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THE CONTENT OF SATURATED, MONOUNSATURATED AND POLYUNSATURATED FATTY ACIDS IN THE SEEDS OF DIFFERENT *CANOLA VARIETIES*

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INTRODUCTION

Canola was developed in the early 1970s using traditional plant breeding techniques by Canadian plant breeders to remove the anti-nutritional components (erucic acid and glucosinolates) from rapeseed to assure its safety for human and animal consumption. The canola plant also produced seeds with a very low level of saturated fat, seven percent or below [1]. There is an internationally regulated definition of canola that differentiates it from rapeseed, based upon its having less than two percent erucic acid ($C_{22:1}$) and less than 30 μmol/g glucosinolates [2]. Oilseed products that do not meet this standard cannot use the trademarked term "Canola." Like soybean, canola contains both high oil content as well as high protein content. It contains about 40% oil and 23% protein compared to 20 and 40%, respectively, for soybean consumption [3].

Commercial varieties of canola were developed from three species: *Brassica napus* L. (Argentine type), *Brassica campestris* L. (Polish type) and *Brassica juncea* L. (canola quality brown mustard). There are considerable differences in agronomic characteristics, yield, and fatty acid (FA) composition of seed oil between species and between varieties. In order to develop herbicide tolerance of the canola plant, and to improve quality of the canola seed, some different innovations have been established. *Roundup Ready* and *Liberty Link* canola varieties were developed using a traditional plant breeding technique called mutagenesis. Another innovation is the development of hybrid canola varieties. Hybrids can increase yields and are increasing in acreage [4].

In the Republic of Macedonia, two different hybrids of canola varieties have been developed in the past decade. The main objective of our work was identification and determination the FA composition of the seed oil of the two canola varieties, which were grown in the Republic of Macedonia, during 2012. For that purpose, a total of hundred samples of the seeds of the two types of canola varieties were analyzed for the presence of total saturated fatty acid (SFA), total monounsaturated fatty acids (MFA), and total polyunsaturated fatty acids (PUFA). The values of polyunsaturated / saturated indexes (P/S) were calculated for the both canola varieties.

MATERIAL AND METHODS

A total of hundred seed samples of two different hybrids of canola varieties were collected from the local producers during the 2012 (canola variety type 1, n=45; canola variety type 2, n=55).

Sample preparation

5 g of grinded samples was mixed with 5 g of anhydrous sodium sulphate (Na_2SO_4). An aliquot of 2 g was placed in Soxlet extractor system, and extraction was performed with petroleum – ether (40-69) within 24 hours. After cooling at room temperature, the solvent was evaporated to dryness in the stream of nitrogen.

Preparation of fatty acid methyl esters (FAMEs)

Fatty acid (FA) composition of the oils and fats was determined as their corresponding methyl esters. Preparation of FAMEs was carried out according to the modified ISO method (5). 0.1 - 0.2 g of oil was dissolved in 10 mL 0.2 mol/L H_2SO_4 prepared in anhydrous methanol. Esterification was performed by refluxing for 30 minutes at 100 ^{0}C in tightly sealed Pyrex tubes. After cooling at room temperature, 10 mL of petroleum ether (40 - 60) was added followed by 10 mL of deionized water, mixed gently and allowed to settle until the upper petroleum ether layer becomes clear. The distinct upper layer of methyl esters in petroleum ether was separated carefully in a capped vial and used for analysis. 2 μL of the petroleum ether aliquots were injected into the chromatographic column and peaks were recorded for their respective retention times and areas by the data processor unit of the GC. Identification of each individual fatty acid methyl ester was achieved by comparison with authentic reference standards. All solvents and standards were of analytical grade (Merck, Fluka).

Chromatography

HP model 5890 series II (plus) gas chromatograph equipped with an HP automatic liquid sampler and a flame-ionization detector (FID) was used either with a nonpolar fused silica capillary column (30 m x 0.32 mm id. x 1 µm film thickness) coated with 100% poly (dimethylsiloxane), commercially available as SPB^{TM-1} obtained from Supelco (USA). The carrier gas (nitrogen) flow rate was 1.5 mL/min and the split ratio was 1:10. The injection port was maintained at 250 ^{0}C and the FID at 280 ^{0}C. Oven temperature was set at 200 ^{0}C (1 minute) increasing for 5 ^{0}C/min. The final oven temperature was maintained at 250 ^{0}C (20 minutes). For confirmation of identified and determined FAMEs in oils and fats, a polyethylene glycol TPA modified polar column commercially available as HP-FFAP (25 m x 0.32 mm id x 0.52 µm) was used with the same HP model 5890 series II (plus) gas chromatograph. The carrier gas (nitrogen) flow rate was 1.5 mL/ min and the split ratio was 1:10. The injection port was maintained at 230 ^{0}C and the FID at 260 ^{0}C. Oven temperature was set at 180 ^{0}C increasing for 2 ^{0}C/ min. The final oven temperature was maintained at 230 ^{0}C (4 minutes).

