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A comparison of fatty acid compounds in winter and spring rapeseed varieties

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ABSTRACT

To assess the two most momentous seed's biochemical characteristics including seed oil content and fatty acid compounds in different winter and spring rapeseed varieties, an experiment was conducted on randomized complete block design with four replications at experimental field of Seed and Plant Improvement Institute, Karaj, Iran in 2005-2007 cropping seasons. 12 varities of rapeseed that involved 5 spring and 7 winter varieties were investigated. The output results showed that there was significant variation among varieties in aspect of fatty acid compounds and seed oil content. In general, spring varieties by seed oil content about 44.35% were superior to winter varieties with 43.14%. Among rapeseed varieties, the spring one 'Option500' had the highest seed oil content (45.54%) and the winter variety 'Talaye' had the lowest seed oil content (42.15%). In this research, detection of seed oil fatty acid compounds in studied varieties by Gas chromatography revealed that oleic, linoleic, linolenic, palmitic and stearic acid were the main fatty acids in seed oil content of rapeseed varieties. Arachidic, erucic, gadoleic and palmitoleic acid were also portion of fatty acids that detected in insignificant amounts in evaluated varieties. The highest amount of two unsaturated fatty acids namely, oleic and linoleic acid detected in 'Hyola420' and 'Option500' varieties, respectively. The spring variety 'Sarigol' had the highest linolenic acid (9.76%), and the winter varieties 'Opera' (4.50%) and 'Orient' (4.47%) had the highest level of palmitic acid.

Key words: *Brassica napus* L., variety, gas chromatography, linoleic acid, oleic acid.

INTRODUCTION

Oil is one of the necessary nutrients for the human body, which is supplied by animal and plant sources [1]. Herbal oils are the main sources of fats and fat-soluble vitamins, which have a substantial role in the human diet [2]. After cereals, oily seeds are the second food sources throughout the world, whose oil is of rich fatty acid types [3]. Rapeseed (*Brassica napus* L.) is considered as one of the most important oil seeds in the world such that after soybean and oily palm, it is the third source of the vegetable oil [4]. In average, this plant contains 40 to 45% oil in

its seeds [5] and generally, seed oil percentage is of great significance in the profitability of rapeseed production [6]. Rapeseed oil has a high nutritional quality due to its lowest levels of saturated fatty acid, balanced amount of unsaturated fatty acids and being free from cholesterol [7]. Experiments conducted by Modares Sanavi and Daneshgar [8] showed that, with the considerable amounts of unsaturated fatty acids in the seed oil of the studied rapeseed varieties and also their less than 1% erucic acid content taken into account, the obtained oil can be a good replacement for animal fat or even other herbal oils in the human diet. Usually, the qualitative properties of each oil type depends on its fatty acid compounds and one of the main breeding objectives regarding rapeseed besides the oil quantity is to increase its oil quality [9]. During the past fifteen years, most of the breeding programs for rapeseed were focused on oleic and erucic unsaturated fatty acids as well as medium-chain fatty acids [10]. Mainly, the quality of rapeseed oil is determined based on its oleic, linoleic and erucic fatty acid contents and is highly affected by the variety type [11,12,13]. Moreover, from their experiments, Pospišil *et al*. [14] concluded that the fatty acid compounds in rapeseed hybrids and its double low varieties were affected by varieties to a great extent. In the new rapeseed varieties, instead of erucic acid, the level of other fatty acids such as oleic acid (more than 60%) and linoleic acid (10-20%) increased, while the level of linolenic acid had decreased (less than10%) [14]. Results of Gas chromatography and the analysis of the seed oils of 8 studied rapeseed varieties in the experiment conducted by Khayami *et al*. [15] showed that seed oils of the said plants had high levels of oleic and linoleic acid contents. From the nutritional perspective, linoleic acid is the most important unsaturated fatty acid. Since this fatty acid is not synthesized in the body, it has to be supplied through meals. In addition, oleic acid is among unsaturated fatty acids whose antioxidant effects have been proved [16]. High levels of this acid in oil increases its resistance to oxidation and makes the taste desirable [17]. Lee *et al*. [11] observed that in the fatty acid compound of rapeseed and turnip mustard (*Brassica rapa*), besides fatty acids that were identified in soybean and sesame seed oils, contain arachidic acid, gadoleic acid and erucic acid as well. Furthermore, these researchers reported that in terms of palmitic acid content, rapeseed varieties were considerably different. Results obtained from the study of Pospišil *et al*. [14] on fatty acid compounds of seed oils of new rapeseed hybrids and also rapeseed double low varieties showed that among seven hybrids and eight studied varieties, the level of erucic acid content was below 2%. They also reported 61.8-62.5% for oleic acid, 19.6-20.5% for linoleic acid, 5.1-5.5% for palmitic acid and 1.4 to 1.5% for stearic acid [14]. Unlike Röbbelen and Nitsch [19] who reported a difference between linoleic and linolenic acid contents in rapeseed varieties, Rucker and Röbbelen [18] reported a difference in terms of palmitic and linolenic acid levels. Results of a study by Javidfar *et al*.[13] showed that lines of the studied rapeseeds in terms of all the investigated fatty acids were significantly different. In the study by Nasr *et al*. [12], five important fatty acids, i.e. oleic acid, linoleic acid, linolenic acid, stearic acid and palmitic acid were commonly found in 10 rapeseed varieties which oleic acid and stearic acid had the highest and lowest percentages, respectively. Oleic acid levels in different rapeseed varieties were 51% to 62%, while there was 18-32% linoleic acid, 2-16% linolenic acid, 0.15-2.2% stearic acid and 4-8% palmitic acid [12]. In addition, Nasr *et al*. [12] reported a difference in terms of seed oil percentages among rapeseed varieties and mentioned the mean variation of seed oil percentages to be 37-42 in them.
Considering the importance of oily seeds cultivation and the growing trend of its cultivated area, the objective of this research was evaluating two main biochemical properties of the seeds in the said plant (oil and fatty acid contents in several winter and spring rapeseed varieties) along with identifying the difference which exists between fatty acid compounds in these varieties.

