Fatty acid composition of seed oil obtained from different canola varieties

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Sastav masnih kiselina u ulju sjemenki dobivenom iz različitih sorti kanole

S a ž e t a k – Kanola je ime koje se upotrebljava za jestivu uljanu repicu. Biljka pripada porodici gorušica (Brassicaceae) zajedno s 3000 drugih vrsta. Biljke srodne ovom usjevu uzgajaju se za hranu od najranijih razdoblja čovječanstva. Komercijalne sorte kanole razvijene su iz tri vrste: Brassica napus L. (argentinski tip), Brassica campestris L. (poljski tip) i Brassica juncea L. (smeđa gorušica). Između vrsta i sorti postoje značajne razlike u agronomskim svojstvima, prinosu te sastavu masnih kiselina u ulju sjemenki.

Glavni cilj ovog istraživanja bio je odrediti sastav masnih kiselina u ulju sjemenki dvaju sorti kanole uzgojenih u Republici Makedoniji 2012. godine. U tu svrhu, u ukupno sto uzoraka sjemenki ove dvije sorte kanole određen je sadržaj ukupnih zasićenih masnih kiselina (SFA), ukupnih mononezasićenih masnih kiselina (MFA) te ukupnih polinezasićenih masnih kiselina (PUFA).

Rezultati istraživanja pokazali su različite sadržaje masnih kiselina u ove dvije sorte kanole. U sorti kanole tipa 2 utvrđen je prosječni sadržaj linolenske kiseline ($C_{18:3}$) od 44,0 % ± 2,02 dok je sorta kanole tipa 1 imala prosječni sadržaj oleinske kiseline ($C_{18:1}$) od 59,5 % ± 1,91. Prosječni sadržaj erucične kiseline ($C_{22:1}$) bio je ispod 0,2 % u obje sorte.

Uz razlike u sastavu masnih kiselina, kao i u ukupnom sadržaju SFA, MUFA i PUFA, obadvije sorte kanole imale su slične vrijednosti polinezasićenih/zasićenih indeksa (P/S), 3,2 odnosno 3,4, što upućuje na to da je ulje obadviju sorti bilo iste hranidbene vrijednosti.

Ključne riječi: *kanola, masna kiselina, plinska kromatografija, polinezasićeni/zasićeni indeks*

INTRODUCTION

Canola was developed in the early 1970s by Canadian plant breeders using traditional plant breeding techniques 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 of 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 a high oil content as well as a 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 the quality of canola seed, some innovations have been applied. *Roundup Ready* and *Liberty Link* canola varieties were developed using the 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 were developed in the past decade. The main objective of our work was identification and determination the FA composition of seed oil from the two canola varieties grown in the Republic of Macedonia in 2012. For that purpose, a total of one hundred samples of seeds of the two canola varieties were analyzed for the presence of total saturated fatty acids (SFA), total monounsaturated fatty acids (MFA), and total polyunsaturated fatty acids (PUFA). The values of polyunsaturated/saturated indexes (P/S) were calculated for both canola varieties.

MATERIALS AND METHODS

Materials

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

Methods

Sample preparation

A total of 5 g of ground samples was mixed with 5 g of anhydrous sodium sulphate (Na_2SO_4). An aliquot of 2 g was placed in a Soxhlet extractor system, and extraction was performed with petroleum ether (40–60) within 24 hours. After cooling at room temperature, the solvent was evaporated to dryness in a nitrogen stream.

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 °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 became 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 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

A Shimadzu 2010 gas chromatograph equipped with an automatic liquid auto injector (AOC 20i, Shimadzu) and a flame-ionization detector (FID) was used with a polyethylene glycol TPA modified polar column commercially available as HP-FFAP (25 m x 0.32 mm id x 0.52 µm). 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 °C and the FID at 280 °C. Oven temperature was set at 200 °C (1 minute) increasing by 5 °C/ min. The final oven temperature was maintained at 250 °C (20 minutes). For confirmation of identified and determined FAMEs in oils and fats, 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$^{TM–1}$ and obtained from Supelco (USA) was used with the same Shimadzu 2010 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 °C and the FID at 260 °C. Oven temperature was set at 180 °C, increasing by 2 °C/ min. The final oven temperature was maintained at 230 °C (4 minutes). The total run time of the chromatographic analysis was 35 minutes.

