Grasas y Aceites
Vol. 50. Fasc. 5 (1999), 351-354

Lipid composition of *Evonimus japonicus L., Piracantha coccinea L.* and *Amelanchier cannadensis L.* seed oils

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RESUMEN

Composición lipídica de aceites de semilla *Evonimus japonicus L., Piracantha coccinea L.* y *Amelanchier cannadensis L.*

El contenido en aceite de las semillas *Evonimus japonicus L., Piracantha coccinea L.* y *Amelanchier cannadensis L.* fue del 45,4%, 3,7% y 7,2% respectivamente. Los ácidos oleico, linoleico y palmítico predominaron en los triacilgliceroles. El contenido de fosfolípidos en los aceites, principalmente fosfatidilcolina, fosfatidilinositol, fosfatidiletanolamina y ácidos fosfatídicos fue del 1,1%, 7,5% y 2,8% respectivamente. Las cantidades de esteroles encontradas fueron del 0,4% en el aceite de *Evonimus japonicus L.*, 0,6% en el aceite de *Piracantha coccinea L.* y 0,9% en el aceite de *Amelanchier cannadensis L.* El componente principal en todos los aceites fue el β-sitosterol. Se identificaron también en pequeñas cantidades campesterol, estigmasterol, brasicasterol, colesterol, Δ^7-estigmasterol, $\Delta^{7,25}$-estigmasterol, Δ^5-avenasterol y Δ^7-avenasterol. Se identificaron además todos los tocoferoles en dichos aceites.

PALABRAS-CLAVE: Aceite de semilla - Amelanchier cannadensis L. - Composición lipídica - Evonimus japonicus L. - Piracantha coccinea L.

SUMMARY

Lipid composition of *Evonimus japonicus L., Piracantha coccinea L.* and *Amelanchier cannadensis L.* seed oils.

The seeds of *Evonimus japonicus L., Piracantha coccinea L.* and *Amelanchier cannadensis L.* contained 45.4%, 3.7% and 7.2% oil respectively. Oleic, linoleic and palmitic acids predominated in the triacylglycerols. The content of phospholipids in the oils, mainly phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine and phosphatidic acids was 1.1%, 7.5% and 2.8% respectively. The sterols amounts were found to be 0.4% in *Evonimus japonicus L.* oil, 0.6% in *Piracantha coccinea L.* oil and 0.9% in *Amelanchier cannadensis L.* oil. β-Sitosterol was the main component in all oils. Campesterol, stigmasterol, brassicasterol, cholesterol, Δ^7-stigmasterol, $\Delta^{7,25}$-stigmasterol, Δ^5-avenasterol and Δ^7-avenasterol were identified in small quantities too. All of the tocopherols were identified in the oils.

KEY-WORDS: Amelanchier cannadensis L. - Evonimus japonicus L. - Lipid composition - Piracantha coccinea L. - Seed oil.

1. INTRODUCTION

Evonimus japonicus L. from fam. *Celastraceae, Piracantha coccinea L.* and *Amelanchier cannadensis L.*, both belonging to *Rosaceae* family are widely grown as ornamental plants in Bulgaria. The fruits of *Evonimus japonicus L.* are used in traditional medicine and the seeds are employed to obtain oil for technical purposes. The fruits of *Piracantha coccinea L.* and *Amelanchier cannadensis L.* contain a significant amount of carbohydrates (about 10,0%) and vitamin C (400 - 500 mg/kg) and are used as a source of preparation of jam, fruit wine etc. (Stoianov and Kitanov, 1960). However, knowledge of the nature and content of lipid constituents present in the seeds is still fragmentary. On the other hand, the information about the content composition of triacylglycerols, phospholipids, sterols and tocopherols is interesting from the scientifical and practical point of view. In the present investigation we have attempted to characterise the fatty acid composition of the triacylglycerols, the content and composition of the above *mentioned* biological active components of the oils.

