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Gas chromatography analysis of blackcurrant oil in relation to its stability

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Abstract

Samples of blackcurrant oil of different age and mode of storage were subjected to gas chromatography (GC) analysis in order to determine the stability of the fatty acid mixture. It was found, that the decrease in the amount of γ-linolenic acid and α-linolenic acid, the priority importance polyunsaturated acids, is represented by the value of about 10% in 10 years. On the other hand, the sum of the composition of linoleic acid, γ-linolenic acid and α-linolenic acid remains almost constant during that period. The study shows the value of GC in designing simple, efficient and highly reproducible analytical technique in performing this study. The designed analytical method may be used for routine analyses of plant oils of different origin.

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Keywords: *Ribes nigrum*; Blackcurrant oil; Triacylglycerol; Fatty acid methyl ester; Gas chromatography

1. Introduction

Polyunsaturated fatty acids (FAs) and their derivatives are important essential nutritive additives in mammals, especially in humans (Christophe, 1996; Kamal-Eldin & Yanishlieva, 2002; Makrides & Gibson, 1998; Okuyama et al., 1998; Phillips & Huang, 1996; Rafflenbeul, 2001; Wille & Wang, 1996; Ziboh, Cho, Mani, & Xi, 2002). We have been dealing with studies of blackcurrant (*Ribes nigrum*) oil, which is important source of polyunsaturated FAs. Blackcurrant oil can be obtained from blackcurrant seeds, which are the waste product in the production of blackcurrant juice, jelly or jam, but contain additional important quantity of essential FAs. Recently, we have studied enzymic methods applicable to perform enzymic transformations of triacylglycerol (TAG) fraction of blackcurrant oil in order to find methodologies for enrichment of different biotransformation fractions by α-linolenic acid and γ-linolenic acid, with special focus on the composition and the ratio of these two unsaturated FAs (Vacek et al., 2000a; Vacek et al., 2000b; Vacek et al., 2001; Zarevúcka et al., 2003). After having used the quantity of blackcurrant oil, which had been prepared for performing all those experiments, we have paid attention to the problem of stability of TAGs in blackcurrant seeds stored at different conditions. Because we have had blackcurrant seeds available from several sources for our studies, which have been of different age, having been stored at different conditions, we have decided to perform a unique analysis of blackcurrant oil samples to study their stability and compositions of FAs. The present study can contribute to the definition of potential stability of blackcurrant oil, and, possibly, can amplify the impact of recent studies, if long-lasting stability of blackcurrant oil is found.

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2. Materials and methods

2.1. Preparation of samples of TAG mixtures

2.1.1. Sample 1

Fresh blackcurrant seeds were received from an agricultural producer in Chelčice, Czech Republic. The seeds were air-dried at room temperature during 4 weeks. The seeds (10 g) were ground, covered by the extraction medium, i.e., chloroform–methanol (1:1, v/v) mixture, in a glass column, and left to stand overnight for 20 h. Extraction was done twice with the same extraction mixture (250 ml each extraction). The combined extracts were evaporated to dryness under reduced pressure, affording 1.693 g (16.93%) of crude blackcurrant oil. The whole seeds, extracted by the same way gave only 3.10% of the oil.

2.1.2. Sample 2

Blackcurrant seeds were stored at +6 °C for 14 months, and then taken for the extraction. The seeds (10 g) were ground, and the extraction was made as described above for Sample 1, affording 1.678 g (16.78%) of blackcurrant oil. If the seeds were left to stay in the column in the extraction mixture of solvents described above for additional 5 days, the final yield of blackcurrant oil increased to 2.473 g (24.73%).

2.1.3. Sample 3

Blackcurrant seeds were stored at room temperature for 14 months. The seeds (10 g) were ground, and their extraction was made as described above (Sample 2), affording 2.583 g (25.83%) of blackcurrant oil.

2.1.4. Sample 4

An amount of raw blackcurrant oil produced from blackcurrant seeds 10 years ago was stored at room temperature since that time. To produce this raw blackcurrant oil that time, blackcurrant seeds, containing pulp, resulting from the juice squeezing, were air-dried to the remaining water content of 6.5% (w/v), and ground in a disc mill. The unsieved ground product was extracted by supercritical carbon dioxide (SC–CO_2) at 27.5 MPa at 45 °C, in a continuous cycle, for 5 h to give clear yellow oil in a yield 15.6% (w/w).

2.1.5. Sample 5

A fraction containing only TAGs was isolated from the above blackcurrant oil (Sample 4) 5 years ago by the column chromatography on silica gel. This crude blackcurrant oil (100 g) afforded 77.55 g (77.55%) of a mixture of TAGs, which were adjusted to several flasks containing approximately 10 g of a mixture in each flask, and stored under the argon atmosphere at −18 °C since that time.

