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ORIGINAL ARTICLE

Lipid Composition and Oxidative Stability of Oils in Safflower (*Carthamus Tinctorius* L.) Seed Varieties Grown in Iran

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Mahboobeh Vosoughkia, Mehrdad Ghavamib, Maryam Gharachorloo, Mohammad Sharrifmoghaddasi, Amir Hasan Omidi: Lipid Composition and Oxidative Stability of Oils in Safflower (*Carthamus Tinctorius* L.) Seed Varieties Grown in Iran.

ABSTRACT

The present study is a preliminary evaluation of the total oil, fatty acid, tocopherol and sterol composition and oxidative stability of the oil of four potentially most useful varieties of safflowers (Padideh, Zendehrood, KF72 and Bacum92) that can be grown in Iran. The fatty acid composition of the oils indicated that the predominant fatty acid was linoleic acid (74.84-77.86%) followed by oleic (12.57-15.75%), palmitic (6.16-7.07%) and stearic acid (2.39-2.83%) while trace amounts of myristic, palmitoleic, arachidic, behenic and linolenic fatty acids in oil of different varieties were presented. The sterol marker, β-sitsterol, constituted about 49.16-53.51% of the total sterol content (1248.34-2976.25 mg/kg) followed by Δ7-stigmasterol (17.65-20.19%), campesterol (6.45-14.17%) and stigmasterol (4.78-6.44%). Significant ($p<0.01$) differences were found among four samples for phytosterols. Among the total tocopherol, α-tocopherol was the most abundant and comprised approximately about 94-96% of the total tocopherols amounting to 192.05-439.64 mg/Kg oil and was followed by γ-tocopherol (5.59-14.68 mg/Kg) and δ-tocopherol (3.06-11.50 mg/Kg). The stability of oil varied from 3.41 h to 3.83h and The results showed that safflower seed oil has unique quality and could be used in pharmaceutical, food and cosmetic products.

***Key words*:** *Carthamus tinctorius* L., fatty acids, safflower oil, oxidative stability, sterols, tocopherols.

Introduction

Carthamus tinctorius L commonly named as safflower is one of the world's oldest crops belonging to the Asteraceae family and is actually native to the Middle East. Safflower is a tap rooted annual crop which can tolerate environmental (drought, salinity) stresses [6]. It is a minor underutilized crop with 615,214 tons world production during 2008, respectively. At present India with 225,000 tons production is the major safflower producer in the world, followed by USA, Mexico and Kazakhstan [7]. Iran is one of the richest germplasm of safflower due to its favorable climatic and agricultural conditions [14].

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Traditionally, safflower was mainly grown for its flowers, which were used as dye sources for coloring foods and textiles as well as medicinal purposes. Nowadays, this crop cultivated mainly for its seed, which is used as edible and industrial oils and as birdfeed. The whole safflower seeds (technically an achene) are normally white to cream in appearance and it is made up of 33- 45% tough hull and 55-65% kernel. A mature seed of common types constitutes 27-32% oil, 5-8% moisture, 14-15% protein, 2-7% ash and 32-40% crude fiber [8]. Safflower oil is golden yellow color and has a nutty flavor. In crude safflower oils, triglycerides are the main constituents, making up to about 92-99%. Other constituents like phospholipids (0.4-0.6%), free fatty acids (1-2%), and unsaponifiable matter (0.6%) are present in minor amounts [9]. Interest in cultivating safflower as edible oil (cooking, salad oil, margarine) has been stimulated since it is identification as a rich source of polyunsaturated essential fatty acids (PUEFA) linoleic acid (70-87%) which is claimed to offer diverse nutritional and therapeutic advantages such as prevention and treatment of coronary heart disease, arteriosclerosis, high blood pressure and hyperlipaemia [5,10]. Nonetheless, it is considered high quality edible oil and general concern about this matter made safflower as an important crop for vegetable oil.