2. MATERIAL AND METHODS

Material

Fruit material. The fruits of the investigated plants were obtained from the Plovdiv region in South Bulgaria, crop 1996.

Methods

The fruits were dried, milled and extracted in Soxhlet apparatus with n-hexane for 8 h. After removing of the solvent under reduced pressure in a rotary film evaporator the extracted oils were weighed.

Fatty acid composition. The fatty acid composition of triacylglycerols was determined by gas liquid

chromatography of their methyl esters. The esterification was carried out by Metcalfe and Wang technique (1981). The methyl esters were purified by thin layer chromatography (TLC) on plates covered with Silica gel 60 G «Merck» and mobile phase n-hexan: diethyl ether 97:3. The determination was performed on a HP 5890 A unit provided with FID and 30 m capillar column «Innowax» impregnation (Scotia Pharmaceuticals Ltd, Carlisle, UK) and conditions as follows:

- column temperature 165 - 225 °C, 4 °C/min.
- 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.

Phospholipid composition. The lipids were extracted from the seeds by Folch procedure (1957). The polar lipids were divided from the unpolar lipids by column chromatography (Kates, 1972). The phospholipid constituents were separated by two-directional thin-layer chromatography on Silica gel 60 G «Merck», impregnated with 1% $(NH_4)_2SO_4$ water solution (Beshkov, 1972). The first direction was carried out in chloroform:methanol:ammonia 65:25:5 v/v/v and 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 spraing with specific reagents (Kates 1972). In addition Rf and standard were used for definitive identification. The quantitative evaluation was carried out spectrophotometrically at 700 nm on the base of free phosphorus after mineralisation with perchloric acid: sulphuric acid 1:1 (Beshkov, 1972).

Sterol composition. The free sterols and sterol esters were separated from the other oil constituents by preparative TLC on Silica gel 60 G «Merck» and mobile phase n-hexane:diethyl ether 1:1. The sterol esters were saponified with ethanolic KOH, extracted and purified by TLC. The evaluation of the general content of both sterol fractions was carried out spectrophotometrically at 597 nm. The quantity of sterols was valued on base of standard solution. (Ivanov,1972). The individual composition was identified by gas chromatography, using HP 5890 A unit with FID, 25 capillar column impregnated with OV-17 and conditions as follows:

- column temperature 260 - 300 °C, 6 °C/min
- detector temperature 320 °C, injector temperature 300 °C
- gas carrier - nitrogen

The identification was confirmed by comparing the retention time of the individual constituents with those of the authentic samples.

Tocopherol composition. Tocopherols and tocotrienols were analysed directly in the oils by HPLC with fluorescence detection (ISO 1989, Ivanov,1995). «Merck-Hitachi» unit fitted with column «Nucleosil» Si 50-5 250 x 4 mm provided with Fluorescent detector «Merck-Hitachi» F 1000 was used. The operating conditions were as follows: λ exc 295, λ em 330 nm, mobile phase n-hexane: dioxane 94:4, rate of mobile phase 1 ml/min. The peaks were identified using authentic individual tocopherols and tocotrienols as standards.

All values are average from three parallel determinations.

3. RESULT AND DISCUSSION

The data about the general composition of the investigated samples presented in Table I showed that *Evonimus japonicus L.* fruits were found to be the richest in vegetable oil: 45,8% in the dried seeds. The highest content of phospholipids (7,5%) was observed in the oil of *Piracantha coccinea L.* The content of tocopherols and tocotrienols in all of the oils was unsignificant - from 8,3 mg/kg in *Evonimus japonicus L.* seed oil to 13,9 mg/kg in *Amelanchier cannadensis L.* seed oil. Similar quantities of sterols (0,4-0,9%) were detected in the oils.

