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Comparison of Winter and Spring Rapeseed Cultivars Considering their Oil Content and Fatty acids composition

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Abstract: In order to evaluate two of the most important biochemical characteristics of seed, such as seed oil content and fatty acids composition in different winter and spring rapeseed cultivars, an experiment was conducted on randomized complete block design with four replications at experimental field of Seed and Plant Improvement Institute, Karaj-Iran in 2004-2006 cropping seasons. In this study, 12 cultivars of rapeseed that involved 5 spring and 7 winter cultivars were studied. The output results showed that, there was significant variation among cultivars in aspect of fatty acids composition and seed oil percentage. In general, spring cultivars by seed oil percentage about 44.35% were superior to winter cultivars with 43.14%. Among rapeseed cultivars, the spring one 'Option 500' had the highest seed oil content (45.5%) and the winter cultivar 'Talaye' had the lowest seed oil content (42.2%). In this study, detection of seed oil fatty acids composition in studied cultivars by Gas Chromatography revealed that oleic, linoleic, linolenic, palmitic and stearic acids were the main fatty acids in seed oil of rapeseed cultivars. Arachidic, erucic, gadoleic and palmitoleic acids were also portion of fatty acids, that detected in insignificant amounts in evaluated cultivars. The highest amount of two unsaturated fatty acids namely oleic acid and linoleic acid detected in 'Hyola420' and 'Option500' cultivars, respectively. Among studied cultivars, the spring cultivar 'Sarigol' had the highest lonoleic acid (9.7%) and the winter cultivars 'Opera' and 'Orient' had the highest level of palmetic acid(4.5%).

Key words: Fatty acid · Gas chromatography · Linoleic acid · Oleic acid · Rapeseed

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]. Due to having the lowest saturated fatty acid levels, a balanced amount of unsaturated fatty acids and not having any cholesterol, rapeseed oil has a high nutritional quality [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 cultivars 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 compositions 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 saturated 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 cultivar type [11, 12,13]. In addition, Pospisil *et al*. [14]

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concluded that the composition of fatty acids in rapeseed hybrids and its double low varieties were affected by cultivars to a great extent. In recent rapeseed cultivars, 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 cultivars 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 and as we all know, 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 cause its resistance to oxidation to increase and its taste to become desirable [17]. Lee *et al*. [11] observed that in the fatty acid composition of rapeseed and turnip mustard (*Brassica rapa*), besides fatty acids that were identified in soybean and sesame seed oils, there were Arachidic acid, gadoleic acid and erucic acid as well. Furthermore, these researchers reported that in terms of Palmitic acid content, rapeseed cultivars were considerably different. Results obtained from the study of Pospisil *et al*. [14] on fatty acid compositions of seed oils of new rapeseed hybrids and also rapeseed double low cultivars showed that among seven hybrids and eight studied cultivars, 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 1.4-1.5% for Stearic acid [14]. Moreover, Rucker and Röbbelen [18] reported a difference in terms of Palmitic and linolenic acid levels, while Robbelen and Nitsch [19] reported a difference, which was found between linoleic and linolenic acid contents in rapeseed cultivars. 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 of 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 cultivars, with oleic acid and stearic acid having the highest and lowest percentages, respectively.

Also, 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 cultivars 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 study was evaluating two main biochemical properties of the seeds in the said plant (i.e., oil and fatty acid contents in several winter and spring rapeseed cultivars) along with identifying the difference, which exists between fatty acid compositions in these cultivars.

MATERIALS AND METHODS

The present experiment was laid out with the purpose of evaluating two biochemical properties of seeds that is the seed oil content and fatty acid compositions in winter and spring rapeseed cultivars during the 2005-2006 and 2006-2007 cropping years in the Research Field of Seed and Plant Improvement Institute, Karaj, Iran. The site is situated at 35°59' E longitude and 50°75' N latitude with semi-arid climate (warm and dry summers). Monthly precipitation rate at the experiment site during the years 2005-2007 has been given in Table I. The soil texture of the studied area was clay-loam consisting of 44% organic carbon (OC), pH=7.8 and an electrical conductivity (EC) of 1.70 mmhos/cm. Phosphorus and potassium contents of the experimental site were 3.3 and 175 ppm, respectively.

This study was conducted in a randomized complete block design with four replications in which 12 rapeseed cultivars including 5 spring (RGS003, Hyola420, Option500, Sarigol, Hyola401) and 7 winter cultivars(Licord, Okapi, SLM046, Zarfam, Orient, Opera, Talaye) were evaluated in terms of their seed oil contents and fatty acid compositions (Table 2).