MATERIALS AND METHODS

Site description

The present study was conducted at research field of Seed and Plant Improvement Institute, Karaj, Iran (latitude 35°59′ N, longitude 50°75′ E, elevation 1315 m above mean sea level) during 2005-2006 and 2006-2007. This region has a semi-arid climate (warm and dry summers). The monthly precipitation data at the experimental site during the years 2005-2007 is delineated in Table1. The soil texture was clay loam with a clay type montmorillonite, low in nitrogen (0.07-0.08%), low in organic matter (0.44%), alkaline in reaction, phosphorus and potassium content of 3.3 and 175 mg kg^{-1} in order with a pH of 7.8 and EC =1.70 mmhos cm^{-1}.

Table 1. Monthly amount of precipitation in Karaj research station in 2005-6 and 2006-7

Year	SEPT	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	Total
						(mm)				
2005/6	0.0	30.3	5.6	49.3	75.2	3.1	42.4	6.7	2.5	215.1
2006/7	48.5	37.2	18.2	24.1	34.6	25.0	118.3	62.5	5.4	373.8

Experimental design

This study was conducted in a randomized complete block design(RCBD) with four replications in which 12 rapeseed varieties including 5 spring (RGS003, Hyola420, Option500, Sarigol, Hyola401) and 7 winter varieties (Licord, Okapi, SLM046, Zarfam, Orient, Opera, Talaye) were evaluated (Table2).

Table 2. Growth type, origin and different phenological stages in winter and spring rapeseed varieties

Type	Varieties	Origin	DVP	DRP	SFD	S-F	F-M	FGD
					(Days)			
Spring	RGS003	Germany	139.3	85.0	55.0	16.0	69.0	224.3
	Hyola420	Canada	140.6	90.6	57.6	18.3	72.3	231.3
	Option500	Germany	144.0	86.0	52.0	20.6	65.3	230.0
	Sarigol	Germany	140.3	88.6	49.6	27.0	61.6	229.0
	Hyola401	Canada	135.3	90.6	58.3	19.0	71.6	226.0
Winter	Licord	Germany	163.6	73.3	46.3	12.3	61.0	237.0
	Okapi	France	164.3	72.6	44.6	14.3	58.3	237.0
	SLM046	Germany	161.0	76.3	47.6	15.0	61.3	236.3
	Zarfam	Iran	150.3	80.3	46.6	19.0	61.3	230.6
	Orient	Germany	161.3	71.6	44.0	14.3	57.3	233.0
	Opera	Sweden	165.6	69.3	41.0	15.6	54.0	235.0
	Talaye	Germany	164.6	68.3	39.0	14.3	53.6	233.0