Fatty acid composition of triacylglycerols is given in Table II. The qualitative fatty acid composition was similar in all investigated oils. In the samples of fatty acid methyl esters a total of 79,8%, 80,0% and 73,8% respectively of unsaturated fatty acids was determined. The percentage of essential fatty acids was 30,1%, 49,3% and 36,9% for the three oils, respectively. Oleic acid (27,5 - 47,8%) and linoleic acid (23,3 - 30,2%) were the main unsaturated acids. A considerable percentage of palmitoleic acid (9,4%) was detected in *Amelanchier cannadensis L.* oil. Palmitic acid predominated as the main saturated fatty acid (13,4 - 23,1%). The fatty acids 14:0, 17:0, 18:0, 20:0, 20:1, 22:0 and 22:1 were identified in separate *oils* in quantities less than 1,0%.

The qualitative analysis based on response to specific spray reagents on thin layer chromatography and comparison with authentic samples indicated the presence of phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine and phosphatidic acids as major phospholipids in all three oils (Table III). Lysophosphatidylcholine, lysophosphatidylethanolamine, mono- and di-phosphatidylglycerols were also detected. *Evonimus japonicus L.* Phosphatidylserine was identified in L. oil only. Phosphatidylcholine predominated in all phospho-lipid fractions: 40,5% in *Evonimus japonicus L.,* 33,3% in *Piracantha coccinea L.* and 31,4% in *Amelanchier cannadensis L.*, followed by phosphatidylinositol: 32,2%, 22,2% and 23,7% respectively. A significant amount of phosphatidic acids were estimated in all of the oils.The other phospholipids were presented in negligible quantities.

Table I
Content of oil in seeds and phospholipids, sterols and tocopherols in oils *

N.°	Botanical name	Content of oil in seeds in dried seeds	Content of main lipid compounds in the oils		
			Phospholipids (% wt)	Sterols (% wt)	Tocopherols (mg/kg)
1	*Evonimus japonicus L.*	45,4	1,1	0,4	8,3
2	*Piracantha coccinea L.*	6,5	7,5	0,6	10,5
3	*Amelanchier cannadensis L.*	3,7	2,8	0,9	13,9

* Values of three determinations.

Table II
Fatty acid composition of triacylglycerols *

N.°	Fatty acids	Content (% wt)		
		Evonimus jap. L.	*Piracantha coc. L.*	*Amelanchier can. L.*
1.	$C_{12:0}$	tr.	tr.	tr.
2.	$C_{14:0}$	0,1	0,1	tr.
3.	$C_{16:0}$	14,4	15,4	23,1
4.	$C_{16:1}$	1,5	0,5	9,4
5.	$C_{17:0}$	0,1	0,1	0,4
6.	$C_{18:0}$	2,7	2,5	2,4
7.	$C_{18:1}$	49,0	30,2	27,6
8.	$C_{18:2}$	24,3	49,2	30,7
9.	$C_{18:3}$	7,0	0,3	6,2
10.	$C_{20:0}$	0,2	0,3	0,2
11.	$C_{20:1}$	0,3	—	—
12.	$C_{22:0}$	0,1	tr.	—
13.	$C_{22:1}$	0,1	tr.	—
14.	Unidentified fatty acids	0,2	1,4	—

* Values of three determinations.

Table III
Phospholipids composition of seed oils *

N.°	Phospholipids	Content (% wt)		
		Evonimus jap. L.	*Piracantha coc. L.*	*Amelanchier can. L.*
1.	Phosphatidylcholine (PC)	40,5	30,5	31,4
2.	Phosphatidylinositol (PI)	32,2	22,2	23,7
3.	Phosphatidylethanolamine (PE)	4,9	16,6	15,3
4.	Phosphatidic acids (PA)	13,8	13,3	13,5
5.	Lysophosphatidylcholine (LPC)	3,5	5,5	—
6.	Lysophosphatidylethanolamine (LPE)	0,6	—	5,8
7.	Phosphatidylserine (PS)	1,2	—	—
8.	Monophosphatidylglycerol (MPGL)	1,3	9,1	4,2
9.	Diphosphatidylglycerol (DPGL)	1,1	—	6,1
10.	Unidentified phospholipids	0,9	—	—

* Values of three determinations.