This investigation was undertaken to obtain information about the chemical composition and oxidative stability of safflower seed oil of four of the potentially most useful varieties of safflowers that can be cultivated in Iran and to determine the oil composition in terms of fatty acid, tocopherol and sterol contents and to evaluate oil composition-oxidative stability relationship. These data can be important for improvement of oil quality and developing new varieties in safflower breeding programs and in the selection of the most useful varieties for future commercial production in the region.

2. Materials and methods

2.1. Materials:

This study was conducted in 2009 with four different safflower varieties (Padideh, Zendehrood, KF72 and Bacum92) provided by Seed and Plant Improvement Institute in Karaj, Iran as plant materials. The seeds were ground by grinder. All the chemicals used in the analysis were of reagent grade and were used without any further purification.

2.2. Methods:

2.2.1. Oil Extraction and Preservation:

Safflower seeds (50g) were extracted with 150 ml hexane at ambient temperature (20°C) under vigorous horizontal shaking for 4 h in dark flasks. The homogenized mixtures were filtered through defatted filter paper under vacuum, and the residues were washed twice with 50ml hexane; thereafter 35 ml of 6.7% sodium sulfate was added and the solvent of upper layer was removed via a rotary vacuum distillation at 40 °C. The pure oils were stored in dark brown glass bottles at 4 °C until further investigation.

2.2.2. 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, 30 m × 0.32 mm 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.

2.2.3. Tocopherol Analysis:

The tocopherol content was measured by high performance liquid chromatography (HPLC) according to the IUPAC method 2.432 [15]. The seed oil 0.5 g was diluted with 5ml hexane and 20µl sample loop were directly injected in to Acme 9000 (Young Lin, Korea) chromatograph equipped with an UV-VIS detector system. Lichosphere (Merck, Darmstdat, Germany) C_{18} reverse-phase column (RP-

100, 200 mm × 4.6 mm × 5 µm) was used with methanol/ acetonitrile/water (42.5/42.5/5, v/v/v) as a mobile phase with a flow rate of 1 ml/min. The separations were carried out at room temperature. Tocopherols were measured at 295 nm. The peaks of the chromatogram were identified by comparison with the retention times of the respective external standard method (Supelco-Sigma, Aldrich Quımica, Mexico).

2.2.4.Oxidative Stability:

Oxidative stability was measured with a Rancimat 743 instrument Metrohm according to the AOCS Official Method Cd 12b-92 [2]. Clean, dry air was passed through the 3 g sample of the oil with 20 L/h air flow while the oil was warmed to 110°C, respectively and the results were expressed in hours as induction time.

2.2.5. Total Sterols Analysis:

For analysis of sterols, the oil samples (5g) and 5a-cholestane as an internal standard (Supelco-Sigma, Aldrich Quımica, Mexico) were saponified by adding 10 M KOH (5ml) in ethanol and heating for 30 min at 70 °C. After cooling, this solution was mixed with 100 ml of distilled water and the unsaponifiable fraction was extracted three times with 100 ml of diethyl ether. The diethyl ether phase dehydrated over anhydrous sodium sulfate, filtered and evaporated under vacuum at 30 °C and then derivatized to trimethylsilyl (TMS) ether for subsequent analyses by GC. 1µl of samples were injected to a Acme 6000 (Young Lin, Korea) gas chromatograph instrument with a flame-ionization detector (FID) and TRB-5 capillary column (60 mm × 0.32 mm ×0.5 µm, Teknokroma) was used. A carrier gas (helium) flow was 1 ml/min. The column was held at 280 °C for 10 min and the temperatures of the injector and detector were 300°C and 320 °C, respectively.

2.2.6. Statistical Analysis:

Each treatment was analyzed as randomized complete block design with three replications and the data were assessed by analysis of variance (ANOVA) and Duncan s multiple range test using MSTAT-C software program. Differences among treatments were tested with least significant difference (LSD) test ($P<0.05$). Besides, correlation analyses were preformed to clarify the relations among parameters considered in this study.