Note. The growth stages including: DVP, duration of vegetative phase; DRP, duration of reproductive phase; SFD, Seed-filling duration; S-F, Stem elongation-flowering stage; F-M, flowering stage-physiological maturity; FGD, full growth duration. The data taken from the recording of Oilseed Crops Research Department, Seed and Plant Improvement Institute, Karaj, Iran.

Agronomic practices

Each experimental plot included 6 rows, 5 m long, and 30 cm row-spacing using a seeding rate of 7 kg ha^{-1}. The experimental fields were mould-board ploughed and seedbed preparation comprised of two passes with a tandem disk. Seeds were planted 1 to 1.5 cm deep at a rate of 100 seeds m^{-2} on 5 October 2005 and 2006. For all treatments, the crop was supplied with the fertilizers at a rate of $N_{150}P_{60}K_{50.}$ Nitrogen fertilizer was used in three splits(pre-sowing, at the beginning of stem elongation and flowering stages).Weeds were controlled by application of

haloxyfop- R-methyl ester (Gallant Super, 10% EC) at 0.6 L ha^{-1}. Broadleaf weeds were also hand weeded during the season.

Note taking of the phenologic stages in rapeseed was done using Sylvester-Bradley and Makepeace method [20]. Proper management practices were adopted throughout the growing seasons to ensure the good growth of crop. Final harvests were carried out on 10 June 2006 and 25 June 2007.

Estimation of fatty acid compounds

At physiological maturity stage after discarding margins, the crop by harvesting 4.8 m^2 of the central part of each plot, subjected to an ambient air temperature until constant weight (12% moisture content) and then transferred to laboratory. The seeds of each plant were manually extracted and dried at room temperature under standard conditions away from sunlight, moisture and microbial contamination. All dried seeds pertaining to each plot were weighed separately. Then, about 3 g of seeds/plot was prepared and the oil content was measured by using a nuclear magnetic resonance (NMR) apparatus (Bruker-Biospin, Karlsruhe, Germany). The said apparatus works based on the magnetic induction of hydrogen nucleus which is a spectrometry method [21]. One of the advantages of this method is its destructiveness which accelerates the speed and accuracy of measuring the seed oil content. Among all spectroscopy methods, determining fatty acid compounds by Gas spectroscopy using their methyl esters gives the most accurate results [22]. Initially, to methyl ester the fatty acids, normal heptane and 2mol/l potassium hydroxide methanol solution (2 N) was used [23]. Then, the obtained methyl esters were injected to Gas chromatography apparatus (Agilent 6890 N, USA) for determining the type and percentages of fatty acids. Column temperature was 175°C, while the detector and injection port temperature was 250°C. The capillary column was 60 m long with the polar silica thickness being 0.32 µm. The applied detector was of the Flame Ionization type with hydrogen fuel and its air oxidation, nitrogen carrier gas, hydrogen pressure and compressed air measure were 15 mm/min. Identification of methyl esters in fatty acids was done by comparing the peaks' retention times with those of the methyl esters of standard fatty acids. The percentage of each fatty acid was measured based on the calculation of the area below the curve by computer stability.

Statistical Analysis

The collected data were processed by the combined analysis of variance using SAS software package (SAS Institute Inc., Cary, North Carolina, United States of America, 2001). Duncan's Multiple Range Test (DMRT) was used to detect the significant grouping among the treatments as well. Effects were considered statistically significant when the probability of Type I error was 0.05 or less.

