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Evaluation of Oil Content and Fatty Acid Composition in Seeds of Different Genotypes of Safflower (*Carthamus tinctorius* L.)

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ABSTRACT

Evaluation of four safflower (*Carthamus tinctorius* L.) genotypes for oil content and fatty acid composition showed that oil content varied from 22.16 to 34.39% and the highest seed oil content was obtained from Isfahan14 genotype (34.39%, respectively). Among those fatty acids were detected, linoleic acid (75.81- 77.86%) was the predominant fatty acid followed by oleic (12.57–13.75%), palmitic (6.09–7.07%) and stearic (2.17–2.62%) acids while trace amounts of other fatty acids were presented and the values of them did not exceed 0.81%. The oil content and fatty acid composition of oil among the genotypes were significantly different ($P<0.01$), indicating that synthesis of them is influenced by genotype and the lipids present in the safflower are rich in premium grade high polyunsaturated essential fatty acids, linoleic acid which makes the oil nutritionally and therapeutically valuable for human consumption.

Keywords: Safflower genotypes; Seed oil; Fatty acid composition.

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INTRODUCTION

Fat and oils are a vital component of the human diet and they are readily acquired from a number of vegetable and animal sources. They play an integral role in foods as nutrients, health improvement and growth promotion. The most important source of vegetable oil is oil-bearing seeds of annual plant. Because of the increasing nutritional interest in polyunsaturated oils, demand for healthier oils which naturally contain a high proportion of polyunsaturated fatty acids such as safflower oil is arising rapidly (Breck & Bhatia, 2008; Camas *et al*., 2007; Sabzalian *et al*., 2008). Carthamus tinctorius L., respectively, named Safflower, is world's oldest crop belonging to the compositae family which originated in the Middle East. Safflower is an annual oilseed crop which is well adapted to dry hot climates and is tolerant to drought and salinity. It has been used traditionally as a medicinal herb and as a natural dye sources for coloring foods and textile. For the last decades, the plant has been cultivated principally for the oil which is extracted from its seeds and has both food and industrial applications. Safflower oil, which is light in color and bland in flavor, is widely used as edible oil for cooking, margarine and salad oil (Breck & Bhatia, 2008; Dordas & Sioulas, 2008; Dordas & Sioulas, 2009; Ekin, 2005; Gecgel *et al*., 2007; Smith, 2005). The whole safflower seeds are normally white to cream in appearance and consist of 33 to 45% tough hull that protects a kernel which forms 55–65% of the seed weight (Gecgel *et al*., 2007).

According to hull types, the seed has an oil content of about 20-45 % (Cosge *et al*., 2007).The whole seeds in normal-hull types contain nearly 27–32% oil of very high quality, 5–8% moisture, 14–15% protein, 2–7% ash, and 32–40% crude fiber (Gecgel *et al*., 2007) and seed oil's richness in the polyunsaturated essential fatty acids (PUEFA) linoleic acid (70–87%). Moreover, safflower seed oil might be regarded as a rich source of α-tocopherols, which demonstrates the highest vitamin E activity (Ekin, 2005). In recent years, considerable attention has been generated in the consumption and development of safflower seed oil as an excellent health care product and the health benefits derived from it include prevention and treatment of hyperlipaemia, arteriosclerosis, coronary heart disease (Han *et al*., 2009).

There are some reports on the oil content and fatty acid composition of safflower (Carvalho *et al*., 2006; Cosge *et al*., 2007; Gecgel *et al*., 2007; Han *et al*., 2009; Sabzalian *et al*., 2008); however, further experimental works are required on the genotypes which have not been investigated previously to improve oil content and fatty acid composition in safflower seed. Therefore the objective of the present research was to evaluate the oil content and fatty acid composition of four domestic and exotic safflower (C. tinctorius L.) genotypes. These data can be important for improvement of oil quality and developing new genotypes in safflower breeding programs and in the selection of the most useful genotypes for future commercial production in the region.

MATERIALS AND METHODS

The study was carried out from four safflower (*C. tinctorius* L.) genotypes originated from 2 provinces of Iran and one genotype from Mexico. The genotypes of the safflower were, Isfahan14 (Isfahan province), Goldasht, Mahalie-Ajabshir (East Azarbaijan province) and Bacum92 from Mexico. All the chemicals used in the analysis were of analytical grade produced by Merck Company.

Oil Extraction

The safflower seeds with hull were dried at 40 °C for 4 hours under vacuum to less than 5% moisture content and then milled to desired particle size by a mortar. Oil was extracted from 15 grams of each seed powder in Soxhlet extractor for 6 hours using hexane as a solvent, following the AOCS method Ba 3-38 (AOCS 1993). Oil content of the samples is expressed on a percent basis, based on whole seed.

