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Lipid composition of mustard seed oils (*Sinapis alba L.*)

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Lipid composition of five accessions *Sinapis alba* L. seeds was investigated. The amount of glyceride oil in the seeds was 22.4 – 38.9%. The main components in triacylglycerols were erucic (28.0 – 53.2%), oleic (13.7 – 25.1%), palmitic (3.9 – 5.2%), gadoleic (9.4 – 14.2%) and linoleic acid (4.9 – 17.4%). The total content of phospholipids was 3.6 – 6.9% and the major representatives were phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine. In the sterol fraction (0.37 – 0.51%) the main component was β-sitosterol (51.9 – 55.9% in free sterols and 52.8 – 59.7 % in sterol esters), followed by campesterol (19.1 – 30.5% and 31.1 – 33.5%, respectively) and brassicasterol (11.9 – 22.5% and 3.7 – 8.9%). The fatty acid composition of sterol esters was similar to triacylglycerols with exception of the palmitic acid which amount was found to be higher in esterified sterols. The total tocopherol content was 456 – 1025 mg/kg and the main representatives were α-tocopherol and γ-tocopherol. The oxidative stability of all examined accessions of *Sinapis alba* L. seed oil was 14.8 – 25.1h.

***Key words**: Sinapis alba L., seed oil, fatty acids, phospholipids, sterols, tocopherols*

INTRODUCTION

Yellow mustard (*Sinapis alba* L.) is cultivated as an important oil crop and its total production is about 43 million tons per annum, mainly in Bangladesh and India [1, 2]. The oil content in the seeds of species from family *Brassica* ranges from 40.0 to 42.0% [3] and those of mustard seeds is 28.0 – 42.0% [1,4,5]. Only George *et al*., 1985 [6] established that the oil content of the latter is 26.8%. Mustard seed oil is one of the edible vegetable oils in human diet and has a pungent flavor and aroma as well as high amount of selenium and magnesium which gives it anti-inflammatory properties [7]. It is valuable industrial oil but contains high levels of erucic acid (40.0 – 50.0%) which make it less desirable as a dietary fat [6]. Despite that, significant amounts of linoleic, oleic and linolenic acid were also established [1, 4]. Many researchers have developed the genetic control of fatty acid composition of *Brassica* species and *Sinapis alba* in order to obtain a seed oils with reduced amount of erucic acid [8-12].

There are some studies on biologically active components in mustard seed oils. Phospholipids in the oil are investigated by Parti *et al*., 2003 [4] and their amount is 6.44% but according to Chhokar *et al*., 2008 [1] it is 1.32%. Nevertheless, there is no information about the individual composition of phospholipid fraction as well as fatty acid composition. Total sterol content in mustard oil is about 0.80% where the free sterols consist of 0.32% and esterified sterols are 0.48% [3]. The predominant components of the sterol fraction are β-sitosterol, brassicasterol, campesterol and Δ5-avenasterol [2, 3]. Mustard oil contains a high level of tocopherols which amount is 410 mg/kg and γ-tocopherol predominates [13]. α-, β-, γ- and δ-Tocopherols are also established in mustard oil by Mortuza M., 2006 [2].

On the whole, the aim of the study is to be determined the lipid content, fatty acid composition, individual composition of phospholipids, sterols and tocopherols as well as oxidative stability of the oil isolated from five different accessions of mustard seeds.

MATERIALS AND METHODS

Samples

Five accessions of *Sinapis alba* L. with different origin has been analyzed. Two Russian (*Zuzanka* and *Stalingradskaya*), two Bulgarian (*BG431* and *BG290*) and one landrace from Romania (*RO268*) have been evaluated ex situ on the experimental field of the Institute of Plant Genetic Resources, Sadovo. Seeds were harvested at 10 % moisture, stored at room temperature and analyzed.

Isolation of glyceride oil and determination of oil content

Mustard oil was extracted from grounded seeds using n-hexane in a Soxhlet apparatus for 8 h [14].

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Fatty acid composition

Fatty acid composition of triacylglycerols (TAG) and sterol esters was determined by gas chromatography (GC) [15]. Fatty acid methyl esters (FAMEs) were prepared by pre-esterification of the triacylglycerols, esterification of free fatty acids isolated from sterol esters after saponification with sulfuric acid in methanol [16]. Determination of FAMEs was performed on HP 5890 gas chromatograph equipped with a 60 m x 0.25 mm x 25 μm capillary DB-23 column and a flame ionization detector. The column temperature was programmed from 130ºC (hold 1 min), at 6.5ºC/min to 170ºC, at 3ºC/min to 215ºC (hold 9 min), at 40ºC/min to 230ºC (hold 1 min); the injector and detector temperature were 250ºC. Hydrogen was the carrier gas at a flow rate 0.8 mL/min. Identification was performed by comparison of the retention times with those of a standard mixture of FAME subjected to GC under identical experimental conditions.