RESULTS AND DISCUSSION

Based on the combined variance analysis of the studied traits, the effect of year on the seed oil content and all measured fatty acids was highly significant (P<0.01) except for linolenic acid. These results could be attributed to climate factors because the precipitation rate during the second year of experiment was 74% more than the first year and the distribution of rainfall during the spring and winter of the second year was more suitable than the first year (Table1). The studied varieties were significantly differed in terms of their fatty acid compounds and seed oil contents. Moreover, the interaction effect of year × variety on all measured fatty acids was highly significant (P<0.01). The seed oil content was not affected by Y× V interactions (Table3).

Table 3. The mean squares of ANOVA for palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, arachidic acid, gadoleic acid, linolenic acid, eurocic and oil content in combined analysis of 2005-2006 and 2006-2007 data.

S.O.V	*df*	Palmitic acid	Palmitoleic acid	Stearic acid	Oleic acid	Linoleic acid	Arachidic acid	Gadoleic acid	Linolenic acid	Eurocic acid	Oil content
Y	1	560.154**	93.298**	3919.075**	8.333**	962.025**	746.724**	138.404**	22.058ns	786.256**	339.649**
E_a (R×Y)	4	0.363	0.626	4.593	0.035	0.294	0.719	0.077	36.260	6.055	0.421
V	11	56.310**	176.117**	2549.803**	10.722**	461.981**	826.721**	272.874**	261.547**	577.204**	5.188**
Y × V	11	64.166**	45.710**	2429.053**	4.271**	202.843**	680.475**	109.152**	173.985**	212.122**	1.807ns
E	44	0.507	0.209	1.741	0.040	0.564	1.398	0.169	34.787	4.616	1.130
CV (%)		1.74	3.32	0.70	0.31	0.43	2.14	2.65	7.06	9.23	2.43

*Note. ***** – P < 0.05, ****** – P < 0.01, ns – P > 0.05; Y – year effect, V – variety effect; Y×V represent interaction terms between the treatment factors*

Table 4. Means for palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, arachidic acid, gadoleic acid, linolenic acid, eurocic and oil content as affected by rapeseed varieties in combined analysis of 2005-2006 and 2006-2007 data.

Type	Varieties	Palmitic acid	Palmitoleic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Arachidic acid	Gadoleic acid	Eurocic acid	Seed oil content
		16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	22:1	16:0
							(%)				
	RGS003	4.347 b	0.1493 d	1.883 f	64.28 e	17.58 f	8.320 b-e	0.621 b	0.2366 b	0.2253 de	43.52 cd
	Hyola 420	3.740 g	0.1408 e	2.041 c	67.38 a	15.87 i	8.522 bcd	0.562 d	0.1461 d	0.1358 h	43.98 bc
Spring	Option500	3.721 g	0.1419 e	1.698 k	64.39 e	19.06 a	8.763 bc	0.576 d	0.1091 g	0.1656 g	45.54 a
	Sarigol	3.554 h	0.0065 h	2.300 a	63.62 f	17.99 d	9.761 a	0.619 b	0.1405 e	0.3906 a	43.80 bc
	Hyola 401	4.064 e	0.0643 g	2.085 b	67.17 ab	16.07 h	8.060 cde	0.688 b	0.1475 d	0.1250 h	44.94 ab
	Licord	3.958 f	0.1273 f	1.917 e	65.51 c	17.85 e	7.645 e	0.680 a	0.1655 c	0.3772 a	42.97 cd
	Okapi	4.233 cd	0.1594 c	1.542 l	67.07 b	17.87 e	7.545 e	0.343 g	0.0964 h	0.1155 h	43.46 cd
	SLM 046	3.986 ef	0.1787 b	1.794 h	63.70 f	17.78 e	7.898 de	0.604 c	0.3335 a	0.3374 b	43.87 bc
Winter	Zarfam	4.299 bc	0.1247 f	2.017 d	65.71 c	17.79 e	8.299 b-e	0.311 h	0.1000 h	0.1841 fg	43.87 bc
	Orient	4.472 a	0.1887 a	1.778 i	64.65 d	18.09 c	7.592 b	0.570 d	0.1465 d	0.2843 c	42.99 cd
	Opera	4.509 a	0.1881 a	1.829 g	64.43 de	18.21 b	8.893 b	0.536 e	0.1121 g	0.2485 d	42.73 cd
	Talaye	4.469 d	0.1819 b	1.761 j	65.53 c	17.33 g	8.894 b	0.509 f	0.1307 f	0.2047 ef	42.15 d