Each experimental plot included six planting rows, 5 meters long and 30cm row spacing with in-row spacing of 4 centimeters, while two lateral rows were considered as margins and the distance between two adjacent blocks was 6 meters. Wet planting was done on October 5 and

Table 1: Amount of precipitation (mm) in 2005-2006 and 2006-2007 cropping seasons at Karaj research station, Iran

Year	Month	Sept	Oct	Nov	Dec	Jan	Feb	March	April	May	Total
2005	06	0.0	30.3	5.6	49.3	75.2	3.1	42.4	6.7	2.5	215.1
2006	07	48.5	37.2	18.2	24.1	34.6	25.0	118.3	62.5	5.4	373.8

Table 2: Growth type, origin and mean of phenological stages in winter and spring rapeseed cultivars

cultivars		Origin	VSD	RSD	SFD	S-F	F-M	GD
			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	Swede	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: Vegetative stage duration; VSD, Reproductive stage duration; RSD, Seed filling duration; SFD, Stem elongation-Flowering; S-F, Flowering-Maturity; F-M, Growth duration; GD

all crop management-related operations were similarly performed for each plot in accordance with the regional custom. Note taking of the developmental stages (phenologic stages) in rapeseed was done using Sylvester-Bradley and Makepeace [20] method, whose results are given in Table 2.

In order to measure the seed oil percentage of each experimental plot, about 3 grams of seed was prepared and using an NMR apparatus, the oil percentage was measured. The said apparatus works based on the magnetic induction of hydrogen nucleus which is a spectrometry method. One of the advantages of this method is its being destructive which accelerates the speed and accuracy of measuring the seed oil content.

Among all spectroscopy methods, determining fatty acid compositions by gas spectroscopy using their methyl esters gives the most accurate results [21]. This method was used in the present research as well. Initially, to methyl ester the fatty acids, normal heptane and 2mol/l potassium hydroxide methanol solution (2 N) was used [22, 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 meters long with the polar silica thickness being 0.32 micrometers. 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 millimeters per minute. 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.

Finally, data collected from the experiment were processed by the combined analysis of variance using SAS statistical software. Means comparison of the data was done by Duncan's multi-range test (DMRT) at the probability level of 5%.

RESULTS AND DISCUSSION

Results of the combined variance analysis of the studied traits are presented in Tables 3 and 4. Based on these results, the effect of year on the seed oil percentage and all measured fatty acids except for linolenic acid was highly significant ($p<0.01$). The significance of year's effect on most of the studied traits in this experiment could be due 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 (Table 1). In addition, the studied cultivars were significantly different in terms of their fatty acid compositions and seed oil percentages. Moreover, the interaction effect of year × cultivar on all measured fatty acids was highly significant ($p<0.01$) except for the seed oil percentage. In this study, identified fatty acids in the seed oil of spring and winter rapeseed cultivars were oleic acid (63.7-67.4%), linoleic acid (15.9-19.1%), linolenic acid (7.5-9.7%), palmitic acid (3.7- 4.5%) and stearic acid (1.7-2.3%), respectively.

Table 3: Combined Analysis of variance (ANOVA) for studied traits in winter and spring rapeseed cultivars for 2005-2006 and 2006-2007 cropping seasons

		MS				
S.O.V	df	Palmitic acid	Palmitoleic acid	Stearic acid	Oleic acid	Linoleic acid
Y	1	560.154**	93.298**	3919.075**	8.333**	962.025**
E_a (R×Y)	4	0.363	0.626	4.593	0.035	0.294
V	11	56.310**	176.117**	2549.803**	10.722**	461.981**
Y × V	11	64.166**	45.710**	2429.053**	4.271**	202.843**
E	44	0.507	0.209	1.741	0.040	0.564
CV (%)		1.74	3.32	0.70	0.31	0.43

* = Significant (P<0.05), ** = Significant (P<0.01), ns = non-significant. Y = year effect, V =variety effect,

Y× V= represent interaction terms between the treatment factors

Table 4: Combined Analysis of variance (ANOVA) for studied traits in winter and spring rapeseed cultivars for 2005-2006 and 2006-2007 cropping seasons

		MS				
S.O.V	df	Arachidic acid	Gadoleic acid	Linolenic acid	Eurcic acid	Oil
Y	1	746.724**	138.404**	22.058ns	786.256**	339.649**
E_a (R×Y)	4	0.719	0.077	36.260	6.055	0.421
V	11	826.721**	272.874**	261.547**	577.204**	5.188**
Y × V	11	680.475**	109.152**	173.985**	212.122**	1.807ns
E	44	1.398	0.169	34.787	4.616	1.130
CV (%)		2.14	2.65	7.06	9.23	2.43