2.2. Thin layer chromatography

2.2.1. Analytical thin layer chromatography

Analytical thin layer chromatography (TLC) was carried out on glass plates (76 mm × 36 mm) coated with silica gel Adsorbosil-Plus, layer thickness 0.2 mm (Applied Science Division) with gypsum (12%, w/w). Detection of the spots of the fractions was achieved by spraying the TLC plates with concentrated sulfuric acid, and subsequent heating at 150 °C for several minutes. The values of the retardation factor (R_F = 0.52) in light petroleum–diethyl ether (85:15, v/v) were found for the spots corresponding to TAG fractions.

2.2.2. Preparative thin layer chromatography

Preparative TLC was carried out on glass plates (76 mm × 60 mm) precoated with silica gel Adsorbosil-Plus, layer thickness 0.2 mm (Applied Science Division) with gypsum (12%, w/w), using a mixture of solvents, light petroleum–diethyl ether (85:15, v/v), both freshly distilled, as mobile phase. The TLC plates were sprayed with Rhodamine 6G (0.05% solution in ethanol). The chromatographic bands, consisting of TAGs, were scraped off and transferred into small columns (8 mm, i.d.) filled with silica gel (0.3 g, particle size 25–50 μm), eluted with freshly distilled diethyl ether (15 ml), and evaporated to dryness.

2.3. Gas chromatography

Gas chromatography (GC) was performed with a HP 5890A gas chromatograph (Hewlett–Packard, USA) equipped with a flame ionization detector and split/splitless injector. Chromatographic conditions: (a) isothermal: injector temperature, 240 °C; detector temperature, 250 °C; oven temperature, 200 °C; carrier gas, hydrogen, 85 kPa, $\bar{u} = 40.2\ \mathrm{cm\,s^{-1}}$; split ratio, 40:1; (b) temperature program: 140 °C (0 min), then a rate 5 °C $\mathrm{min^{-1}}$ to 230 °C (25 min); carrier gas, hydrogen, 90 kPa, $\bar{u} = 46.2\ \mathrm{cm\,s^{-1}}$ at 140 °C; split ratio, 46:1. Nitrogen was used as make-up gas (30 ml $\mathrm{min^{-1}}$). Data were collected with a HP 3393A integrator (Hewlett–Packard, USA). Fused silica column DB-WAX (30 m × 0.25 mm × 0.25 μm; J&W Scientific, USA) was used for analyses.

2.4. Transesterification of TAGs to fatty acid methyl esters

The reaction followed the procedure described by Stránský and Jursík (1996a, 1996b). The fractions from preparative TLC, only consisting of TAGs (3.0 mg), were heated with a mixture of methanol–chloroform (3:2, 250 μl) and acetyl chloride (29.5 μl) in sealed ampoules at 70 °C for 90 min. Neutralization was made with silver carbonate (57.3 mg). The reaction mixture

was subjected to centrifugation, and the supernatant, containing fatty acid methyl esters (FAMEs), was analyzed directly using GC. The samples 1–5 were analyzed in that way.

2.5. Calculation of equivalent chain length values

Following inner reference compounds were used for calculation of equivalent chain length (ECL) values: The FAs 14:0, 16:0 and 18:0 were naturally present in the Samples 1–5, however, the natural quantity of 18:0 was low, and, therefore, its quantity was increased by its additional amount. The FAs 20:0 and 22:0 (not naturally present in the Samples 1–5) were used as added reference compounds, injected together with the analyzed samples of transesterified TAGs (i.e., FAMEs). Calculation of the ECL values was described in details earlier (Stránský, Jursík, & Vítek, 1997; Stránský, Jursík, Vítek, & Skořepa, 1992).

3. Results

The basic aim of the present study was determination of the stability of the FA composition in the TAG fraction of blackcurrant oil of different origin and treatment. Table 1 summarizes the results from the GC analyses. Regarding the age of the blackcurrant oil samples, the samples of TAGs can be sorted into two basic groups. Samples 1–3 represent composition of FAMEs obtained by transesterification of up to 14 months old samples of blackcurrant seeds, while Samples 4 and 5 were prepared from the oil extracted from blackcurrant seeds about 10 years ago. Following acids represent 5 unsaturated FAs of priority interest in our study:

Oleic acid [(9*Z*)-octadec-9-enoic acid; 18:1 $n-9$]: It made up around 11% of the composition of the samples of young blackcurrant oil (Samples 1–3), and it increased up to 12.9–13.5% in the samples of old blackcurrant oil (Samples 4 and 5).