Fatty acid composition

The fatty acid composition was determined by the conversion of the oil to fatty acid methyl esters according to the AOCS method. The fatty acid methyl esters (FAMEs) were prepared by adding 7 ml of 50 µl sodium methoxide (0.5 M) to 350 mg of oil. The mixture was heated in the bath at its boiling temperature for 10 min followed by adding 5ml of boron trifluride (BF_3) as catalyst and heated again for 2 min. Then 6 ml of GC-grade hexane was added and heated for 2 min. Finally, 50 ml of saturated saline water was added and well were shaken at room temperature for 1 min. The top layer (0.5 µl) was injected on to a gas chromatography (Unicam 4600, Cambridge, England). The system was equipped with a flame ionizing detector (FID) and a fused silica capillary column (BPX70, 50ml × 0.32mm i.d) with the film thickness of 0.25µm. This process was operated at an oven temperature of 120 °C which was then raised to 220 °C at a rate of 3.5 °C/min and then kept at 220 °C for 15 min. The injector and detector temperatures were set at 250°C. Helium was used as the carrier gas at a flow rate of 1 ml min^{-1}. Identification and quantification of FAMEs were performed by comparing the relative retention times with individual standard FAME of behenic ($C_{22:0}$), arachidic ($C_{20:0}$), linolenic ($C_{18:3}$), linoleic ($C_{18:2}$), oleic ($C_{18:1}$), stearic ($C_{18:0}$), palmitoleic ($C_{16:1}$), palmitic, ($C_{16:0}$), myristic ($C_{14:0}$) acids purchased from Merck (Darmstadt, Germany). The relative in percentage of the fatty acid was calculated on the basis of the peak area of a fatty acid species to the total peak area of all the fatty acids in the oil sample.

Statistical Analysis

Each treatment was analyzed in triplicate and the figures were then averaged. Data were assessed by analysis of variance (ANOVA) using MSTAT-C software program. The least significant difference (LSD) test with a probability $P<0.05$ was applied to the results and in order to calculate the correlation coefficients between the oil content and fatty acids using the same statically software program.

RESULTS AND DISCUSSION

Oil Content

The important economic trait for safflower genotypes is the oil content of seeds which affecting the success of safflower introduction in new areas (Bassil & Kaffka, 2002). Many factors such as genotype, ecology, morphology, physiology and agronomic practices influence the oil content and fatty acid synthesis of crops (Cosge *et al*. 2007). The results of analysis for oil content in Table 1 showed that there were significant differences among four genotypes for seed the oil content ($P < 0.01$) and the oil content of four different genotypes varied from 22.16 to 34.39% are listed in Table 2, the highest seed oil content was obtained from Isfahan14 genotype (34.39%, respectively). On the other hand, the lowest (22.16%) was obtained from the Goldasht genotype (Table 2). The oil content of the other genotypes ranged among these values. Bayraktar & Ülker (1992) reported that the Oil content of safflower cultivars varied between 34.55 to 38.99%. Camas *et al*., (2005) also explained that the oil content of safflower varied between 24.5 to 27.2%. Our finding established that our results are lower than Bayraktar & Ülker (1992), but it is higher than Camas *et al*., (2005).

Table 1: Analysis of variance for oil content and main fatty acids in four genotypes of safflower

Source of variation	df	Oil	C16:0	C18:0	C18:1	C18:2	C18:3	Totala	Othersb	SAFAc	USFAd	U/Te	(C18:1/ C18:2)
Replication	2	1.481^{ns}	0.03^{ns}	0.028^{ns}	0.023^{ns}	0.049^{ns}	0.001^{ns}	0.005^{ns}	0.001^{ns}	0.008^{ns}	0.004^{ns}	0.001^{ns}	0.001^{ns}
Between genotypes	3	80.79**	0.65**	0.10**	0.843**	2.37**	0.007**	0.19**	0.12**	0.758**	1.62**	0.001^{ns}	0.001^{ns}
Within genotypes	6	0.534	0.026	0.005	0.021	0.034	0.001	0.003	0.001	0.012	0.014	0.001	0.001

[ns] not significant. ** Significant at 0.01 levels of probability, respectively
[a] Sum of stearic ($C_{16:0}$), palmitic ($C_{18:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids
[b] Sum of myristic ($C_{14:0}$), palmitoleic ($C_{16:1}$), arachidic ($C_{20:0}$) and behenic ($C_{22:0}$) acids
[c] Sum of Saturated fatty acids of stearic ($C_{16:0}$), palmitic ($C_{18:0}$)
[d] Sum of unsaturated fatty acids of oleic ($C_{18:1}$) and linoleic ($C_{18:2}$)
[e] The ratio of unsaturated to total fatty acids

