Bulgarian Journal of Agricultural Science, 20 (No 1) 2014, 112-116
Agricultural Academy

CHANGES OF COMPOSITION IN TRIACYLGLYCEROLS, STEROLS AND TOCOPHEROLS OF FLAX DURING THE VEGETATION

O. TENEVA[1], M. ZLATANOV[1*], G. ANTOVA[1] and M. MARCHEVA[2]
[1] *University of Plovdiv "P. Hilendarski", Department of Chemical Technology, BG - 4000 Plovdiv, Bulgaria*
[2]*Agricultural University of Plovdiv, BG – 4000 Plovdiv, Bulgaria*

Abstract

TENEVA, O., M. ZLATANOV, G. ANTOVA and M. MARCHEVA, 2014. Changes of composition in triacylglycerols, sterols and tocopherols of flax during the vegetation. *Bulg. J. Agric. Sci.,* 20: 112-116

The changes of content and composition of flax seed fatty acids, sterols and tocopherols during vegetation in period April - July were investigated. The quantity of glyceride oil increased from 174 - 327g.kg^{-1} between 21 - 35 day after flowering. In the triacylglycerol fraction the content of oleic acid decreased from 383 g.kg^{-1} to 322 g.kg^{-1} at the expense increasing of linolenic acid (from 294 to 342 g.kg^{-1}). In sterol esters higher content of monounsaturated oleic acid was detected – from 392 g.kg^{-1} at 21 day after flowering to 517 g.kg^{-1} at 35 day after flowering. The quantity of stearic acid decreased from 167 g.kg^{-1} to 107 g.kg^{-1}. The content of sterols was 4 g.kg^{-1} in whole period. β - Sitosterol was the main component in free (454 – 523 g.kg^{-1}) and esterified sterols (553 – 566 g.kg^{-1}) during vegetation, followed by campesterol. The percentage of tocopherols was highest in the first stage of development (975 mg/kg vs 898 mg/kg) in the last period.

Key words: flax seeds, vegetation, fatty acids, tocopherols, sterols

Introduction

Flax (*Linum usitatissimum* L.) is a member in *Linaceae* family. Flax is one of the oldest and multi–purpose oilseed crops cultivated in Europe and Asia (Beltagi et al., 2007). In recent years, the flax seeds are used as a source for obtaining of glyceride oil rich of essential fatty acids, mainly linolenic acid and as food supplement in order to improve health status. Because fatty acids other compounds as sterols and tocopherols were found to be important biologically active substances with substantial role for food value of the oils. Significant contents of carbohydrates, dietary fibers, proteins were found to be in the seeds (Bozan and Temelli, 2008; Piłat and Zadernowski, 2010). These compounds accumulate with different intensity in the oils depending of agrometeorological conditions as temperature, humidity, season etc. (Przybylski, 2005). According Herchi et al. (2012) the oil content increased gradually from 93.5 g.kg^{-1} to 426 g.kg^{-1} in 42 day after flowering (DAF) and after that the quantity decreased to 350 g.kg^{-1} in 56 DAF.

Unsaturated fatty acids mainly oleic acid ($C_{18:1}$) and linolenic ($C_{18:3}$) are the major components (85%) in triacylglycerol fraction. Gunstone (2002) reported that linolenic acid ($C_{18:3}$) was about 474 g.kg^{-1}, followed by linoleic ($C_{18:2}$) – 241 g.kg^{-1} and oleic ($C_{18:1}$) – 190 g.kg^{-1}. Bozan and Temelli (2008) and Choo et al. (2007) reported about 705 g.kg^{-1} linolenic acid in the oil, while saturated fatty acids ranged from 90 g.kg^{-1} to 120 g.kg^{-1}. Phytosterols were presented in the flax seed oil in a relatively lower amount (about 2 – 4 g.kg^{-1}) as β-sitosterol was the main component (Gunstone, 2002).

The level of tocopherols is 400 – 500 mg/kg in flaxseed oil. γ -Tocopherol predominated in tocopherol fraction (Herchi et al., 2011b).

Information about changes in composition of triacylglycerols, sterols and tocopherols as well as distribution of free and esterified sterols and fatty acids of sterol esters is very scanty. In this connection, the aim of this study was to determine the changes of content of glyceride oil in the seeds and content as well changes of individual composition of triacylglycerols, tocopherols and sterols during vegetation of flax seeds for establishing of optimal harvest conditions of plants and prognosis of lipid composition.

