Lipid composition of Bulgarian chokeberry, black currant and rose hip seed oils

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Abstract: The lipid composition of chokeberry, black currant and rose hip seeds was investigated. The seeds contain 19.3 g kg^{-1}, 22.0 g kg^{-1} and 8.2 g kg^{-1} glyceride oil respectively. The content of phospholipids, mainly phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine, was 2.8 g kg^{-1}, 1.3 g kg^{-1} and 1.4 g kg^{-1}, respectively. The total amounts of sterols were 1.2 g kg^{-1}, 1.4 g kg^{-1} and 0.4 g kg^{-1}. The main component was β-sitosterol, followed by campesterol and Δ^5 -avenasterol. In the tocopherol fraction (55.5 mg kg^{-1} in chokeberry oil, 249.6 mg kg^{-1} in black currant oil and 89.4 mg kg^{-1} in rose hip oil), α-tocopherol predominated in chokeberry oil (70.6 mg kg^{-1}). γ-Tocopherol was the main component in black currant oil (55.4 mg kg^{-1}) and rose hip oil (71.0 mg kg^{-1}). The fatty acid composition of triacylglycerols, individual phospholipids and sterol esters was also identified. In the phospholipids and sterol esters, the more saturated fatty acids, mainly palmitic, stearic, and long chain fatty acids predominated.

Keywords: chokeberry; black currant; rose hip; fatty acids; phospholipids; sterols; tocopherols

INTRODUCTION

The fruits of chokeberry (*Aronia melanocarpa* L, fam, Rosaceae), black currant (*Ribes nigrum* L, fam, Saxifragaceae) and rose hip (*Rosa canina* L, fam Rosaceae) contain significant amounts of anthocyans, flavonoids and vitamin C, and are used to produce jam, fruit wine, syrups etc. These products also contain glyceride oils and other biologically active compounds and as phospholipids, sterols and tocopherols. Knowledge of the nature and composition of those lipid constituents is still fragmentary. The fatty acid composition of black currant seed oil has been reported by several authors.[1–8] The Sterol composition of the black current seed oil was identified by Artaud.[1] The tocopherol composition of black currant and rose hip fruits was investigated by Pironen *et al.*[9]

In the present investigation we have attempted to characterise the fatty acid composition of the triacylglycerols, the main individual phospholipids and sterol esters; and the content and composition of free and esterified sterols, phospholipids and tocopherols of the glyceride oils recovered from the seeds of chokeberry, black currant and rose hip fruits.

MATERIALS AND METHODS

Fruit material

The fruits of the investigated plants (1995 crop) were provided from the Plovdiv region in South Bulgaria. The investigations were carried out in air-dried fruits at technical ripeness.

Extraction

The oils were extracted in a Soxhlet apparatus with hexane for 8 h. After rotation vacuum distillation of the solvent, the extracted oils were weighed.

Fatty acid composition

The composition of the fatty acids was determined by gas–liquid chromatography of their methyl esters. The esterification was carried out by the technique of Metcalfe and Wang.[10] Methyl esters were purified by thin layer chromatography on plates covered with Silica gel 60 G 'Merck' employing a mobile phase of hexane–diethyl ether 97:3 (w/v). The analysis was performed on an HP 5890 A unit provided with FID and 30 m capillary column 'Innowax' impregnation (Scotia Pharmaceuticals Ltd, Carlisle, UK). The conditions were:

Column temperature: 165–225 °C, 4 °C min^{-1}
Detector temperature: 320 °C, injector temperature, 300 °C
Gas carrier: nitrogen

The peaks were identified using authentic fatty acid methyl esters as standards. The area percentages were considered as weight percentages.

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Table 1. Content of oil in seeds and content of phospholipids, sterols and tocopherols in the oils[a]

Species	Content of oil in seeds ($g\,kg^{-1}$ wt)	Content of main lipid compounds in the oils		
		Phospholipids ($g\,kg^{-1}$ wt)	Sterols ($g\,kg^{-1}$ wt)	Tocopherols ($mg\,kg^{-1}$)
Chokeberry (*Aronia melanocarpa* L)	19.3	2.8	1.2	55.5
Black currant (*Ribes nigrum* L)	22.0	1.3	1.4	249.6
Rose hip (*Rosa canina* L)	8.2	1.4	0.4	89.4

[a] Mean of three replicates.