RESULTS AND DISCUSSION

A total of 100 seed samples of two different hybrids of canola varieties were analyzed on the composition of fatty acids using gas chromatographic method. The content of following saturated and unsaturated fatty acids was tested in the samples: caproic acid ($C_{6:0}$), caprylic acid ($C_{8:0}$), capric acid ($C_{10:0}$), lauric acid ($C_{12:0}$), myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), arachidic acid ($C_{20:0}$), behenic acid ($C_{22:0}$) , lignoceric acid ($C_{24:0}$), oleic acid ($C_{18:1}$), linoleic ($C_{18:2}$), linolenic acid ($C_{18:3}$), and erucic acid ($C_{22:1}$). The fatty acid percent composition of tested seeds is shown in Table 1 and Table 2, respectively. The mean of total saturated fatty acid (SFA), monounsaturated fatty acids (MFA), polyunsaturated fatty acids (PUFA) and the values of polyunsaturated/saturated indexes (P/S) are shown in Table 3.

The results of the determination of FA composition (Table 1) indicate that the seeds of both canola varieties have low content of saturated FA with the predominant presence of $C_{16:0}$ and $C_{18:0}$. The level of $C_{18:0}$ in the seeds of the canola variety type 1 was 4.4%±1.4 of the total FA, and 6.9%±1.6 of the total FA in seeds of canola variety type 2. Although it is classified as a SFA, data accumulated during the past years indicate that $C_{18:0}$ is unique among the SFAs in the food supply [6]. Unlike other predominant long-chain SFA: $C_{16:0}$, $C_{14:0,}$ and $C_{12:0}$ which increase blood cholesterol levels, $C_{18:0}$ has been shown to have a neutral effect on blood total and low density lipoprotein (LDL) cholesterol levels [6,7]

Table 1. Saturated fatty acid composition of canola seeds of two varieties

Type of Canola variety	**Mean ±SD**									
	$C_{6:0}$ (%)	$C_{8:0}$ (%)	$C_{10:0}$ (%)	$C_{12:0}$ (%)	$C_{14:0}$ (%)	$C_{16:0}$ (%)	$C_{18:0}$ (%)	$C_{20:0}$ (%)	$C_{22:0}$ (%)	$C_{24:0}$ (%)
Canola type 1 (n=45)	<0.1	<0.1	<0.1	<0.1	<0.1	5.2± 0.6	4.4± 1.4	<0.2	<0.2	<0.1
Canola type 2 (n=55)	<0.1	<0.1	<0.1	<0.1	<0.1	10.5 ±2.5	6.9± 1.6	<0.2	<0.2	<0.1

The obtained results for the unsaturated FA (Table 2) composition of the seed oil, showed the predominant presence of $C_{18:1}$ in the seed oil of canola variety type 1 which was found to be 59.5% ± 1.91 of the total FA. It is known that the consumption of $C_{18:1}$ is effective in lowering LDL cholesterol level. The predominant presence of $C_{18:3}$ (44.0% ± 2.02) was found in the seed oil of canola variety type 2. One of the most interesting yet controversial dietary approaches has been the possible prophylactic role of dietary γ-linolenic acid (GLA) in treating various chronic diseases states. This strategy is based on the ability of diet to modify cellular lipid composition and eicosanoid (cyclooxygenase and lipoxygenase) biosynthesis [8].

$C_{22:1}$ is a MUFA which is a major constituent of certain oils, such as rapeseed oil. Because it has been linked to cardiac muscle damage, oil such as canola oil that are low in $C_{22:1}$ was developed. In our studies, the seeds of the both canola varieties had low content of $C_{22:1}$.

Table 2. Unsaturated fatty acid composition of canola seeds of two varieties

Type of Canola variety	Mean ±SD			
	$C_{18:1\ (\%)}$	$C_{18:2\ (\%)}$	$C_{18:3\ (\%)}$	$C_{22:1\ (\%)}$
Canola type 1 (n=45)	59.5 ± 1.91	18.8 ± 3.5	11.9 ± 1.1	0.11±0.05
Canola type 2 (n=55)	23.2 ± 2.9	15.2 ± 3.6	44.0 ± 2.02	0.18±0.09

All samples presented total SFA content less than one fourth of the total FA content (Table 3). It is known that the excessive consumption of SFA is related to the increase of the plasmatic cholesterol and the obesity [9]. On the other hand, the consumption of PUFA and MUFA has been recommended to improve the lipid profile in relation to the saturated SFA. Yu-Poth *et al*. indicate that the rich diets in PUFA may provoke an increase in the LDL - cholesterol oxidation and the reduction of the HDL -cholesterol levels [10]. There is a tendency in increasing the recommendations of MUFA consumption, that seems not to affect the HDL levels, and also it may reduce the LDL and triacylglycerols blood levels, that make it more effective in prevention of hearth diseases. Canola oil variety 1 showed high content of MUFA (59.5 ± 1.91) and canola oil variety 2 showed hight content of PUFA (59.2 ±1.1).