Fatty Acid Composition

The fatty acid composition of the oil is the major factor determining its best commercial uses such as nutritional, industrial or pharmaceutical and it is influenced by the variety, climate and the area of production (Camas *et al*., 2007, Murphy, 1994; Sabzalian *et al*., 2008). The results of variance analysis for fatty acid in Table 1 revealed that there were significant differences among four genotypes for fatty acid composition ($P< 0.01$). The most principal fatty acids of safflower samples analyzed were linoleic (75.81- 77.86%), oleic (12.57–13.75%), palmitic (6.09–7.07%) and stearic (2.17–2.62%) in respecting decreasing order and these fatty acids together composed about 98.86–99.47% of the total fatty acids in all Safflower genotypes analyzed (Table 2). Minor amount of myristic ($C_{14:0}$), palmitoleic ($C_{16:1}$), arachidic ($C_{20:0}$) and behenic ($C_{22:0}$) were present and the values of them did not exceed 0.81% of the total fatty acids (Table 2). The nutritional properties of safflower, like most fat and oils, are depend on its fatty acid composition, particularly for the amount of oleic and linoleic which makes it desirable in point of nutritional. In this study, the unsaturated fatty acids of linoleic and oleic made up about 89.39-91.01% of the total fatty acids in all samples analyzed (Table 2). Linoleic acid was the most abundant fatty acid which comprised 75.81-77.86% of the total fatty acids. According to Table 3, there was an inverse relationship between linoleic and oleic acid content in all genotypes ($r = -0.616$, $p<0.01$). The highest linoleic acid (77.86%) was found in the Bacum92 genotypes. On the other hand, the lowest value for oleic acid content (12.57%) was obtained for this genotype too (Table 2). The results are in agreement with the findings by Gecgel *et al*., (2007), who revealed that the highest amount of linoleic acid was found in the lowest oleic acid content. Palmitic acid was the principal saturated fatty acid followed by stearic acid and these fatty acids together comprised about 8.35-9.46% of the total fatty acids. Palmitic acid content varied between 6.09-7.07% and the highest values for the palmitic acid content were determined in Isfahan14 genotype. Also, the palmitic acid was negatively associated with linoleic acid ($r = -0.858$, $p<0.01$). Stearic acid content ranged from 2.17-2.62% and Bacum92 demonstrated higher mean than the other genotypes. The results showed that a negligible amount of linolenic acid ($C_{18:3}$) was detected (0.14 – 0.25%) and It was observed that there was a positive correlation between linolenic acid and linoleic acid. ($r = +0.474$, $p<0.01$). These finding were in accordance with that reported by Gecgel *et al*., (2007). The ratio of unsaturated to total fatty acids (U/T) varied from 0.90 to 0.91 (Table 2). The ratio of unsaturated to saturated fatty acids (U/S) varied from 9.44 to 10.86 (Table 2). The oil stability ($C_{18:1}/C_{18:2}$) of samples varied from 0.15 to 0.17 and there was an inverse relationship ($r = -0.758$, $p<0.01$) between linoleic acid and oil stability while observed significant positive correlation ($r = +0.980$, $p<0.01$) between oleic acid and oil stability and palmitic acid and oil stability ($r = +0.383$, $p<0.01$). (Table 3). These results are in agreement with those of similar studies on the fatty acid composition of safflower oils reported in the literature (Arslan, 2007; Carvalho *et al*., 2006; Cosge *et al*., 2007; Garcia-Martinez *et al*., 2007; Gecgel *et al*., 2007; Lee *et al*., 2004).

The quality of safflower oil is high due to its fatty acids composition. As known, the fatty acid composition of vegetable oil is a main factor affecting its commercial uses and it influenced by a lot of factors (Cosge *et al*., 2007; Gecgel *et al*., 2007). Genotype during oil formation exert the major effect on the proportions of oleic and linoleic acids. Safflower normally produces a seed oil rich in linoleic acid which is being utilized as premium edible oil in view of its role in reducing blood cholesterol levels while linoleic content was negligible. High-oleic safflower oil because of its bland flavor and remarkable stability on heating is excellent frying oil (Gecgel *et al*., 2007). The seed oil of all four genotypes was rich in the polyunsaturated essential fatty acids (PUEFA) linoleic acid which makes the oil nutritionally and therapeutically valuable for human consumption, because it is essential for normal growth, health promotion, and disease prevention such as coronary heart diseases, atherosclerosis and high blood pressure (Han *et al*., 2009; Sabzalian *et al*., 2008). Our findings from the present study indicated that the oil content of seed and fatty acid composition of safflower have been affected significantly by the genotypes and the oil content and fatty acid profiles of oil in all

four genotypes were different. Genotype such as Isfahan14 which has the high oil content and high oil quality is the most useful cultivar for future commercial production in the region. Safflower is a rich source of many important nutrients such as essential polyunsaturated fatty acid and lipid soluble bioactive which have a very positive effect on human health. So the production of oil from safflower seed for industrial applications provides the use of a renewable resource, and at the same time adding value to agricultural products.