Means in each column followed by the different letters are significantly different (P < 0.05) according to Duncan test

In this study, identified fatty acids in the seed oil of studied spring and winter rapeseed varieties were oleic acid (63.62-67.38%), linoleic acid (15.87-19.06%), linolenic acid (7.55-9.76%), palmitic acid (3.55-4.51%) and stearic acid (1.54-2.3%), respectively. Moreover, arachidic acid, erucic acid, gadoleic acid and palmitoleic acid were among fatty acids with the lowest percentages (Less than 1%) of rapeseed varieties (Table 4). This finding is in line with Nasr *et al*. [12] who reported that oleic acid, linoleic acid, linolenic acid, palmitic acid and stearic acid are the most important and essential fatty acids in rapeseed oil.

Means comparison of Gas chromatography results are demonstrated in Table 4. According to results, there was a significant difference between the studied varieties in terms of their fatty acid compounds. These findings are in conformity with the results by Nasr *et al*. [12], Javidfar *et al*. [13] and Pospišil *et al*. [14] who found a salient diversity among selected varieties of *Brassica napus*. In the present research, of all the studied varieties, the highest levels of two important unsaturated fatty acids (oleic acid and linoleic acid) were observed in spring varieties of 'Hyola420' (67.38%) and 'Option500' (19.08%), respectively. 'Sarigol' (spring variety), also, having the highest linolenic acid content (9.76%) was superior to other varieties (Table 4). Gas chromatography results and the seed oil analysis of 8 studied rapeseed varieties in the trial conducted by Khayami *et al*. [15] revealed that the obtained seed oils had the highest oleic and linoleic acid contents and nutritionally, the latter is the most important unsaturated fatty acid. Since it is not synthesized in the body, it should be supplied through meals. Moreover, oleic acid is one of the main unsaturated fatty acids which besides the important role in nutrition, it comprises of an oil that is greatly resistant to oxidation and is suitable for all uses. Generally, the type and amount of fatty acids in the studied varieties' oils is an indication of the oil quality. The oil obtained from the studied rapeseed varieties in this study had a desirable amount of oleic and linoleic acid. Linoleic and linolenic are among unsaturated fatty acids which form long Omega 3 and Omega 6 chains. Usually, the linoleic-linolenic acids ratio affects the onset of arteriosclerosis through the synthesis of eicosenoic acid. Therefore, it can be said that by having linoleic acid, rapeseed oil is effective in reducing cholesterol and preventing arteriosclerosis [24]. Among the studied varieties, 'Opera' and 'Orient' (winter varieties) had the highest palmitic acid contents (4.5% in average), while 'Sarigol' (spring variety) had the highest level of stearic acid content (2.3%). On the other hand, 'Licord', 'SLM046' and 'Orient' were the most superior varieties in terms of their arachidic, gadoleic and palmitoleic acid contents [3]. In the studied varieties, erucic acid content was below 2%; however, this harmful fatty acid content in 'Sarigol' (spring variety) and 'Licord' (winter variety) was more than other varieties (Table 4). In contrast, 'Okapi' (winter variety) and 'Hyola420' (spring variety) had the lowest levels of this acid, which was very desirable. This 22-carbon fatty acid is usually harmful to the human health; thus, those varieties, which do not have it, are nutritionally, placed at the highest level [3]. Pospišil *et al*. [14] reported lower erucic acid content in new rapeseed varieties, which is fully coincide with findings of this experiment. Oil content is one of the important components, which play a crucial role in the seed quality [25]. Results from the means comparison of the oil content revealed that as a whole, spring varieties with a mean value of 44.35% were superior to winter varieties with a mean value of 43.14% (Table 4). 'Option500' (spring variety) and 'Talaye' (winter variety) had the highest and lowest oil content, respectively. The difference in the oil content of rapeseed varieties might be due to genetic variations which existed among them [26].

CONCLUSION

The results of the present study revealed that spring varieties of 'Hyola420' and 'Option500', with appropriate qualitative and quantitative properties in their seed oil contents, were suitable for cultivation in the agro-climatic condition of Karaj-Iran.

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