Materials and Methods

Reagents and standards

All solvents and reagents (reference fatty acids, tocopherols and sterols) are with analytical grade provided from Mer-

Corresponding author: magzlat@uni-plovdiv.bg

ck (Darmstadt, Germany). Thin layer chromatography plates (TLC), (0.2 mm, 20 x 20 cm) for purification of fatty acids, methyl esters and sterols were prepared in laboratory using silica gel 60 G (Merck, Darmstadt, Germany).

Samples

The flax seeds variety *A900012* were grown on plantation in Plovdiv region, Bulgaria in April – July, 2012. The seeds were studied in period 35 days after flowering (10 June 2012) when the oil was accumulated in seeds.

Determination of oil content

The seeds (20 g sample) were air-dried (10% humidity). The humidity was determined by drying at 105^{0}C to constant weight. Flaxseed oil from finely grounded seeds was extracted with hexane in a Soxhlet apparatus for 8h (ISO 659, 2009). After extraction, the solvent was removed by a rotary vacuum evaporator and oil was determined by weight.

Determination of fatty acid composition

Fatty acid composition of triacylglycerols and sterol esters was determined by gas chromatography (GC) of fatty acid methyl esters (FAME) (ISO 5508, 1990). FAME were prepared by pre-esterification with sulfuric acid in methanol as catalyst (Christie, 2003) and were purified by TLC on silica gel 60 G with mobile phase hexane: acetone = 100:8 (by volume). The operating conditions were as follows: GC HP 5890 A equipped with 60 m x 0.25 mm x 0.25 μm DB – 23 column and FID detector. The temperature gradient was from 130^{0}C for 1.0 min, 130 - 170^{0}C at 6.5^{0}C/min, 170 - 215 ^{0}C at 30^{0}C/min, 215^{0}C for 9 min, 215 - 230^{0}C at 40^{0}C/min to 230^{0}C for 1 min. Hydrogen was the carrier gas, split 100:1. The temperature of detector and injector was kept at 270^{0}C. Identification of fatty acids was performed by comparison with standard mixture of FAME.

Determination of sterols

Free sterols and sterol esters were isolated by preparative thin layer chromatography (TLC) on neutral 0.2 mm, 20x20 cm silica gel 60 G plates (Christie, 2003). Sterol esters were hydrolysed with ethanolic KOH (ISO 12228, 1999). Free sterols and sterols obtained after saponification of sterol esters were purified by TLC on 0.2N NaOH silica gel 60 G plates, mobile phase hexane : diethyl ether (1:1) and were analysed by GC with HP 5890 A equipped with 30 m x 0.25 mm DB-5 column. A temperature gradient from 90^{0}C (2 min) to 290^{0}C at 15^{0}C/min and increased to 310^{0}C at 4^{0}C/min and held at this temperature for 10 min was applied. The injector and detector temperatures were 300^{0}C and 320^{0}C respectively. Hydrogen was the carrier gas, split 100:1. Total content of sterols was determined spectrophotometrically at 597 nm (Ivanov et al., 1972).

The obtained and isolated free fatty acids from sterol esters were methylated, purified and determined as fatty acids of triacylglycerols.

Determination of tocopherols

Qualitative and quantitative composition of tocopherols was determined directly in the oil by high performance liquid chromatography (HPLC) (ISO 9936, 2006) on a Merck-Hitachi (Merck, Dormstadt, Germany) equipped by 250 x 4 mm Nucleosil Si 50-5 column (Macherey - Nagel, Germany) and fluorescent detector Merck – Hitachi F 1000. The operating conditions were as follows: mobile phase hexane : dioxane = 96:4, excitation 295 nm, emission 330 nm, flow rate 1 ml/min. Tocopherols were identified by comparison with standard mixture and determined quantitatively on the basis of tocopherol peak areas in the samples versus the peak area of standard α-tocopherol solution.

Statistics

All analyses were made in triplicate. Statistical differences between samples were tested using ANOVA. Dates were expressed as mean ± SD. The level of significance was set at $p<0.05$.

Results and Discussion

The changes of total content of oil and biologically active substances during flaxseed development are presented in Table 1.

