	3.5	15.3
9134-7	15.0	14.2	3.2	198	0.72	3.3	17.0
9139-8	15.8	14.8	3.8	170	0.96	2.6	17.8
9141-6	16.6	13.0	3.2	135	0.89	3.1	15.6
9143-1	14.9	11.8	2.7	254	0.41	3.1	16.3
9148-9	13.3	15.8	3.2	158	1.10	3.1	19.5
9152-3	16.1	11.1	2.5	133	0.68	2.7	18.0
9161-9	**20.2**	**12.7**	**3.6**	**198**	**0.75**	**2.9**	**16.2**
9165-6	**18.1**	**12.8**	**3.2**	**85**	**0.59**	**4.0**	**15.2**
9165-7	13.9	12.8	2.8	159	0.75	3.5	15.2
9170-1	**22.7**	**11.1**	**3.5**	**128**	**0.45**	**3.9**	**13.9**
9170-11	**20.1**	**12.4**	**3.5**	**143**	**0.68**	**3.4**	**16.6**
9179-13	13.0	13.1	2.6	155	0.88	3.6	17.2
9197-2	13.9	15.4	3.3	216	0.66	3.3	17.2
9198-1	12.1	16.0	2.7	317	0.90	3.7	18.3
9198-2	14.9	12.8	2.8	177	0.49	4.2	15.9
9199-4	15.6	14.2	3.2	373	0.67	3.1	15.9
91108-1	12.5	14.8	2.8	157	0.90	3.7	15.3
91110-1	**20.7**	**11.1**	**3.2**	**189**	**0.68**	**3.5**	**19.0**
91129-1	**18.6**	**12.3**	**3.2**	**376**	**0.57**	**3.3**	**14.2**
91153-1	17.4	13.8	3.6	208	0.54	4.1	16.1

in the previous study (*15*). These genotypes displayed the lowest ALA contents of all genotypes. As observed in our previous study (*15*) there was the suggestion of a weak negative correlation ($r = -0.614$) between GLA and ALA contents (and to a lesser extent a positive correlation between ALA and SA; $r = 0.468$) across all genotypes. The total fatty acid content of the higher GLA genotypes was in the middle range.

Table 3. GLA, ALA, SA (Weight Percent of Total Fatty Acids) and Oil Contents (Weight Percent in Seeds) in Seeds of Selected Black Currant Genotypes by the Regular Procedure and Total Fatty Acid Contents (Weight Percent in Seeds) by the Regular (a) and Small-Scale Procedures (b)

genotype	GLA	ALA	SA	oil	total fatty acids (a)	total fatty acids (b)
9165-6	17.9	13.7	3.3	16.8	13.8	16.9
91129-1	18.6	12.6	3.3	21.4	16.9	16.8
9170-11	20.0	12.9	3.5	18.3	15.3	15.2
9161-9	19.7	13.2	3.6	12.9	8.2	13.2
91110-1	20.1	11.5	3.2	18.1	15.4	14.9
9170-1	22.1	11.8	3.6	17.2	13.3	14.4

A possible relationship was found between parentage and high GLA content, although the association did not always hold. Three (9165-6, 9170-1, and 9170-11) of the four genotypes with C2-1-62 × Ben More and Ben Connan as parents had high GLA contents (18.1−22.7%). However, the other genotype (9165-7) exhibited a GLA content of only 13.9%. Genotypes 91110-1 and 91129-1 shared one common parent (B1834-120) and had high GLA contents (20.7 and 18.6%, respectively). In contrast, 9161-9 and 9111-14 shared the same parentage (Ben Alder and B1610-68), but the former had a high GLA content (20.2%) and the latter a low content (15.7%). In our earlier study (*15*) no relationship of GLA content with parentage was found. It is clear that further extensive investigations of the heritability of GLA content are required. Examination of the data, including plots of the scores from principal component analysis, did not reveal any strong parental effects for any of the other variables examined in the study.

Table 3 shows GLA, ALA, and SA percentages as well as oil content obtained by using the conventional procedure. The total fatty acid contents obtained by the conventional (a) and rapid (b) procedures are also included in **Table 3**. With regard to GLA, ALA, and SA contents only slight variations between the rapid (**Table 2**) and regular (**Table 3**) procedures could be found. Similarly, no remarkable differences concerning total fatty acid content (**Table 3**) were observed except for 9161-9, and 9165-6 to a lesser degree, which displayed considerably higher values when the rapid method was used.