Phospholipid composition

Lipids were extracted from the seeds by the procedure of Folch *et al.*[11] Polar lipids were separated from non-polar lipids by column chromatography.[12] The phospholipid constituents were separated by two-directional thin-layer chromatography on Silica gel 60 G 'Merck', impregnated with $1\,g\,kg^{-1}$ $(NH_4)_2SO_4$ water solution.[13] The first direction was carried out in chloroform:methanol:ammonia 65:25:5 (v/v/v) and the second in chloroform:methanol:ammonia:acetic acid:water 50:20:10:10:5 (v/v/v/v/v). The spots of the separated individual phospholipids were identified by spraying with specific reagents.[12] In addition R_f and standard spots were used for definitive identification. Quantification was carried out spectrophotometrically at 700 nm.[13]

Sterol composition

The free and esterified sterols were separated from the other oil constituents by preparative TLC on Silica gel 60 G 'Merck' employing a mobile phase of hexane: diethyl ether 1:1. The esterified sterols were saponified with ethanolic KOH, extracted, and purified by TLC. Quantification was carried out spectrophotometrically.[14] Individual composition was identified by gas chromatography,[21] using an HP 5890 A unit with FID, 25 m capillar column impregnated with OV^- 17. The conditions were:

Column temperature: 260–300 °C, $6\,°C\,min^{-1}$

Detector temperature: 320 °C, injector temperature; 300 °C

Gas carrier: nitrogen

Identification was confirmed by comparing retention time of the individual constituents with those of authentic standards.

Tocopherol composition

Tocopherols and tocotrienols were analysed directly in the oils by HPLC with fluorescence detection.[15,16] A 'Merck–Hitachi' unit fitted with a 'Nucleosil' Si 50-5 250×4 mm column and a fluorescent detector ('Merck–Hitachi' F 1000) was used. The operating conditions were as follows: excitation 295 nm, emission 330 nm, mobile phase hexane:dioxane 94:4, rate of mobile phase $1\,cm^3\,min^{-1}$. The peaks were identified using authentic individual tocopherols.

RESULTS AND DISCUSSION

Data on the composition of investigated samples are presented in Table 1. which shows that chokeberry and black currant seeds contain significant amounts of glyceride oil. The same oils also have a high content of phospholipids and sterols. The highest quantity of tocopherols was found in black currant oil. This value is close to the content of tocopherols in some germ oils, which are the richest sources.

Comparative results from the analyses of phospholipids are presented in Table 2. Almost all phos-

Phospholipids	Content ($g\,kg^{-1}$ fresh wt)		
	Chokeberry	Blackcurrant	Rose hip
Phosphatidylcholine (PC)	19.9	34.7	46.3
Phosphatidylinositol (PI)	29.8	20.6	20.7
Phosphatidylethanolamine (PE)	16.6	11.2	12.2
Phosphatidic acids (PA)	15.8	7.5	4.3
Lysophosphatidylcholine (LPC)	10.8	8.3	1.2
Lysophosphatidylethanolamine (LPE)	1.4	6.4	1.8
Phosphatidylserine (PS)	ND	1.9	1.7
Monophosphatidylglycerol (MPGl)	0.8	6.9	1.2
Diphosphatidylglycerol (DPGL)	2.0	ND	0.9
Sphingomieline	ND	3.3	0.9
Unidentified phospholipids	2.9	ND	8.8

Table 2. Phospholipid composition of chokeberry black currant and rose hip seed oils[a]

[a] Mean of three replicates.
ND – not detected.

Table 3. Sterol composition of chokeberry, black currant and rose hip seed oils[a]

	Content ($g kg^{-1}$ wt)					
	Chokeberry		Black currant		Rose hip	
Sterols	Free	Esterified	Free	Esterified	Free	Esterified
Cholesterol	0.6	5.3	0.9	4.1	0.5	4.4
Campesterol	6.0	5.0	1.5	1.0	1.8	1.2
Brassicasterol	ND	ND	ND	ND	5.4	4.6
Stigmasterol	2.6	5.1	6.8	3.0	3.5	1.6
β-Sitosterol	89.8	73.8	87.3	85.7	81.5	81.6
Δ^5-Avenasterol	0.5	3.2	1.3	1.3	4.6	6.6
Δ^7-Stigmasterol	0.5	3.1	0.9	1.0	ND	ND
$\Delta^{7.25}$-Stigmasterol	tr	3.6	1.1	1.6	1.8	tr
Δ^7-Avenasterol	tr	0.9	tr	2.3	0.9	tr
Ratio sterols free:esterified		83.5:16.5		78.3:21.7		95.0:5.0