Determination of phospholipids

Ground seeds were subjected to Folch extraction [17]. Individual phospholipid classes were isolated by two-dimensional thin-layer chromatography (TLC) [18]**.** Identification was performed by comparing the respective R_f values with those of authentic standards. The quantification was carried out spectrophotometrically at 700 nm after scrapping the phospholipid spot and mineralization of the substance with a mixture of perchloric and sulphuric acid, 1 : 1 (v/v) [19].

Determination of sterols

Free (R_f = 0.4) and esterified sterols (R_f = 0.8) were isolated from the glyceride oil by TLC with mobile phase n-hexane: acetone, 100: 8 (v/v). Then free sterols were subjected to GC without derivatization while sterol esters were hydrolyzed with 2N KOH in ethanol and sterols were extracted with n-hexane and purified by TLC [20]. Sterol composition was determined on HP 5890 gas chromatograph equipped with a 30 m x 0.25 mm DB - 6 capillary column and flame ionization detector. The temperature gradient was 90ºC (hold 2 min) up to 290ºC at a rate of change 15ºC/min and then up to 310ºC at a rate of 4ºC/min (hold 10 min); the injector and detector temperature was 300ºC and 320ºC. Hydrogen was the carrier gas at a flow rate of 0.8 mL/min. Identification was confirmed by comparison of retention times with those of a standard mixture of sterols.

Determination of tocopherols

Determination of tocopherols was carried out by high performance liquid chromatography (HPLC) [21] on 250 mm x 4 mm Nucleosil Si 50-5 column and fluorescent detection at 295 nm excitement and 330 nm emission. The operating conditions were mobile phase of n-hexane: dioxane, 96:4 (v/v) and flow rate 1 mL/min.

Oxidative stability

The oxidative stability (induction period in hours) was determined by Rancimat apparatus Methrom 679 (at 100 ºC and an air flow rate 20 L/h) [22].

RESULTS AND DISCUSSION

Total oil content of different accessions of mustard seeds, the quantity of biologically active substances of the oils as well as their oxidative stability were established (Table 1).

Oil content in all examined oils differed in small range (22.4 – 29.4%) apart from accession *Zuzanka* where its quantity was higher (38.9%). Similar results were observed in previous studies where the oil content was 28.0 – 42.0% [1, 4, 5, 6]. Phospholipid content in the oils varied from 3.6 to 6.9% where their amount in *Stalingradskaya* was found to be similar to those presented by Parti *et al*., 2003 [4] (6.44%).

On the other hand, these values were much higher than results reported by Chhokar *et al*., 2008 [1] where the amount of phospholipids in mustard oil was significantly lower (1.32%). Total sterol content in all samples was about 0.4 – 0.5% which was lower than data reported by Nagaraj, 2009 (0.8%) [3].

Table 1. Lipid composition of mustard seeds

Compounds	***Accessions***				
	BG 431	***BG 290***	***RO 268***	***Zuzanka***	***Stalingradskaya***
Oil content, %	26.7	29.4	26.8	38.9	22.4
Phospholipids, %	3.6	4.4	5.6	4.2	6.9
Sterol content, %	0.51	0.44	0.47	0.37	0.48
- free	0.42	0.34	0.38	0.30	0.38
- esterified	0.09	0.10	0.09	0.07	0.10
Tocopherols, mg/kg	705	465	1025	353	456
Oxidative stability, h	25.1	14.8	16.4	17.0	22.3

Fraction of free sterols was comprised by approximately 80.0 % of total sterols. The amount of free sterols in all samples (0.30 – 0.42%) was approximately four times higher than this of esterified sterols (0.07 – 0.10%). Nonetheless, according to Nagaraj, 2009 [3] the fraction of free sterols was presented in lower quantity (0.32%) than esterified sterols (0.48%). Tocopherol content in seed oil from Romania (*RO 268*) was found to be in high level (1025 mg/kg) followed by Bulgarian accession *BG 431* (705 mg/kg). Tocopherol content of the others seed oils were from 353 to 465 mg/kg which was in a good agreement with Vaidya and Choe, 2011 [13]. Oxidative stability of all seed oils was 14.8 – 25.1h. This results were higher than that observed by Ciubota-Rosie *et al*., 2013 [23] and Jham *et al*., 2009 [24] (7h). The seed oils from *BG431* and *Stalingradskaya* were found to be more stable against oxidation than the others samples.