Linoleic acid [(9*Z*, 12*Z*)-octadeca-9,12-dienoic acid; 18:2 $n-6$]: Its content also increased. The samples of young blackcurrant oil (Samples 1–3) contained about 43–43.6% of linoleic acid, while the samples of old blackcurrant oil (Samples 4 and 5) contain more than 46% of linoleic acid.

γ-Linolenic acid [(6*Z*, 9*Z*, 12*Z*)-octadeca-6,9,12-trienoic acid; 18:3 $n-6$]: Its content showed the opposite development than found for oleic acid and linoleic acid. Higher quantity of this acid (15.6%) was found in the young blackcurrant oil (Samples 1–3), and its lower amount (13.4–12.8%) was found in the samples of old blackcurrant oil (Samples 4 and 5).

α-Linolenic acid [(9*Z*, 12*Z*, 15*Z*)-octadeca-9,12,15-trienoic acid; 18:3 $n-3$]: Its content showed the same scheme as the content of γ-linolenic acid, however the differences among the samples from the young and old blackcurrant oil were not so remarkable. The samples of young blackcurrant oil (Samples 1–3) contain 14.2–13.9% of α-linolenic acid, and the samples of old blackcurrant oil (Samples 4 and 5) contain 12.7–12.2% of α-linolenic acid.

Stearidonic acid [(6*Z*, 9*Z*, 12*Z*, 15*Z*)-octadeca-6,9,12,15-tetraenoic acid; 18:4 $n-3$]: Its content showed

Table 1
Content of fatty acids and other compounds in triacylglycerols in the tested blackcurrant oil samples

Compound	Calculated ECL_c values	Reference ECL_r values	Difference (Δ)[a]	Sample 1 (%)	Sample 2 (%)	Sample 3 (%)	Sample 4 (%)	Sample 5 (%)
14:0[b]	–	14.0000	–[c]	0.11	–	0.10	0.10	0.10
Unknown	14.7001	–	–	–	–	0.51	0.56	0.63
16:0[b]	–	16.0000	–[c]	6.75	6.62	6.48	5.91	6.38
Unknown	16.7417	–	–	–	–	–	–	0.14
Glycerol [d]	17.1002	17.1103	−0.0101	1.74	2.05	2.03	1.78	1.94
18:0[b]	–	18.0000	–[c]	1.84	1.83	1.93	1.88	1.99
18:1 $n-9$[b]	18.2343	18.2334	+0.0009	11.34	10.96	11.15	12.88	13.50
18:1 $n-7$[b]	18.3022	18.3028	−0.0006	0.77	1.24	1.15	1.14	1.14
18:2 $n-6$[b]	18.7126	18.7093	+0.0033	42.79	43.01	43.60	46.37	46.18
18:3 $n-6$[b]	19.0248	19.0252	−0.0004	15.61	15.59	15.58	13.40	12.82
18:3 $n-3$[b]	19.3584	19.3598	−0.0014	13.64	14.17	13.93	12.74	12.19
18:4 $n-3$[b]	19.6740	19.6773	−0.0033	3.09	3.10	2.92	2.37	2.19
20:1 $n-9$[b]	20.2128	20.2157	−0.0029	0.66	0.67	0.62	0.87	0.85
20:2 $n-6$[b]	20.6916	20.6980	−0.0064	0.25	+ [e]	+ [e]	+ [e]	+ [e]

[a] $\Delta = ECL_c - ECL_r$.
[b] Fatty acid.
[c] The fatty acids 14:0, 16:0 and 18:0 were present in the Samples 1–5 in the quantities given in the Table 1, and used for calculation of the ECL_c values.
[d] We have calculated the ECL_r and ECL_c values for glycerol, because it is one of the components of the reaction mixtures after transesterification of TAG fractions; higher Δ value for glycerol is caused by the chromatographic properties of glycerol.
[e] This fatty acid was present in the Samples 2–5 in the concentrations lower than the threshold concentration.

the same development as found for γ-linolenic acid and α-linolenic acid. Higher quantity of this acid (2.92–3.10%) was found in the young blackcurrant oil (Samples 1–3), and its lower composition (2.59–2.19%) was found in the samples of old blackcurrant oil (Samples 4 and 5).

Other FAs found in the forms of their methyl esters showed only mild variations in their quantities in the tested samples of blackcurrant oil. Moreover, combined amounts of the above 5 unsaturated FAs represent the sum of 87.2–86.5% of total peak areas obtained in the GC analyses (Table 2). Therefore, the impact of other FAs is not so important in this study.