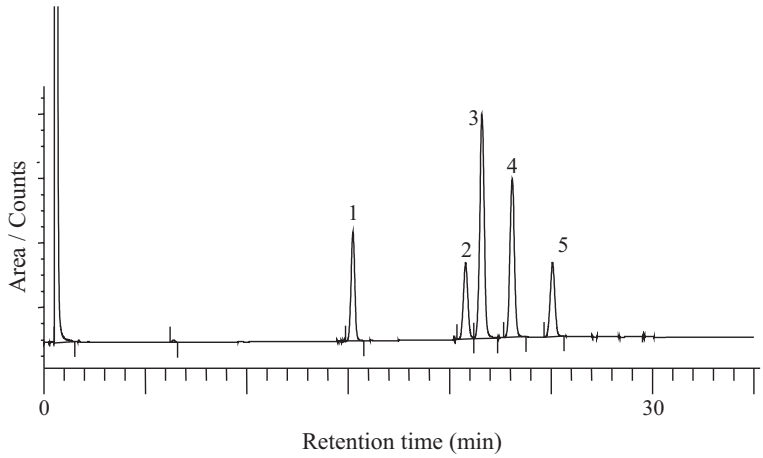

Figure 1. Representative GC-FID chromatogram of the most abundant FAMEs obtained on the HP-FFAP column for canola oil type 1: $C_{16:0}$ (1); $C_{18:0}$ (2); $C_{18:1}$ (3); $C_{18:2}$ (4); $C_{18:3}$ (5).

RESULTS AND DISCUSSION

A total of 100 seed samples of two different hybrids of canola varieties were analyzed for the composition of fatty acids using the gas chromatographic method. The content of the following saturated and unsaturated fatty acids in the samples was tested: 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 Tables 1 and 2. The means of total saturated fatty acids (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 a low content of saturated FA with the predominant

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

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

presence of $C_{16:0}$ and $C_{18:0}$. The level of $C_{18:0}$ in seeds of the canola variety type 1 was 4.4 % ± 1.4 of total FA, and 6.9 %±1.6 of total FA in seeds of the 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 food supply (6). Unlike other predominant long-chain SFAs: $C_{16:0}$, $C_{14:0,}$ and $C_{12:0}$ that increase blood cholesterol levels, $C_{18:0}$ has been shown to have a neutral effect on total blood and low density lipoprotein (LDL) cholesterol levels (6,7).

The results obtained for unsaturated FA (Table 2) composition of seed oil showed the predominant presence of $C_{18:1}$ in seed oil of the canola variety type 1, which was found to be 59.5 % ± 1.91 of total FA. It is known that the consumption of $C_{18:1}$ is effective in lowering the LDL cholesterol level. The predominant presence of $C_{18:3}$ (44.0 % ± 2.02) was found in seed oil of the 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 disease states. This strategy is based on the ability of the diet to modify cellular lipid composition and eicosanoid (cyclooxygenase and lipoxygenase) biosynthesis (8).

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

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

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

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

Table 3. SFA, MUFA, PUFA contents and P/S indexes of two canola varieties

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 the P/S index. This value is an important parameter for determination of the nutritional value of certain oils. Oils and fats with P/S index higher than 1 are considered to have a nutritional value. Several studies indicate that higher P/S index means lower deposition of lipids in the body (11). The results of our investigations (Table 3) show that 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 both oil varieties had the same nutritional value.

ABSTRACT

Canola is the name applied to edible oilseed rape. This plant belongs to the *Brassicaceae* (mustard) family along with 3000 other species. Close relatives of this crop have been cultivated for food since the earliest recordings of man. 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 to identify and determine the FA composition of seed oil of two canola varieties grown in the Republic of Macedonia in 2012. For that purpose, a total of one hundred samples of seeds of the two canola varieties were analyzed for the presence of total saturated fatty acids (SFA), total monounsaturated fatty acids (MFA) and total polyunsaturated fatty acids (PUFA).

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

Besides the differences in FA composition, as well as in total SFA, MUFA, and PUFA contents, 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 both oil varieties had the same nutritional value.

Keywords: *canola, fatty acid, gas chromatography, polyunsaturated/saturated index*

CONCLUSIONS

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

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

Both canola varieties had similar values of polyunsaturated/saturated indexes (P/S), which means that both oil varieties had the same nutritional value.

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