Fatty acid composition of triacylglycerols of mustard seed oils is shown in Table 2.

Table 2. Fatty acid composition of triacylglycerols of mustard oils

Fatty acids, %	***Accessions***				
	BG 431	***BG 290***	***RO 268***	***Zuzanka***	***Stalin-gradskaya***
C 12:0	0.2	0.2	0.2	0.3	0.2
C 14:0	0.1	0.2	0.2	0.4	0.2
C 14:1	0.3	0.4	0.4	0.6	0.4
C 16:0	4.0	4.5	4.8	5.2	3.9
C 16:1	0.3	0.3	0.3	0.3	0.2
C 18:0	1.6	2.0	2.3	2.3	1.3
C 18:1	13.7	23.8	20.9	25.1	21.2
C 18:2	11.5	17.4	14.7	13.5	4.9
C 18:3	3.6	5.9	5.4	4.5	2.1
C 20:0	1.3	1.4	1.7	1.1	0.9
C 20:1	9.4	13.9	14.2	11.1	10.6
C 20:2	0.8	0.9	0.7	0.6	0.2
C 22:0	1.8	1.1	1.5	1.0	0.7
C 22:1	51.4	28.0	32.7	34.0	53.2
SFA	**9.0**	**9.4**	**10.7**	**10.3**	**7.2**
UFA	**91.0**	**90.6**	**89.3**	**89.7**	**92.8**
MUFA	**75.1**	**66.4**	**68.5**	**71.1**	**85.6**
PUFA	**15.9**	**24.2**	**20.8**	**18.6**	**7.2**

As can be seen, erucic, oleic, linoleic and gadoleic fatty acids were the major components in triacylglycerol fraction in all examined seeds oils. Erucic acid predominated in all of them and varied from 28.0 to 53.2% considering that it was higher in the seed oils from accessions *BG 431* and *Stalingradskaya*. The latter was in a good agreement with results reported by George *et al*., 1985 [6]. The presence of erucic acid in accession *BG 290* (28.0%) was the same as the data reported by Vaidya and Choe, 2011 [13]. Moreover, its amount in *RO 268* was almost similar to this in *Zuzanka.* The quantity of oleic acid for all investigated oils was from 13.7 to 25.1%, which was in a good agreement with results reported by Vaidya and Choe, 2011 [13] and Chhokar *et al*., 2008 [1] apart from accession *BG 431* where it was lower and similar to data observed by Parti *et al*., 2003 [4]. Linoleic acid was found to be 11.5 – 17.4% in all samples except the oil from *Stalingradskaya* where its amount was lower (4.9%). The data was close to this reported by Parti *et al*., 2003, Chhokar *et al*., 2008 and Vaidya and Choe, 2011 [1, 4, 13]. Interestingly, there was higher level of gadoleic acid (9.4 – 14.2%) in all investigated mustard seed oils and the amount of linolenic acid was about 5.0% apart from accessions *BG 431* and *Stalingradskaya* where its quantity was 3.6 and 2.1%, respectively. Unsaturated fatty acids (UFA), especially monounsaturated (MUFA), predominated in all the oil of accessions. The content of UFA was higher in *Stalingradskaya* (92.8%) and lower in *RO 268* and *Zuzanka* (89.7%). The quantity of PUFA varied from 7.2 (*Stalingradskaya*) to 24.2% (*BG 290*) and those of MUFA – from 66.4 (*BG 290*) to 85.6% (*Stalingradskaya*). The higher level of UFA was at the expense of the lower amount of SFA which were presented mainly by palmitic acid.

Composition of free and esterified sterols of different accessions of mustard seed oils is presented in Table 3.

The composition of free sterols and sterol esters was similar in all mustard oils but there were differences in the quantity for all their representatives. β-Sitosterol was the main component in all samples and its amount was approximately the same in both sterol fractions (51.9 – 55.9% in free sterols and 52.8 – 53.9% in esterified sterols) except Stalingradskaya where it was found to be 59.7% in esterified sterols.

The percentage of campesterol was higher in sterol esters (31.1 – 33.5 % vs. 19.1 – 30.5 % in free sterols) while the content of stigmasterol was close in both fractions (1.1 – 1.5% in free sterols and 0.9 – 2.1% in esterified sterols). There was low content of cholesterol in both fractions but in the sterol esters it was several times higher than free ones.

Fatty acid composition of sterol esters is shown in Table 4.

Erucic acid was the major component in all samples (26.7 – 46.2%), followed by oleic acid (19.4 – 32.0%).