In contrast to data reported by Herchi et al. (2012) it was observed that the accumulation of glyceride oil increased

Table 1
Content of oil, tocopherol and sterol during flaxseed vegetation

Compounds		Day after flowering		
		21 DAF	28 DAF	35 DAF
Oil content in the seeds, g.kg^{-1}		174 ± 5.0	291 ± 12.0	327 ± 9.0
Content of tocopherols in the oil, mg/kg		975 ± 39.0	890 ± 44.5	898 ± 35.9
Sterol content in the oil, g.kg^{-1}		4 ± 0.1	4 ± 0.1	4 ± 0.2
Sterol ratio, g.kg^{-1}	Free sterols	757 ± 23.0	730 ± 29.0	769 ± 23.0
	Esterified sterols	243 ± 8.0	243 ± 12.0	231 ± 9.0

*Average of three determinations

continuously in whole period of development from 174 g.kg^{-1} in the first stage to 327 g.kg^{-1} in the last period. The content of tocopherols was decreased insensibly in accordance of other authors Herchi et al. (2012) while the percentage of sterols was constant in whole period. The main part of sterols was presented in free form (more than 700 g.kg^{-1}) and the ratio between both free and esterified sterol fractions was not changed. Individual composition of tocopherols was similar to data reported early (Herchi et al., 2011a).

γ-Tocopherol and γ-tocotrienol predominated and their percentage was relatively constant in all period of development. On the other hand higher content of γ-tocotrienol was found to be in investigated oils (Table 2).

The changes of individual sterol composition were presented in Table 3.

Both sterol fractions were found to be qualitatively identical. The main component was β-sitosterol, followed by campesterol and Δ^5-avenasterol. In free sterol fraction higher quantities of stigmasterol, Δ^5-avenasterol, Δ^7-stigmasterol were detected at the expence of lower content of β-sitosterol, campesterol and cholesterol. The content of campesterol, cholesterol, stigmasterol in free sterol fraction decreases during vegetation at the expense of increasing quantities of β-sitosterol. The content of cholesterol and brasicasterol decreased in sterol esters while the level of campesterol increased insensibly from 269 g.kg^{-1} to 297 g.kg^{-1}. Changes in quantity of β-sitosterol were not observed.

The main constituents in triacylglycerol fraction are oleic, linoleic and linolenic acids (Figure 1).

During vegetation, the content of oleic acid decreased gradually from 383 g.kg^{-1} in 21 DAF to 322 g.kg^{-1} in 35 DAF. On the other hand, the content of linolenic acid increases from 294 g.kg^{-1} (21 DAF) to 342 g.kg^{-1} (35 DAF). Total content of polyunsaturated fatty acids increased from 440 g.kg^{-1} to 507 g.kg^{-1} while the level of monounsaturated acids decreased from 386 g.kg^{-1} to 323 g.kg^{-1}.

The qualitative composition of fatty acids isolated from sterol esters after hydrolisation was similar to these of triacylglycerols, but the quantitative composition is significantly different. Higher quantities of saturated palmitic and stearic acids were detected in sterol fraction at the expense of polyunsaturated linoleic and linolenic acids. The content of oleic acid during development of the plants increased (from 392 g.kg^{-1} to 517 g.kg^{-1})

Table 2
Tocopherol composition of flaxseed oils during vegetation

Individual tocopherol composition, g.kg^{-1}	Day after flowering		
	21 DAF	28 DAF	35 DAF
α-tocopherol	21 ± 1.0	32 ± 1.0	32 ± 1.0
γ-tocopherol	619 ± 19.0	622 ± 19.0	602 ± 18.0
γ-tocotrienol	360 ± 11.0	346 ± 14.0	367 ± 11.0

*Average of three determinations

Table 3
Changes of sterol composition during vegetation

Composition, g.kg^{-1}		Day after flowering		
		21 DAF	28 DAF	35 DAF
Cholesterol	free	10 ± 0.2	6 ± 0.2	3 ± 0.1
	esterified	22 ± 0.4	9 ± 0.3	6 ± 0.2
Brasicasterol	free	7 ± 0.2	16 ± 0.4	13 ± 0.4
	esterified	15 ± 0.4	3 ± 0.1	3 ± 1.1
Campesterol	free	162 ± 5.2	129 ± 4	124 ± 4.2
	esterified	269 ± 5.1	276 ± 11	297 ± 12.3
Stigmasterol	free	79 ± 2.4	47 ± 1.1	33 ± 0.9
	esterified	30 ± 0.6	38 ± 1.3	29 ± 0.9
Δ^7 -Campesterol	free	26 ± 0.8	36 ± 0.7	40 ± 2.6
	esterified	10 ± 0.3	8 ± 0.2	3 ± 0.1
β -Sitosterol	free	454 ± 9.1	500 ± 20	523 ± 21.1
	esterified	553 ± 17.0	566 ± 17.1	555 ± 17.3
Δ^5 -Avenasterol	free	182 ± 5.1	193 ± 4.2	198 ± 6.5
	esterified	66 ± 1.2	73 ±1.4	79 ± 3.2
Δ^7 -Stigmasterol	free	76 ± 3.1	69 ± 2.2	62 ± 2.4
	esterified	7 ± 0.1	10 ± 0.2	12 ± 0.4
Δ^7 -Avenasterol	free	4 ± 0.1	6 ± 0.1	4 ± 0.1
	esterified	28 ± 0.8	17 ± 0.5	16 ± 0.5

