LIPID COMPOSITION OF FLAX SEED OIL

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Abstract: The composition of seven varieties of flax seeds was investigated. 34.2 - 44.4% wt vegetable oil in the seeds was found to be. Fatty acids composition of the triacylglycerols was determined by capillary gas chromatography. Linolenic (35.3 - 42.0%) and linoleic (14.9 - 19.0 %) acids are the main unsaturated fatty acids. Palmitic acid (8.7 - 12.1%) predominated in fraction of saturated acids, followed by stearic acid. The quantity of tocopherols determined by HPLC with fluorescence detection was 602 - 788 mg/kg. Phospholipids were isolated by column chromatography and quantifiated spectrophotometrically at 700 nm. Their percentages in the oil were found to be 0.1 - 1.0% wt. Total sterol content determined by gas chromatography was 0.2-0.4%.
Keywords: Linum usitatissimum L.; glyceride seed oil; phospholipids; sterols; tocopherols; fatty acids.

Introduction

The flax plant (*Linum usitatissimum* L.), fam. *Linaceae* is not a new crop and native to West Asia and the Mediterranean. As the source of linen fibre, flax has been cultivated since at least 5000 BC. Today it is grown for its glyceride oil and for fibres. Linseed (Flax) seed oil is a kind of high quality edible oil and is used in cosmetic, pharmaceutical industry and as food additive. It is rich in α-linolenic acid and various unsaturated fatty acids. It plays an important role in the promotion of human intelligence, physical brain, preventing cardiovascular disease, and the suppression of disease genes (10). On the other hand the presence of polyunsaturated acids makes the oil unstable to oxidation. In view of this reason fatty acid profile of the oil is an important parameter in evaluating the quality of the flax oil. The oxidative stability of the oils is greatly affected and by minor components such as tocopherols, sterols and phospholipids.

This study focuses on the determination of the total content of glyceride oil in the 7 varieties of flaxseeds (*Linum usitatissimum* L.) cultivated in Bulgaria, the general content of biologically active substances as sterols, phospholipids and tocopherols in the oils. Fatty acid composition of triacylglycerols was investigated too.

Materials and methods

All solvents and reagents were analytical grade and were used without additional purification. Reference phospholipids and fatty acid methyl esters were purchased from Fluka (Chemie Gmbh, Switzerland). Reference tocopherol isomers and individual sterols were purchased from Merck (Darmstadt, Germany). TLC plates were prepared in the laboratory using Silica gel 60 (Merck, Darmstadt, Germany).

***Samples*.**

The Flaxseeds were growed and obtained from the Institute of Plant Genetic Resources, Sadovo, Bulgaria, crop 2010.

Isolation of glyceride oil and determination of oil content

The seeds (50g sample) were air-dried and the oil was extracted with n-hexane in *Soxhlet* unit for 8 h. The solvent was partly removed in rotary vacuum evaporator, the residue was transferred in pre-weight glass vessels and the rest of the solvent was removed under stream of nitrogen to a constant weight to determine the oil content in the seeds [1].

***Phospholipids*.**

Another part (10 g) of air-dried seeds was subjected to Folch extraction according to Christie [8]. Polar lipids were isolated from the total lipids by column chromatography [8]. The quantification was carried out spectrophotometrically against a standard curve by measuring the phosphorous content at 700 nm. Etalon – 10 μkl/cm^3 water solution of KH_2PO_4 as P. Content of phospholipids in the sample – 1-125 mkg/kg as P.

***Sterols*.**

The oil was hydrolyzed with ethanolic KOH [8], sterols were extracted with light petroleum ether and purified by silica gel TLC on 20x20 cm plates covered with 0.2 mm Silica gel 60 G layer (Merck, Darmstadt, Germany), impregnated by 0.2% NaOH water solution with mobile phase n-hexane: diethyl ether 1:1 (by volume). Sterol content was determined by Gas chromatography [3] on a HP 5890 (Hewlett Packard GmbH, Austria) gas chromatograph equipped with a 30 m x 0.25 mm (I.D.) capillary DB-5 column, Hewlett Packard GmbH, Austria) and a FID.

***Tocopherols*.**

Tocopherols were determined directly in the oil by high performance liquid chromatography (HPLC) on a "Merck-Hitachi" (Merck (Darmstadt, Germany) instrument equipped with 250 mm x 4 mm Nucleosil Si 50-5 column (Merck (Darmstadt, Germany) and fluorescent detector "Merck-Hitachi" F 1000 [2].

***Fatty acids*.**

The total fatty acid composition was determined by GC after transmethylation of the respective sample with 2N methanolic KOH at 50^{o}C according to *Christie* [8]. Fatty acid methyl esters (FAME) were purified by silica gel TLC on 20x20 cm plates covered with 0.2 mm Silica gel 60 G layer (Merck, Darmstadt, Germany) with mobile phase n-hexane:acetone 100:8 (by volume). GC was performed on a HP 5890 (Hewlett Packard GmbH, Austria) gas chromatograph equipped with a 30 m x 0.25 mm (I.D.) capillary InnoWax column (cross-linked PEG, Hewlett Packard GmbH, Austria) and a FID.