* = Significant (P<0.05), ** = Significant (P<0.01), ns = non-significant. Y = year effect, V =variety effect,

Y× V= represent interaction terms between the treatment factors

Table 5: Mean comparison of seed oil fatty acids composition in winter and spring rapeseed cultivars in combined analysis of 2005-2006 and 2006-2007 data
Means followed by similar letters in each column are not significantly different at 5% probability level-using Duncan Multiple Range Test

		Palmitic acid 16:0	Palmitoleic acid 16:1	Stearic acid 18:0	Oleic acid 18:1	Linoleic acid 18:2	Linolenic acid 18:3	Arachidic acid 20:0	Gadoleic acid 20:1	Eurcic acid 22:1	Seed oil
Cultivars		(%)									
Spring	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
	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
Winter	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
	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

Also, arachidic acid, erucic acid, gadoleic acid and palmitoleic acid were among fatty acids with the lowest percentages (Less than % 1) in rapeseed cultivars (Table 5). In addition, Nasr *et al*. [12] mentioned oleic acid, linoleic acid, linolenic acid, palmitic acid and stearic acid as the most important and essential fatty acids in rapeseed oil, a finding which was consistent with the results of this research.

Means comparison related to Gas Chromatography results have been given in Table 5, according to which there was a significant difference between the studied cultivars in terms of their fatty acid compositions (Table 5). These results are similar to the findings of Nasr *et al*. [12], Javidfar *et al*. [13] and Pospisil *et al*. [14] in this regard.

In the present research, of all the studied cultivars, the highest levels of two important unsaturated fatty acids, i.e. oleic acid and linoleic acid were observed in spring cultivars of 'Hyola420' (67.4%) and 'Option500' (19.1%), respectively. 'Sarigol' (spring cultivar), also, having the highest linolenic acid content (9.7%) was superior to other cultivars (Table 5). Gas Chromatography results and the seed oil analysis of 8 studied rapeseed cultivars in the study conducted by Khayami *et al*. [15] revealed that, the obtained seed oils had the highest oleic acid 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. In addition, oleic acid is one of the main unsaturated fatty acids, which besides its important role in nutrition, the oil that contains it, is greatly resistant to oxidation and is suitable for all uses. Generally, the type and amount of fatty acids in the studied cultivars' oils is an indication of the oil quality. The oil obtained from the studied rapeseed cultivars in this study had a desirable amount of oleic and linoleic acid. Moreover, 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 cultivars, 'Orient' and 'Opera' (winter cultivars) had the highest palmitic acid contents (4.5%), while 'Sarigol' (spring cultivar) had the highest level of stearic acid content (2.3%). On the other hand, 'Licord', 'SLM046' and 'Orient' were the most superior cultivars in terms of their arachidic acid, gadoleic acid and palmitoleic acid contents (Table 5). The results are in conformity with Siavash *et al*. [3]. In all the studied cultivars, erucic acid content was below 2%; however, this harmful fatty acid's content in 'Sarigol' (spring cultivar) and 'Licord' (winter cultivar) was more than others (Table V). On the other hand, 'Okapi' (winter cultivar) and 'Hyola420' (spring cultivar) had the lowest levels of this acid, which was very desirable (Table 5). This 22-carbon fatty acid is usually harmful to the human health; thus, those cultivars, which do not have it, are nutritionally, placed at the highest level [3]. Results conducted by Pospisil *et al*. [14] in terms of erucic acid content in the recent rapeseed cultivars are completely in agreement with findings of this experiment.

Oil content is one of the important components, which play a crucial role in the rapeseed seed quality [25]. Results from the means comparison of the oil percentages revealed that as a whole, spring cultivars with a mean value of 44.35% were superior to winter cultivars with a mean value of 43.14% (Table 5). The results indicated that, 'Option500' (spring cultivar) and 'Talaye' (winter cultivar) had the highest and lowest oil percentages, respectively (Table 5). Probably, the difference in the oil percentage of rapeseed cultivars was due to genetic variations, which existed among them. Naeemi *et al*. [26] reported a significant difference in terms of seed oil percentage of these cultivars.

CONCLUSION

The results of the present study revealed that spring cultivars of 'Hyola401' and 'Option500' having appropriate qualitative and quantitative properties in terms of their seed oils were suitable for cultivation in the agro-climatic condition of Karaj-Iran.

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