[a] Mean of three replicates.
tr – less than 0.1 $g kg^{-1}$; ND – not detected.

pholipid classes have been identified in the oils. Phosphatidylserine and sphingomieline have not been detected in chokeberry seed oil. Phosphatidylinositol is the main component in the phospholipid fraction of chokeberry followed by phosphatidylcholine and phosphatidylethanolamine. High levels of phosphatidic acids and lysophosphatidylcholine were also detected. Phosphatidylcholine predominates in both black currant and rose hip oils, followed by phosphatidylinositol and phosphatidylethanolamine. The other phospholipids are present in insignificant amounts in all the oils.

The quantitative and qualitative composition of the free and esterified sterols is given in Table 3. The greatest part of sterol is in free form. β-Sitosterol predominates in all the sterol fractions. The β-sitosterol content of black currant oil ($87.3 g kg^{-1}$ in free form and $85.7 g kg^{-1}$ in esterified form) is higher than reported ($79.3 g kg^{-1}$) by Artaud.[1] This higher quantity was balanced mainly by a lower level of campesterol – only $1.5 g kg^{-1}$ in free form and $1.0 g kg^{-1}$ in esterified form, in comparison with $7.9 g kg^{-1}$ reported by Artaud.[1] Brassicasterol was only detected in rose hip oil. High levels of stigmasterol derivatives (total content $11.4 g kg^{-1}$) have been determined in esterified sterols of black currants. A marked difference was established in the cholesterol content between free and esterified sterols. In the sterol esters fraction, a significantly higher percentage of cholesterol was observed than in the free form. Similar results were reported earlier for glyceride oils of Apiaceae[17] and for tomato seed oils.[18,19] The other sterol constituents were present in insignificant quantities or in trace amounts in all investigated oils.

The tocopherol and tocotrienol composition of the oils is shown in Table 4. Tocopherols are found to be the major components of all the tocopherol and tocotrienol fractions: the ratio tocopherols:tocotrienols was 99.2:0.8, 99.4:0.6 and 91.8:8.2 in chokeberry, black currant and rose hip respectively. α-Tocopherol is the predominant component in chokeberry seed oil,

Table 4. Tocopherol composition of chokeberry, black currant and rose hip seed oil[a]

	Content ($g kg^{-1}$ wt)		
Tocopherols (T) and tocotrienols (T-3)	Chokeberry	Black currant	Rose hip
α-Tocopherol	70.6	36.9	19.0
α-Tocotrienol	ND	0.1	3.0
β-Tocopherol	28.2	0.2	ND
β-Tocotrienol	ND	0.3	0.7
γ-Tocopherol	0.2	55.4	71.0
γ-Tocotrienol	0.8	0.2	2.5
δ-Tocopherol	0.2	6.9	1.8
δ-Tocotrienol	ND	ND	2.0
Ratio T:T-3	99.2:0.8	99.4:0.6	91.8:8.2

[a] Mean of three replicates.
ND – not detected.

followed by β-tocopherol. γ-Tocopherol is the main constituent in the tocopherol fractions of black currant and rose hip. Those fractions also contain high levels of α-tocopherol. The results obtained for tocopherol composition of black currant and rose hip glyceride oils are similar to the values reported by Pironen *et al.*,[9] for tocopherol composition of fruits of those plants.

All of the tocotrienols have been detected in rose hip oil but only, in negligible amounts.