Table 3. The content of SFA, MUFA, PUFA and the values of P/S indexes in two types of canola varieties

Type of Canola variety	Mean ±SD			P/S index
	SFA (%)	MUFA (%)	PUFA (%)	
Canola type 1 (n=45)	9.6 ± 0.56	59.5 ± 1.91	30.7 ± 1.7	3.2
Canola type 2 (n=55)	17.4 ± 0.67	23.2 ± 2.9	59.2 ± 1.1	3.4

The relationship between saturated and polyunsaturated FA content is expressed as P/S index. This value is an important parameter for determination of nutritional value of certain oil. Oils and fats with higher value of P/S index than 1 are considered to have nutritional value. Several studies indicate that higher value of P/S index means a smaller deposition of lipids in the body [11]. The results of our investigations (Table 3) showed that the both canola varieties had similar values of polyunsaturated/saturated indexes (P/S), which were found to be 3.2, and 3.4, respectively. This means that the both varieties of the oil had the same nutritional value.

CONCLUSIONS

The canola plant was developed in order to produce edible oil that contains much lower levels of erucic acid ($C_{22:1}$) than rapeseed oil. Canola oil derived from the seeds of the canola plant, the genetically engineered rapeseed plant or the canola hybrids, has been considered for human consumption. The types of fatty acids determine whether a vegetable oil is used for edible or industrial purposes.

In our investigations the FA composition of the seed oil obtained from two types of canola hybrids was analyzed. The canola variety type 2, was found to be high linolenic with the average content of linolenic acid ($C_{18:3}$) 44.0% ± 2.02 (n=55). The canola variety type 1, was found to be high oleic with the average content of oleic acid ($C_{18:1}$) 59.5% ± 1.91 (n=45). The average content of ($C_{22:1}$) was below 0.2% in the both types.

The canola variety type 1 contained lower mean value of the total SFA (9.6%±0.56) in comparison with canola variety type 2, which had higher mean value of the total SFA (17.4% ±0.67). The canola variety type 1, had higher content of the total MUFA (59.5%±1.91), unlike the canola variety type 2 (23.2% ±2.9). Besides the differences in the FA composition, as well as, the total SFA, MUFA, and the PUFA content, the both canola varieties had similar values of polyunsaturated/saturated indexes (P/S), which were found to be 3.2, and 3.4, respectively. This means that the both varieties of the oil had the same nutritional value.

ABSTRACT

Canola is a name applied to edible oilseed rape. This plant belongs to the *Brassicaceae* (mustard) family along with 3,000 other species. Close relatives of this crop have been cultivated for food since the earliest recordings of man. The name "canola" was registered in 1979 by the Western Canadian Oilseed Crushers Association to describe "double-low" varieties. Double low indicates that the processed oil contains less than 2% erucic acid ($C_{22:1}$). Like soybean, canola contains both high oil content as well as high protein content. It contains about 40% oil and 23% protein compared to 20 and 40%, respectively, for soybean consumption.

Commercial varieties of canola were developed from three species: *Brassica napus* L. (Argentine type), *Brassica campestris* L. (Polish type) and *Brassica juncea* L. (canola quality brown mustard). There are considerable differences in agronomic characteristics, yield, and fatty acid (FA)

composition of seed oil between species and between varieties.

The main objective of this work was identification and determination the FA composition of the seed oil of the two canola varieties grown in the Republic of Macedonia, during 2012. For that purpose, a total of hundred samples of the seeds of the two types of canola varieties were analyzed for the presence of total saturated fatty acid (SFA), total monounsaturated fatty acids (MFA), and total polyunsaturated fatty acids (PUFA). After the Soxlet extraction of the seeds, methylation of the oil was performed, and fatty acids methyl esters (FAMEs) were characterized by gas chromatography (GC-FID) on HP-FFAP and SPB^{TM-1} column, respectively.

The results of the study, showed different FA content among the two canola varieties. The canola variety type 2, was found to be high linolenic with the average content of linolenic acid ($C_{18:3}$) 44.0% ± 2.02 (n=55). The canola variety type 1, was found to be high oleic with the average content of oleic acid ($C_{18:1}$) 59.5% ± 1.91 (n=45). The average content of erucic acid ($C_{22:1}$) was below 0.2% in the both varieties.

The canola variety type 1 contained lower mean value of the total SFA (9.6% ± 0.56) in comparison with canola variety type 2, which had higher mean value of the total SFA (17.4% ± 0.67). The canola variety type 1, had higher content of the total MUFA (59.5% ± 1.91), unlike the canola variety type 2 (23.2% ±2.9). Besides the differences in the FA composition, as well as, the total SFA, MUFA, and the PUFA content, the both canola varieties had similar values of polyunsaturated/saturated indexes (P/S), which were found to be 3.2, and 3.4, respectively. This means that the both varieties of the oil had the same nutritional value.

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