All genotypes examined in this study had been selected from many other genotypes over the three previous seasons. They were all assessed in the field for yield, height, habit/branch, strength, berry size, pest/disease resistance, fruit set, and overall agronomic worth. Most genotypes were selected on the basis of their overall performance rather than one or two outstanding characteristics. Notable exceptions include S36-1-100, considered an Elite parent for good fruit-setting characters,which has been used in the production of mapping populations for molecular mapping studies, 9148-9, which is highly rated within the breeding program for yield and disease resistance, and 9199-4 and 91129-1, which are both putative gall-mite-resistant genotypes. There appears to be no obvious or simple relationship of GLA content with any agronomic trait; however, further studies using entire segregating populations or diallel crosses are necessary to confirm this, and such work is in progress.

Parameters of commercial interest for juice production are also depicted in **Table 2**. Anthocyanin content is an important feature from a commercial standpoint, because it is responsible for color, the higher values being the most favorable. The

absorbances ranged from 0.41 to 1.14, and values of $\sim \geq 0.8$ are sought within the breeding germplasm. Ascorbic acid content is of prime commercial and nutritional importance, and in the present study, values ranged from 75 to 403 mg/100 mL of juice. Contents of 0.9−3.6 mg/mL have been reported in the literature (*17*), and values of >2 mg/mL are valued in potential new cultivars. Some genotypes gave highly acceptable values in both anthocyanin and ascorbic acid contents, although the correlation ($r = -0.023$) between the parameters was not significantly different from zero.

With regard to total acidity and percent soluble solids, values between 2.6 and 4.7% and between 13.4 and 19.5%, respectively, were found. Acidity is usually used as an indicator of fruit maturity. A high value has been generally associated with an unpleasant character (*17*), although industrial processors of commercial juices vary in their requirements regarding acidity in the raw materials. A wide range is generally acceptable in juice samples; values from 3.4 to 5.5% have been described as normal (*17*), so the values for all genotypes are acceptable. A high content of soluble solids is considered to be a positive attribute, and in some territories, for example, New Zealand, growers are paid partly according to the soluble solids content of the fruit. The negative correlation ($r = -0.516$) between percent soluble solids and acidity (and between acidity and pH) was significantly different from zero but was not strong.

One aim of this study was to determine whether genotypes can be produced with both favorable fatty acid (particularly GLA) contents and juice properties and whether genetic linkages between these traits were evident. For all 29 genotypes there were no significant pairwise correlations between any of the fatty acids and any of the juice parameters (or indeed between any of the juice parameters, except for the weak correlation already noted between acidity and percent soluble solids and pH). A principal component analysis was carried out on all eight variables, and the first two components accounted for just over 50% of the total variation. In the loading plot for component 1 there was a high positive value for acidity and high negative values for pH and percent soluble solids, whereas in component 2, ALA was highly positive and GLA was highly negative. Apart from these observations, which had been confirmed by pairwise correlation analysis, no other strong relationships between variables was observed in the loading plots.

Although there were no strong positive correlations between GLA content and any of the juice parameters, it is noteworthy that there were also no strong negative correlations, and indeed it was possible to produce individual genotypes with both favorable GLA contents and juice qualities. Unfortunately, the genotype (9170-1) with the highest GLA content had some of the lowest anthocyanin and ascorbic acid contents (0.45 and 128 mg/100 mL, respectively), whereas 9161-9 and to a lesser extent 91110-1 had high GLA contents and acceptable anthocyanin and ascorbic acid contents. The former genotype also had one of the lowest total acidities, whereas the latter genotype had a high soluble solids content. However, if ascorbic acid content is considered to be the most important juice parameter, then genotype 91129-1 is highlighted because, as well as a high GLA content, it has one of the highest ascorbic acid contents of all genotypes, although the anthocyanin content was reasonably low. One genotype (S36-5-115-7) exhibited both the highest ascorbic acid content (403 mg/100 mL) and one of the highest anthocyanin contents (0.94), but GLA content was modest (16.3%).

In conclusion, it has been shown that, although GLA content was not strongly correlated to favorable juice characteristics, black currant genotypes can be developed with both high GLA contents and highly acceptable juice characteristics, that is, with greater added value. Selection of appropriate genotypes is in progress within the SCRI germplasm and breeding program, and the possible relationship of high GLA content to parentage may assist in targeting genotypes with high GLA content. Selection of genotypes for commercial purposes will depend on the weight given to different parameters, and at the present time anthocyanin and particularly ascorbic acid content of the juice would be weighted heavily (if other juice parameters were acceptable). Although GLA content in the seed would be some way down the list at present, the requirements of the processing and associated industries are under constant review. Black currant seed oils may have other positive attributes. Indeed, in a study of the seed oils of several *Ribes* species, those from *R. nigrum* were richest in tocopherol content (*14*). Obviously, other agronomic factors such as yield, disease resistance, and frost tolerance must also be considered in selection. Our data provide evidence that it is possible to breed black currant for high GLA contents in the seeds and that this trait can be integrated with other breeding objectives relating to fruit quality.