Table 2: Means of oil content (%) and fatty acid compositions (% of total) of four genotypes of safflower

	Isfahan14	Bacum92	Goldasht	Mahalie Ajabshir	LSD
Oil content	34.39[a]	29.68[b]	22.16[d]	26.22[c]	1.46
$C_{16:0}$	7.07[a]	6.16[b]	6.17[b] 6.09[b]	0.32	
$C_{18:0}$	2.39[b]	2.62[a]	2.17[c] 2.37[b]	0.14	
$C_{18:1}$	13.58[a]	12.57[b]	13.75[a]	13.53[a]	0.28
$C_{18:2}$	75.81[d]	77.86[a]	76.99[c]	77.48[b]	0.36
$C_{18:3}$	0.14[c]	0.21[ab]	0.25[a]	0.17[bc]	0.06
Total[a]	98.86[d]	99.21[b]	99.08[c]	99.47[a]	0.10
Others[b]	0.81[a]	0.51[c]	0.65[b]	0.34[d]	0.06
SAFA[c]	9.46[a]	8.79[b]	8.35[c]	8.46[c]	0.21
USFA[d]	89.39[c]	90.43[b]	90.74[a]	91.01[a]	0.29
U/T[e]	0.90[a]	0.91[a]	0.91[a]	0.91[a]	0.06
U/S[f]	9.44[c]	10.28[b]	10.86[a]	10.75[a]	0.28
Oil stability ($C_{18:1}/C_{18:2}$)	0.17[a]	0.15[a]	0.17[a]	0.17[a]	0.06

* In each raw, means with the same superscript letter were not significantly different

[a] Sum of stearic ($C_{16:0}$), palmitic ($C_{18:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids

[b] Sum of myristic ($C_{14:0}$), palmitoleic ($C_{16:1}$), arachidic ($C_{20:0}$) and behenic ($C_{22:0}$) acids

[c] Sum of Saturated fatty acids of stearic ($C_{16:0}$), palmitic ($C_{18:0}$)

[d] Sum of unsaturated fatty acids of oleic ($C_{18:1}$) and linoleic ($C_{18:2}$)

[e] The ratio of unsaturated to total fatty acids

[f] The ratio of unsaturated to saturated fatty acids

Table 3: Correlation coefficient between the oil content and fatty acid compositions of safflower seed

	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	Others	Total	SAFA	USFA	U/T	U/S	Oil	($C_{18:1}$/ $C_{18:2}$)
$C_{16:0}$	1												
$C_{18:0}$	-0.099 ^{ns}	1											
$C_{18:1}$	0.207 ^{ns}	-0.770 **	1										
$C_{18:2}$	-0.858**	0.324 *	-0.616**	1									
$C_{18:3}$	-0.581 **	-0.269 ^{ns}	-0.108 ^{ns}	0.474**	1								
Others	0.759 **	-0.173 ^{ns}	0.308 *	-0.840 **	-0.136 ^{ns}	1							
Total	-0.756 **	0.136 ^{ns}	-0.292 *	0.840 **	0.117 ^{ns}	-0.988**	1						
SAFA	0.920**	0.297*	-0.100 ^{ns}	-0.698**	-0.664 **	0.660 **	-0.673 **	1					
USFA	0.294 *	-0.040 ^{ns}	0.155 ^{ns}	-0.275 *	-0.396 **	0.192 ^{ns}	-0.114 ^{ns}	0.267^{ns}	1				
U/T^{e}	-0.946**	-0.010 ^{ns}	-0.268 ^{ns}	0.896**	0.702**	-0.778 **	0.773 **	-0.914**	-0.306 *	1			
U/S	-0.906**	-0.328*	0.128 ^{ns}	0.682**	0.650 **	-0.663 **	0.676 **	-0.998**	-0.255^{ns}	0.903**	1		
Oil content	0.432**	0.799**	-0.695 **	-0.064 ^{ns}	-0.503 **	0.198 ^{ns}	-0220 ^{ns}	0.728**	0.138 ^{ns}	-0.463**	-0.745**	1	
Stability ($C_{18:1}$/ $C_{18:2}$)	0.383 **	-0.719 **	0.980**	-0.758**	-0.207 ^{ns}	0.464 **	-0.450 **	0.089 ^{ns}	0.197 ^{ns}	-0.444 **	-0.062^{ns}	-0.560**	1

^{ns} not significant

* Significant at 0.05 levels of probability, respectively

** Significant at 0.01 levels of probability, respectively

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