3. Results and Discussion

3.1. Fatty Acid Composition:

The quality and nutritional properties of safflower oil, like most fats and oils, are high due to its chemical characteristics, in particular its fatty acid 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 such as genotype of the variety, environmental conditions, etc [8]. The results of variance analysis for fatty acid (Table 1) revealed significant differences among 4 varieties of safflower ($P<0.01$). The unsaturated fatty acids of linoleic (74.84-77.86%) and oleic (12.57-15.75%) and the saturated fatty acids of palmitic (6.16-7.07%) and stearic (2.39-2.83%) were the most abundant fatty acids in respecting decreasing order, which together composed about 98.86-99.42% of the total fatty acids. A negligible amount of linolenic acid was detected (less than 0.21%) and 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). These results are comparable to data previously reported in the literature by Sabzalian *et al*., [16]. There was an inverse correlation between linoleic and oleic acid content. Similar observations were made by Gecgel *et al*., [8], who revealed that the highest amount of linoleic acid was found in the lowest oleic acid content. Compared to most other common edible oils, relatively safflower oil contains the highest level of the linoleic acid, an essential fatty acid, which is make it as premium edible oil, because of its nutritional advantages and potential therapeutically properties in the prevention of coronary heart disease and cancer but the presence of the large amounts of linoleic acid makes the oil quite sensitive to oxidation [12].

3.2. Tocopherol Composition:

Tocopherols, normally known as vitamin E, are a group of lipid soluble compounds which naturally occurring in oilseeds in four various forms (α-, β-, γ- and δ-tocopherol). Tocopherols are valued for their vitamin E activity and their antioxidant properties to protect polyunsaturated fatty acids against oxidation thus the level of tocopherol in seed oils are extremely important. The nature and content of tocopherol is characteristic and relies on the geographical area, plant cultivar, seasonal effects, the quantity of polyunsaturated fatty acid, oil processing, and storage conditions [13]. The four varieties analyzed in this study contained 201 to 465 mg/kg tocols and the results of variance analysis for tocopherol content revealed significant differences among samples ($P<0.01$) (Table 2). Among the tocopherols identified, α-tocopherol was the most abundant and comprised approximately 94–96% of the total tocopherols amounting to 192.05 - 439. 64

Table 1: Fatty acid composition (% of total) of oil extracted from safflower varieties grown in Iran.

Fatty acid	Padideh	Zendehrood	KF72	Bacum92	LSD
C16:0	6.26[b]*	7.07[a]	6.84[a]	6.16[b]	0.26
C18:0	2.44[c]	2.39[c]	2.83[a]	2.62[b]	0.12
C18:1	15.75 [a]	13.58[b]	13.27 [c]	12.57[d]	0.24
C18:2	74.84 [d]	75.81[c]	76.47 [b]	77.86[a]	0.38
C18:3	0.17[ab]	0.14[b]	0.19[ab]	0.21[a]	0.06
Others[a]	0.41[c]	0.81[a]	0.35[c]	0.51[b]	0.06

* In each raw, means with the same superscript letter were not significantly different.
[a] Sum of myristic (C14:0), palmitoleic (C16:1), arachidic (C20:0) and behenic (C22:0) acids.
[b] The ratio of unsaturated to total fatty acids.

Table 2: Oxidative stability, measured by the Rancimat system, of oil extracted from safflower varieties grown in Iran.

	Padideh	Zendehrood	KF72	Bacum92	LSD
Oxidative stability (h)	3.74[ab]	3.83[a]	3.65[b]	3.41[c]	0.14

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

mg/Kg oil (Table 2). α-tocopherol is recommended for human consumption and it has been suggested to possess a higher biologically activity than other tocopherols. The α-tocopherol contents of the samples were lower than the values reported earlier by Bozan and Temelli, (2000). Only trace amounts of γ-tocopherol and δ-tocopherol were present and values of them ranged from 5.59-14.68 and 3.06-11.50 mg/Kg, respectively. δ-tocopherol were not detected in previously data published by Bozan and Temelli, (2000). The present of β-tocopherol and tocotrienols reported earlier were not confirmed in the present study. These variations can be attributed to differences in the method of lipid extraction and genetic variations (plant cultivar, variety grown). There was a positive relationship between δ-tocopherol content and oxidative stability of oil. The highest level of δ-tocopherol in Zendehrood variety may be the main factor contributing to the highest oxidative stability of it, because δ-tocopherol has the most antioxidant activity among tocopherols homologues. Hence, consumption safflower seed oils, which contain Vitamin E that protect the body's cells from free radical damage, provide potential health benefits.