Table 3. Individual composition of free and esterified sterols of mustard oils

Sterols, %	*Accessions*									
	BG 431		***BG 290***		***RO 268***		***Zuzanka***		***Stalingradskaya***	
	free	esterified	free	esterified	free	esterified	free	esterified	free	esterified
Cholesterol	-*	1.0	0.2	0.7	0.2	0.7	-	0.5	-	-
Brasicasterol	22.5	8.9	22.2	7.0	20.7	5.5	19.3	6.1	11.9	3.7
Campesterol	19.5	31.1	19.6	32.5	19.1	31.9	24.2	33.5	30.5	32.2
Stigmasterol	1.5	0.9	1.3	1.0	1.2	1.3	1.5	2.1	1.1	1.5
β - Sitosterol	52.8	53.8	53.5	52.8	55.9	53.6	51.9	53.9	53.8	59.7
Δ^5 - Avenasterol	0.6	0.9	0.5	1.1	0.4	1.0	0.6	0.3	0.5	0.6
Δ^7 - Stigmasterol	0.3	0.5	0.3	0.5	0.3	0.9	0.4	0.7	0.3	0.3
Δ^7 - Avenasterol	2.8	2.9	2.4	4.0	2.2	4.5	2.1	2.9	1.9	2.0

* - Not identified

Table 4. Fatty acid composition of esterified sterols of mustard oils

Fatty acids, %	*Accessions*				
	BG 431	***BG 290***	***RO 268***	***Zuzanka***	***Stalin-gradskaya***
$C_{12:0}$	0.4	0.4	0.2	1.4	0.9
$C_{12:1}$	1.0	1.0	1.0	1.1	1.0
$C_{14:0}$	1.0	0.6	0.5	1.8	1.2
$C_{14:1}$	1.0	1.2	1.9	1.4	1.8
$C_{15:0}$	0.3	0.2	0.2	0.5	0.3
$C_{16:0}$	10.9	8.1	9.8	16.7	12.9
$C_{16:1}$	0.9	0.5	0.5	1.0	1.2
$C_{17:0}$	0.3	0.2	0.2	0.6	0.4
$C_{18:0}$	3.2	3.0	4.0	5.5	3.7
$C_{18:1}$	19.4	27.8	26.5	27.3	32.0
$C_{18:2}$	3.4	7.0	3.5	3.3	3.5
$C_{18:3}$	0.2	0.5	0.3	0.1	0.3
$C_{20:0}$	1.3	1.6	2.0	1.6	0.9
$C_{20:1}$	8.0	13.7	14.7	9.0	8.2
$C_{20:2}$	0.3	0.4	0.2	0.4	0.4
$C_{22:0}$	2.2	1.2	1.3	1.6	1.0
$C_{22:1}$	46.2	32.6	33.2	26.7	30.3
SFA	**19.6**	**15.3**	**18.2**	**29.7**	**21.3**
UFA	**80.4**	**84.7**	**81.8**	**70.3**	**78.7**
MUFA	**76.5**	**76.8**	**77.8**	**66.5**	**74.5**
PUFA	**3.9**	**7.9**	**4.0**	**3.8**	**4.2**

The highest amount of erucic acid was established in BG 431 (46.2%) at the expense of lower quantity of oleic acid (19.4%).On the other hand, bigger quantity of oleic acid was found to be in seed oil from accession Stalingradskaya (32.0%). Palmitic acid was presented in smaller amounts in BG 431, BG 290 and RO 268 (8.1 – 10.9%) but it was higher in Zuzanka and Stalingradskaya (12.9 – 16.7%). The quantity of stearic acid in all samples varied from 3.0 to 5.5%. The content of linoleic acid varied from 3.3 to 7.0% and those of linolenic and eicosadienoic acid were found to be less than 0.5%.

The qualitative composition was similar to this of triacylglycerols but there were quantitative differences between all accessions. The amount of erucic acid in BG 431, Zuzanka and Stalingradskaya was lower in esterified sterols (26.7 – 46.2%) than in triacylglycerols (34.0 – 53.2%). On the other hand, its quantity in BG 290 and RO 268 was higher in sterol esters (32.6 – 33.2%) than in triacylglycerols (28.0 – 32.7%). There were similar differences in the content of other fatty acids such as oleic, palmitic and gadoleic for all investigated seed oils. Considerably higher percentage of SFA was found in sterol esters than in TAG (15.3 – 29.7% vs. 7.2 – 10.7%). On the other hand, the amount of unsaturated fatty acids in sterol fraction was lower (70.3 – 84.7%) than in TAG (89.7 – 92.8%). SFA in seed oil from Zuzanka were found to be 29.7% but the amount of those in other accessions were significantly lower (15.3 – 21.3%). The quantity of MUFA varied from 66.5 to 77.8% while these of PUFA were 3.8 – 7.9% which was lower than in TAG (7.2 – 24.2%). Individual tocopherol composition is given in Table 5.