Results and discussion

General characteristics of the seeds and oils

General characteristics of the seeds were determined such as: oil content in dry seeds, content of total phospholipids, sterols and tocopherols in the oils. The results are shown in Table 1.

Table 1 Content of glyceride oil in Linum usitatissimum L. and phospholipids, sterols and tocopherols in the oils*

Compounds	Varieties *Linum usitatissimum* L.						
	A900012	A900013	A900014	A900015	A900016	A900017	A900018
Oil content, %	33.8	37.3	38.0	41.6	37.1	36.2	44.5
Tocopherols, mg/kg	731	766	768	770	788	775	602
Phospholipids, %	0.7	0.9	1.0	0.4	0.8	1.0	0.7
Sterols, %	0.3	0.4	0.3	0.3	0.3	0.3	0.2

*Average of three determinations

The content of glyceride oil in the seeds of all cultivars varied in limits of 33.8 - 44.5%. The seeds of A900018 and A900015 were richest in the oil - 44.5% and 41.6%. Those quantities are higher than the results reported earlier by *Beltagy* – 36.0-39.0% [5] and *Bozan* -33.6% [7].

The quantity of phospholipids in the oils (0.4-1.0%) was closed to percentages of other vegetable oils as sunflower and rapeseed (0.7-0.9 % and 0.7-1.0 % respectively (*Gunstone*, *Zlatanov et al.)*[10,3].

Total content of tocopherols in the glyceride oils (602-788 mg/kg) were in closed quantities and higher than date reported by *Przybylski* (347 mg/kg) [12] and *Gunston* (440-588 mg/kg) [10], but lower than percentages established by *Oomah et al*.–845-972 mg/kg [11]. ɣ-tocopherol predominated in the tocopherol fraction, followed by ɣ-tocotrienol and α-tocopherol.

The content of sterols was found to be 0.2-0.4%. Those values were in agreement to data reported by *Przybylski* and *Gunston* (0.23% and 0.42% respectively) [12; 10].

Qualitative fatty acid profile of triacylglycerols was showed in Table 2. Al of investigated triacylglycerol fractions contain a significant number of fatty acid, but the qualitative composition was found to be similar. The predominant constituents in the oils were unsaturated fatty acids as linolenic, linoleic and oleic (81.8-87.6%). Linoleneic acid is the main unsaturated component (35.3-42.0%), followed by linoleic and oleic acids. This lower content was at the expense of higher quantity of monounsaturated oleic acid (26.0-33.5%). Palmitic and stearic acid, the main saturated components, were detected in reasonable amounts.

Table 2 Fatty acid composition of seven varieties of *Linum usitatissimum L.**

Fatty acids,%	Varieties *Linum usitatissimum* L.						
	A900012	A900013	A900014	A900015	A900016	A900017	A900018
$C_{14:0}$ Myristic	0.2	0.2	0.2	0.1	0.1	0.1	0.2
$C_{14:1}$ Myristoleic	0.1	0.1	0.2	0.3	0.1	0.1	0.3
$C_{15:0}$ Pentadecenoic	0.1	-	0.1	-	-	0.1	0.1
$C_{16:0}$ Palmitic	12.1	9.0	10.1	10.7	8.7	9.0	10.5
$C_{16:1}$ Palmitoleic	0.2	0.1	0.1	0.2	0.1	0.1	0.2
$C_{17:0}$ Margaric	0.1	0.1	0.1	0.1	0.1	0.1	0.1
$C_{18:0}$ Stearic	5.7	4.8	4.3	4.2	4.3	3.1	4.0
$C_{18:1}$ Oleic	31.1	30.6	26.0	29.1	33.5	30.3	27.4
$C_{18:2}$ Linoleic	15.1	17.5	19.0	18.9	15.8	16.4	14,9
$C_{18:3}$ Linolenic	35.3	37.6	39.6	36.2	37.3	40.7	42.0
$C_{20:0}$ Arachidic	-	-	0,1	0.1	-	-	0.1
$C_{20:2}$ Eicosadienoic	-	-	0.2	0.1	-	-	0.2
SFA**	18.2	14.1	14.9	15.2	13.2	12.4	15
UFA***	81.8	85.9	85.1	84.8	86.8	87.6	85.0
MUFA****	31.4	30.8	26.3	29.6	33.7	30.5	27.9
PUFA*****	50.4	55.1	58.9	55.2	53.1	57.1	57.1

*Average of three determinations
** Saturated fatty acids
*** Unsaturated fatty acids
**** Monounsaturated fatty acids
***** Polyunsaturated fatty acids

These data are significantly different to fatty acid composition reported earlier by Bhatty [6], Gunston [10], Choo et al. [9], Bozan [7] and Przybylski [12] according them the quantity of linolenic acid was found to be higher - 46.0-58.3%. The results might be explained by the different agrometeoroligical conditions (mainly temperature and humidity) for cultivations of the plants.

Conclusion

The seeds of *Linum usitatissimum* L. cultivated in Bulgaria contain glyceride oils rich in biologically active substances as sterols, phospholipids and tocopherols. Fatty acid composition of triacylglyceros was specific and was characterized with lower content of linolenic acid at the expense of oleic acid.

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