The composition of free sterols and esterified sterols of the investigated oils is presented in Table IV. As it is shown in the Table, the major part of the sterols - 87,1% in *Evonimus japonicus L.*, 81,0% in *Piracantha coccinea L.* and 70,8% in *Amelanchier cannadensis L.* seed oils occur as free sterols.

In the sterol fraction at least 9 constituents were presented in all oils. β-Sitosterol predominated in both free and esterified sterols. Their content was ranged from 71,8% in *Amelanchier cannadensis L.* to 95,9% in *Piracantha coccinea L.* followed by stigmasterol, campesterol and Δ^5-avenasterol. More considerable amounts of campesterol, brassicasterol, stigmasterol and Δ^5-avenasterol were identified in the *Amelanchier cannadensis* oil. $\Delta^{7,25}$-stigmasterol was identified in *Evonimus japonicus L.* only. Marked differences in the quantitative composition between free and esterified sterols were not observed.

The tocopherol and tocotrienol composition of the oils is shown in Table V. δ-Tocotrienol was not detected in any of the samples and it was not included in the Table.

α-Tocopherol - 58,2% was the predominant component in *Piracantha coccinea L.,* followed by β-tocopherol - 31,4%. The other tocopherols and tocotrienols were presented in small quantities.

The qualitative and quantitative composition of the tocopherols of *Evonimus japonicus L.* seed oil was simmilar to that of *Amelanchier cannadensis L.* oil. It was established that the amounts of α-, β-, γ-, δ-tocopherols were closed in both oils.

In *Evonicus japonicus L.* oil was established a significant presence of unsaturated α and γ tocotrienols - 8,3% and 21,1% respectively. *Amelanchier cannadensis L.* oil contained α-tocotrienol 23,6% and β-tocotrienol 17,0%. Tocopherols predominated in all tocopherol fractions: the ratio tocopherols: tocotrienols was 70,5:29,5, 92,1:7,9 and 59,4:40,6 respectively.

Table IV
Sterol composition of seed oils *

N.°	Sterols	Content (% wt)					
		Evonimus jap. L		Piracantha coc. L		Amelanchier can. L	
		free	esterified	free	esterified	free	esterified
1.	Cholesterol	0.9	1,0	0,3	0,5	0,5	1,1
2.	Campesterol	1,5	1,0	1,7	1,3	7,6	8,8
3.	Brassicasterol	tr.	tr.	0,1	0,3	7,8	1,7
4.	Stigmasterol	6,8	1,3	0,4	2,4	6,6	2,8
5.	β-Sitosterol	87,3	86,5	95,9	90,7	71,8	75,9
6.	Δ^5-Avenasterol	1,3	1,3	0,4	1,7	5,6	7,4
7.	Δ^5-Stigmasterol	0,9	1,0	1,0	1,8	0,1	tr.
8.	$\Delta^{7,25}$-Stigmasterol	1,1	1,6	—	—	—	—
9.	Δ^7-Avenasterol	tr.	6,3	0,2	1,3	tr.	2,3
	Ratio sterols free: sterified	87,1:12,9		81,0:19,0		70,8:19,2	

* Values of three determinations.

Table V
Tocopherol composition of seed oils *

N.°	Tocopherols (T) and Tocotrienols (T-3)	Content (% wt)		
		Evonimus jap. L	*Piracantha coc. L*	*Amelanchier can. L*
1.	α-Tocopherol	18,1	58,2	16,0
2.	α-Tocotrienol	8,3	2,1	23,6
3.	β-Tocopherol	10,5	31,4	8,6
4.	β-Tocotrienol	0,4	0,4	17,0
5.	γ-Tocopherol	22,8	0,7	21,6
6.	γ-Tocotrienol	21,1	5,4	—
7.	δ-Tocopherol	18,8	1,8	13,2
	Ratio T:T-3	70,2:29,8	92,1:7,9	59,4:40,6

* Values of three determinations.

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Recibido: Mayo 1998
Aceptado: Diciembre 1998