Individual FAs were identified by their ECL values. The method of determination of the exact ECL values was described in the previous papers (Stránský et al., 1997, 1992), in which the ECL values of 83 defined FAs (as their FAMEs) have been determined and calculated on fused silica columns DB-WAX and DB-1. It should be stressed that reference values (ECL_r), calculated therein (Stránský et al., 1997, 1992), were based on average ECL values (analyses and calculations were repeated at least five times) of the studied standard FAMEs. On the basis of many years of experience in this field, the ECL values of individual FAs, i.e., the reference values (ECL_r) and the values calculated by means of this method (ECL_c), are stable within $\Delta = \pm 0.003$–0.007 units (Table 1), if the chromatographic system is still the same [i.e., all parameters connected with both, the column (producer and chromatographic conditions), and carrier gas]. It means that reliable differentiation of at least 70 additional potential FAs between two homologues can be achieved using this method. The difference value Δ between the ECL_r and the ECL_c values for glycerol results in higher value ($\Delta = -0.0101$) than are the Δ values calculated for FAs, because the chromatographic properties of glycerol differ from those of FAs on a DB-WAX column (ECL values were developed to describe FAs exclusively). The practical value of the reference value (ECL_r), therefore, results in saving time during any analysis of a FAME mixture. When using the same type of a chromatographic system, described for calculation of the reference values (ECL_r), only one successful analysis is needed to assign the composition of the analyzed FAME mixture. The Δ value (Table 1) is then equivalent to standard deviation (SD) usually used in this type of analyses, normally repeated several times.

4. Discussion

Different calculated ratio values (cf. Table 3) are able to assist in answering the basic question, mentioned in the first paragraph of Section 3, in the best way. Calculating the compositions of 3 major polyunsaturated FAs in the tested samples of blackcurrant oil (Samples 1–5), an increase was calculated for each of the ratios of the linoleic acid composition towards the compositions of γ-linolenic acid (Entry 1), α-linolenic acid (Entry 2), stearidonic acid (Entry 3), and towards two different sums of their compositions (Entries 4 and 5), when samples of the old blackcurrant oil (Samples 4 and 5) were compared with the samples of the young blackcurrant oil (Samples 1–3). This calculation gave a proof for a slow degradation of γ-linolenic acid and α-linolenic acid, which resulted in a decrease of their composition by 10 relative percents within the period of 10 years.

To conclude, it should be summarized that blackcurrant oil is relatively stable natural plant oil, which can be stored for a long time even at room temperature. All samples of blackcurrant oil tested in this study, were stored before subjecting to the described GC analysis

Table 2
Combined amounts of the major unsaturated fatty acids in the blackcurrant oil samples (in percents)

Basis for calculation of the combined amounts of fatty acids	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
18:1 $n-9$ + 18:2 $n-6$ + 18:3 $n-6$ + 18:3 $n-3$ + 18:4 $n-3$	86.47	86.83	87.18	87.76	86.88
18:1 $n-9$ + 18:2 $n-6$ + 18:3 $n-6$ + 18:3 $n-3$	83.38	83.73	84.26	85.39	84.69
18:2 $n-6$ + 18:3 $n-6$ + 18:3 $n-3$ + 18:4 $n-3$	75.13	75.87	76.03	74.88	73.38
18:2 $n-6$ + 18:3 $n-6$ + 18:3 $n-3$	72.04	72.77	73.11	72.51	71.19

Table 3
Ratio values calculated among the major polyunsaturated fatty acids from the blackcurrant oil samples

Entry	Ratio	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
1	18:2 $n-6$/18:3 $n-6$	2.741	2.759	2.798	3.460	3.602
2	18:2 $n-6$/18:3 $n-3$	3.137	3.035	3.130	3.640	3.788
3	18:2 $n-6$/18:4 $n-3$	13.848	13.874	14.932	19.565	21.087
4	18:2 $n-6$/(18:3 $n-6$ + 18:3 $n-3$)	1.463	1.445	1.477	1.774	1.846
5	18:2 $n-6$/(18:3 $n-6$ + 18:3 $n-3$ + 18:4 $n-3$)	1.323	1.309	1.344	1.626	1.698
6	18:3 $n-6$/18:3 $n-3$	1.144	1.100	1.118	1.052	1.052

either at laboratory temperature or at the temperature usual for refrigerators (4–10 °C), however, in all cases they were stored with exclusion of UV irradiation. Therefore, it is advisable to keep the productions containing blackcurrant oil in dry atmosphere and in the dark.

The used TLC and GC analyses and transesterification method are of general application for routine, simple and, in particular, reproducible analysis of natural oils, not only of plant origin. The excellent reproducibility of the ECL values, used for characterization of the FAs, gives an easy, convenient and valuable methodology for identification of FAs in their natural sources (cf. Table 1).

It seems that the method of analyzing the FAMEs by GC, described in this paper, gives the results more reproducible than the analytical methods applied according to the IUPAC or ISO rules, presently compulsory in the GC analyses of plant TAGs for nutritive applications (ISO 5508).

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