*Average of three determinations

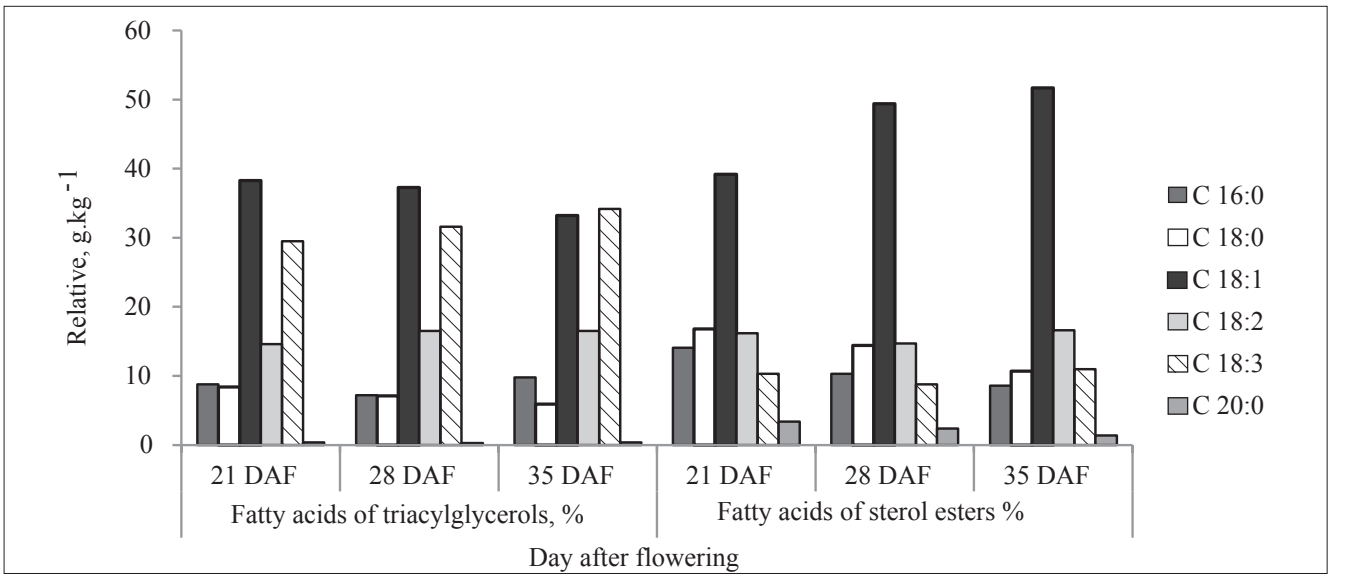

Fig. 1. The changes of fatty acids of triacylglycerols and sterol esters during flaxseed vegetation

while the quantities of palmitic acid (141 – 86 g.kg^{-1}) and stearic acid (167 – 107 g.kg^{-1}) decreased. The levels of linolenic and linoleic acids were not changed significantly. The content of minor compounds as lauric ($C_{12:0}$), myristic ($C_{14:0}$), palmitoleic ($C_{16:1}$), margaric ($C_{17:0}$) and eicosenoic acid ($C_{20:1}$) which percentage was lower than 10 g.kg^{-1} was changed insignificantly in all period of development of the plants.

Generally, the polyunsaturated and monounsaturated fatty acids predominated in triacylglycerols and their content increased gradually in all vegetation period. In sterol esters, more saturated fatty acids were observed. The quantities of monounsaturated fatty acids (mainly oleic acid) increased progressively at the expense of saturated acids. The obtained results about triacylglycerol composition are similar to analogical data announced by Herchi et al. (2011a) (for 0116 variety) but significantly different from changes of other varieties (H52 and P129) reported by the same author where the content of oleic acid increased at the expense of decreasing content of linoleic acid.