Table 5. Tocopherol composition of mustard oils

Tocopherols, %	Accessions				
	BG 431	***BG 290***	***RO 268***	***Zuzanka***	***Stalingradskaya***
α-tocopherol	26.5	26.5	17.4	37.2	16.1
β- tocopherol	-*	-	4.2	-	-
β-tocotrienol	-	-	1.6	-	-
γ- tocopherol	70.7	73.5	60.1	63.8	82.7
γ-tocotrienol	-	-	9.3	-	1.2
δ- tocopherol	2.8	-	7.4	-	-

** - Not identified*

Table 6. Phospholipid composition of mustard oils

Phospholipids, %	Accessions				
	BG 431	***BG 290***	***RO 268***	***Zuzanka***	***Stalingradskaya***
Phosphatidylcholine	51.0	50.2	46.1	37.1	37.2
Phosphatidylinositol	23.8	28.0	29.3	23.7	24.5
Phosphatidylethanolamine	16.8	14.2	17.2	16.5	17.9
Phosphatidic acids	1.1	0.5	0.7	6.0	-*
Phosphatidylserine	0.6	0.2	0.2	3.1	4.2
Sphingomyeline	2.5	3.0	2.6	4.3	5.2
Others	4.2	3.9	3.9	9.3	11.0

** - Not identified*

γ-Tocopherol predominated in all investigated mustard oils and it was over 70.0% except in RO 268 and Zuzanka. The amount of α-tocopherol was found to be higher in BG 431 (26.5%), BG 290 (26.5%) and Zuzanka (37.2%), while in seeds oils from RO 268 and Stalingradskaya were established significantly lower quantities (16.1 and 17.4%). There were minor amounts of β- and δ-tocopherol as well as unsaturated derivatives β- and γ-tocotrienol (observed only in RO 268).

Phospholipid composition of mustard seed oils is shown in Table 6.

The major phospholipid components were phosphatidylcholine (37.1 – 51.0%) followed by phosphatidylinositol (23.7 – 29.3%) and phosphatidylethanolamine (14.2 – 17.9%). Accessions Zuzanka and Stalingradskaya were observed to contain lower quantity of phosphatidylcholine at the expence of higher content of the other phospholipids. The quantity of sphingomyeline was considerably higher (2.5 – 5.2%) which made mustard seed oil different from the other vegetable oils (1.0 – 2.0%) [25].

CONCLUSIONS

All investigated seeds of *Sinapis alba* were found to be rich in glyceride oil which contained great amount of biological active substances. There were some differences in fatty acid composition as well as in sterol, phospholipid and tocopherol content as a result of the genotype of the plants, climatic and agrometeorologic conditions. On the whole, it could be summarized that because of the higher quantity of erucic acid all of the examined seed oils were not suitable for human consumption, but could be used for biodiesel production.

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ЛИПИДЕН СЪСТАВ НА СЕМЕНА ОТ СИНАП (*Sinapis alba* L.)

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(Резюме)

Изследван е липидния състав на семена от пет образци синап (*Sinapis alba* L.). Съдържанието на глицеридно масло в семената е от 22.4 до 38.9%. Основните мастни киселини в триацилглицеролите са ерукова (28.0 – 53.2%), олеинова (13.7 – 25.1%), палмитинова (3.9 – 5.2%), гадолеинова (9.4 – 14.2%) и линолова киселина (4.9 – 17.4%). Общото фосфолипидно съдържание варира между 3.6 и 6.9%, като основните представители са фосфатидилхолин, фосфатидилинозитол и фосфатидилетаноламин. В стероловата фракция (0.37 – 0.51%) основните компоненти са β-ситостерол (51.9 – 55.9% в свободните стероли и 52.8 – 59.7 % в стероловите естери), последван от кампестерол (19.1 – 30.5% и 31.1 – 33.5% съответно) и брасикастерол (11.9 – 22.5% и 3.7 – 8.9%). Мастнокиселинният състав на стероловите естери е подобен на този на триацилглицеролите с изключение на палмитиновата киселина, чието количество е по-високо в свързаните стероли. Съдържанието на токофероли е 456 – 1025 mg/kg, като основните представители са α- и γ-токоферол. Оксидантната стабилност на изследваните масла от семена от *Sinapis alba* L. е сравнително висока (14.8 – 25.1 h).

***Ключови думи**: Sinapis alba L., глицеридно масло, мастнокиселинен състав, фосфолипиди, стероли, токофероли*