LITERATURE CITED

(1) Lister, C. E.; Wilson, P. E.; Sutton, K. H.; Morrison, S. C. Understanding the health benefits of blackcurrants. *Acta Hort.* (ISHS) **2002**, *585*, 443−449.

(2) Taylor, J. Colour stability of blackcurrant (*Ribes nigrum*) juice. *J. Sci. Food Agric.* **1989**, *49*, 487−491.

(3) Simopoulos, A. P. Fatty acids. In *Designer Foods, Pharmafoods, Nutraceuticals*; Goldberg, I., Ed.; International Thomson Publishing: New York, 1994; pp 355−392.

(4) Gunstone, F. D. Gamma-linolenic acid—occurrence and physical and chemical properties. *Prog. Lipid Res.* **1992**, *31*, 145−161.

(5) Leventhal, L. J.; Boyce, E. G.; Zurier, R. B. Treatment of rheumatoid arthritis with blackcurrant seed oil. *Br. J. Rheumatol.* **1994**, *133*, 847−852.

(6) Wu, D.; Meydani, S. N. The biological/nutritional significance of γ-linolenic acid in the epidermis: metabolism and generation of potent biological modulators. In *γ-Linolenic Acid. Metabolism and Its Roles in Nutrition and Medicine*; Huang, Y.-H., Mills, D. E., Eds.; AOCS Press: Champaign, IL, 1996; pp 118−128.

(7) Mills, D. E.; Huang, Y.-S.; Poisson, J.-P. γ-Linolenic acid attenuates blood pressure responses to environmental stimuli: implications for human essential hypertension. In *γ-Linolenic Acid. Metabolism and Its Roles in Nutrition and Medicine*; Huang, Y.-S., Mills, D. E., Eds.; AOCS Press: Champaign, IL, 1996; pp 189−199.

(8) Poisson, J.-P.; Narce, M.; Huang, Y.-S.; Mills, D. E. γ-Linolenic acid biosynthesis and chain elongation in fasting and diabetes mellitus. In *γ-Linolenic Acid. Metabolism and Its Roles in Nutrition and Medicine*; Huang, Y.-S., Mills, D. E., Eds.; AOCS Press: Champaign, IL, 1996; pp 259−272.

(9) Das, U. N. Anti-cancer actions of γ-linolenic acid with particular reference to human brain malignant glioma. In *γ-Linolenic Acid. Metabolism and Its Roles in Nutrition and Medicine*; Huang, Y.-S., Mills, D. E., Eds.; AOCS Press: Champaign, IL, 1996; pp 282−292.

(10) Clough, P. Sources and production of speciality oils containing GLA and stearidonic acid. *Lipid Technol.* **2001**, *13*, 9−12.

(11) Guil-Guerrero, J. L.; Gómez-Mercado, F.; García-Maroto, F.; Campra-Madrid, P. Occurrence and characterization of oils rich in α-linolenic acid (I): *Echium* seeds from Macaronesia. *Phytochemistry* **2000**, *53*, 451−456.

(12) Guil-Guerrero, J. L.; Gómez-Mercado, F.; Rodríguez-García, I.; Campra-Madrid, P.; García-Maroto, F. Occurrence and characterization of oils rich in α-linolenic acid (III): The taxonomical value of the fatty acids in *Echium* (Boraginaceae). *Phytochemistry* **2001**, *58*, 117−120.

(13) Guil-Guerrero, J. L.; Garcia Maroto, F.; Gimenez Gimenez, A. Fatty acid profile from forty-nine plant species that are potential new sources of γ-linolenic acid. *J. Am. Oil Chem. Soc.* **2001**, *78*, 677−684.

(14) 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.

(15) Ruiz del Castillo, M. L.; Dobson, G.; Brennan, R.; Gordon, S. Genotypic variation in fatty a cid content of blackcurrant seeds. *J. Agric. Food Chem.* **2002**, *50*, 332−335.

(16) Christie, W. W. Gas chromatographic analysis of fatty acids derivatives. In *Gas Chromatography and Lipids*; The Oily Press: Dundee, Scotland, 1989; pp 85−128.

(17) Shaklady, J. Fruit juices and fruit juice beverages. In *Food Industries Manual*, 20th ed,; Woollen, A., Ed.; Leonard Hill Books: London, U.K., 1969; pp 249−275.

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