The changes in fatty acid composition of triacylglycerols and sterol esters correspond to different phases of their biosynthesis. The same trend was found to be and about other vegetable oils as sunflower and walnut oils (Zlatanov et al., 2009; Momchilova and Nikolova-Damyanova, 2007).

Conclusion

The accumulation of biologically active substances as fatty acids in triacylglycerols and sterol esters, sterols and tocopherols during plant vegetation was accompanied with insignificant changes in their content. On the other hand, there were some changes of the individual composition of the fatty acids and sterols. These changes can be explained with the different stages of biosynthesis of the fatty acids in triacylglycerols and in sterol esters as well as of sterols and sterol esters. The information about these differences can be useful for the determination of optimal harvest conditions of the flax plant and for the prognosis of the content and composition of the main biologically active substances in the oil.

Acknowledgments

The investigations were carried out with the partial financial support of the Science Research Department to University of Plovdiv, Contract SI 13FC006/2013.

References

ISO 5508, 1990. Animal and vegetable fat and oils – Analysis by gas chromatography of methyl esters of fatty acids.

ISO 12228, 1999. Animal and vegetable fat and oils – Determination of individual and total sterols contents (Gas chromatographic method).

ISO 9936, 2006. Animal and vegetable fat and oils – Determination of tocopherol and tocotrienol contents by Hhigh-Performance Liquid Chromatography.

ISO 659, 2009. Oilseeds – Determination of oil content (Reference method).

Beltagi, H. S., Z. A. Salama, D. M. El – Hariri, 2007. Evaluation of fatty acids profile and the content of some secondary metabolities in seeds of different flax cultivars (*Linum usitatissimum* L.), *Gen. Appl. Plant physiology*, **33**: 187-202.

Bozan, B. and F. Temelli, 2008. Chemical composition and oxidative stability of flax, safflower and poppy seed and seed oils. *Bioresource Technology,* **99**: 6354-6359.

Choo, W. S., J. Birch and J. P. Dufour, 2007. Physicochemical and quality characteristics of cold-pressed flaxseed oils. *Journal of Food Composition and Analysis*, **20:** 202-211.

Christie, W. W., 2003. Lipid Analysis. The Oily Press: Bridgwater (3rd Edition), England.

Gunstone, F., 2002. Vegetable oils in food technology: Composition, Properties and Uses. *The lipid handbook – III edition*, pp. 318-320.

Herchi, W., F. Sakouhi, S. Boukhchina, H. Kallel and C. Pepe, 2011a. Changes in fatty acids, tocochromanols, carotenoids and chlorophylls content during flaxseed development. *JAOCS, Journal of the American Oil Chemists' Society,* pp. 1011-1017.

Herchi, W., F. Sakouhi, S. Boukhchina, H. Kallel and C. Pepe, 2011b. Changes in Fatty Acids, Tocochromanols, Carotenoids and Chlorophylls Content During Flaxseed Development. *J. Am. Oil. Chem. Soc*., **88**: 1011–1017.

Herchi, W., I. Bouali, S. Bahashwan, S. Rochut, S. Boukhchina, H. Kallel and C. Pepe, 2012. Changes in phospholipid composition, protein content and chemical properties of flaxseed oil during development. *Plant Physiology and Biochemistry:* 1-5.

Ivanov, S., P. Bitcheva and B. Konova, 1972. Des phytosterols dans les huiles vegetales et les concentres steroliques. *Rev. Fr. Corps Gras,* **19**: 177-180.

Piłat, B. and R. Zadernowski, 2010. Physicochemical characteristics of linseed oil and flour. *Polish Journal of Natural Sciences*, **25**: 106-113.

Przybylski, R., 2005. Flax Oil and High Linolenic Oils. Bailey's Industrial Oil and Fat Products, Sixth Edition, Six Volume Set. Edited by Fereidoon Shahidi. *Copyright John Wiley & Sons, Inc.*

Momchilova, S. and B. Nikolova-Damyanova, 2007. Quantitative TLC and gas chromatography determination of the lipid composition of raw and microwaved roasted walnuts, hazelnuts, and almonds. *J. Liq. Chromatogr. Relat. Technol.,* **30**: 2267-2285.

Zlatanov, M. D., M. J. Angelova-Romova, G. A. Antova, R. D. Dimitrova, S. M. Momchilova and B. M. Nikolova – Damyanova, 2009. Variations in fatty acids, phospholipids and sterols during the seed development of a high oleic sunflower variety. *J. Am. Oil Chem. Soc.,* **86**: 867-875.

Received April, 2, 2013; accepted for printing December, 2, 2013.