The fatty acid composition of triacylglycerols, sterol esters and four main individual phospholipids: phosphatidylcholine (PC), phospatidyl ethanolamine (PE), phosphatidylinositol (PC) and phosphatidic acids (PA) is presented in Table 5. Linoleic acid predominates in the triacylglycerols of chokeberry and black currant followed by oleic acid. Oleic acid and palmitic acid are the main components in rose hip oil. The Fatty acid composition of the sterol esters is different from that of the triacylglycerols; The content of saturated fatty acids in them is markedly higher. The percentage of saturated, mainly palmitic and stearic, and long chain (more than 20 carbon atoms) fatty

Table 5. Fatty acid composition of triacylglycerols, phospholipids and sterol esters of chokeberry, black currant and rose hip seed oils[a]

Species	Fatty acids ($g kg^{-1}$ wt)											Saturated fatty acids (g per 100 g)
	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{22:0}$	
Chokeberry												
Oil	ND	tr	5.1	tr	ND	1.1	21.4	71.1	0.5	ND	0.8	7.0
PC	ND	ND	24.4	ND	ND	10.3	22.7	38.5	ND	ND	3.1	37.8
PE	ND	ND	46.8	ND	ND	13.2	13.4	22.9	3.7	ND	ND	60.0
PI	ND	ND	29.1	ND	ND	8.2	9.6	16.5	2.3	5.4	29.4	72.1
PA	3.3	0.7	5.7	2.1	12.1	2.1	8.5	24.5	12.5	1.1	23.8	48.8
Sterol esters	2.0	4.4	8.0	0.4	8.1	27.0	3.1	11.0	7.3	7.3	21.7	78.5
Black currant												
Oil	ND	tr	6.4	tr.	ND	1.6	16.1	57.8	13.2	0.2	4.7	12.9
PC	ND	ND	26.4	ND	ND	6.4	2.1	51.6	9.3	ND	4.2	37.0
PE	ND	tr	28.8	ND	ND	3.0	1.3	47.7	9.6	0.9	8.9	41.6
PI	ND	ND	27.4	0.2	ND	2.8	12.8	49.5	5.4	ND	4.8	35.0
PA	ND	tr	31.2	ND	ND	16.6	ND	17.0	17.5	15.6	1.6	65.0
Sterol esters	ND	0.4	8.8	0.3	10.5	17.2	0.5	15.4	13.7	13.2	20.7	70.8
Rose hip												
Oil	2.5	0.4	17.8	2.6	ND	8.8	52.6	2.1	1.6	3.5	8.0	41.0
PC	tr	tr	11.2	ND	0.6	6.2	23.5	17.1	2.3	12.3	26.6	56.9
PE	ND	1.0	15.7	1.6	ND	20.1	33.7	23.3	1.8	2.8	ND	39.6
PI	tr	ND	32.7	1.0	ND	25.2	22.9	12.9	2.7	2.6	ND	60.5
PA	0.6	1.6	17.1	1.4	ND	18.7	ND	32.1	5.8	8.0	14.7	60.7
Sterol esters	ND	1.8	21.1	2.0	ND	19.8	38.6	4.9	0.9	6.9	4.6	53.6

PC – phosphatidylcholine; PE – phosphatidylethanolamine; PI – phosphatidylinositol; PA – phosphatidic acids; ND – not detected
[a] Mean of three replicates.

acids are $78.5 g kg^{-1}$ in chokeberry; $70.8 g kg^{-1}$ in black currant and $53.6 g kg^{-1}$ in rose hip sterol esters. In the corresponding glyceride oils those quantities were $7.0 g kg^{-1}$, $12.9 g kg^{-1}$ and $41.0 g kg^{-1}$ respectively. In phospholipids the content of saturated and long chain fatty acids is also significantly higher. High levels of them were detected in phosphatidylcholine of rose hip oil, in phosphatidylethanolamine of chokeberry oil, and in phosphatidylinositol of chokeberry and rose hip oil. The saturated fatty acids contents in phosphatidic acids of black currant and rose hip oil were $65.0 g kg^{-1}$ and $60.7 g kg^{-1}$ respectively. Those observations are in agremeent with the data reported earlier about the fatty acid composition of the phospholipids and sterol esters of other seed oils.[17–19]. According to Munshi *et al*[20] the differences between fatty acid composition of triacylglycerols, sterol esters and phospholipids may be due to different phases of the biosynthesis of those compounds and the stages of biosynthesis and accumulation of fatty acids. First phospholipids and sterol esters are synthesised, then triacylglycerols are accumulated. The first stage is also characterised by a high concentration of saturated fatty acids, especially palmitic and stearic, which are accumulated in phospholipids and sterol esters.

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