3.3. Oxidative Stability:

Oxidative stability is a paramount parameter in assessing the sensory and nutritive quality of the oils and fats which reflects their susceptibility to oxidative degeneration and it is influenced by the present of unsaturated fatty acids and bioactive constituents such as sterols and tocopherols. Lipid oxidation causing reduction of the nutritive value and functional properties of food products (Bozan and Temelli, 2000). The results of Rancimat test are shown in table 3. Stability of the oils analyzed, expressed as the oxidation induction time, ranged from 3.41 h to 3.83h. The oil extracted from Zendehrood proved to be significantly more stable in an oxidation test than any of the other safflower cultivars. The source of these variations between oils might be due to the different amounts of unsaturated fatty acids and minor constituents such as sterols and tocopherols. According to these results and published data (Bozan and Temelli, 2000), Iranian safflower oils are most stable to oxidation and it might be due to the present of δ-tocopherol which has been suggested to possess a higher antioxidant capacity as compared to the other tocopherol homologues and was not detected in previously data published.

3.4. Sterol Composition:

Another group of minor constituents of vegetable oil are Sterols, which comprise a major portion of the unsaponifiable fraction in most vegetable oils and are widely used to characterize of oils or to detect adulterations [17]. Sterols are very important for human health and nutrition because of their biological properties related to reduce serum total and LDL cholesterol levels. Due to a correlation between Sterols and health benefit, the content of them in foods has received much attention [1]. The results of sterol analysis are given in Table 4. The four varieties analyzed in this study contained 1248 to 2976 mg/kg sterols and the results of variance analysis for sterols content revealed significant differences among samples ($P<0.01$). β-sitosterol was the dominant phytosterol (49.16-53.51% of the total sterol content), followed by Δ7-stigmasterol (17.65-20.19%), Campesterol (6.45-14.17%) and stigmasterol (4.78-6.44%). Other sterols were present in smaller quantities. β-sitosterol content of samples was lower than the values (52-57%) given by Itoh *et al.,* [11] for Japanese safflower oil. Analysis of these results showed that a significant correlation between Δ7-stigmasterol content and linoleic acid content exists. These results are comparable to data previously reported in the literature[3].

4. Conclusions:

This study revealed that Zendehrood variety had the highest level of tocopherol and was more stable in the Rancimat test and it is the most useful variety for future commercial production in the region.

Table 3: Tocopherol composition and content (mg/kg) in safflower seed oils grown in Iran.

Tocopherol	Padideh	Zendehrood	KF72	Bacum92	LSD
α -Tocopherol	307.81[b*]	439.64[a]	192.05[d]	216.30[c]	0.20
β -Tocopherol	ND[**]	ND	ND	ND	-
γ -Tocopherol	8.48[b]	14.68[a]	5.59[c]	5.60[c]	0.10
δ -Tocopherol	5.15[b]	11.50[a]	4.15[c]	3.06[d]	0.06
α- Tocotrienol	ND	ND	ND	ND	-
β - Tocotrienol	ND	ND	ND	ND	-
γ- Tocotrienol	ND	ND	ND	ND	-
δ - Tocotrienol	ND	ND	ND	ND	-
Total Tocopherols	321.48[b]	465.89[a]	201.84[d]	225.00[c]	0.23
α /T [a]	95.47[b]	94.36[d]	95.14[c]	96.13[a]	0.06
δ/T [b] 1.60[c]	2.46[a]	2.05[b]	1.35[d]	0.06	

[a] The ratio of α-Tocopherol to Total Tocopherols
[b] The ratio of δ -Tocopherol to Total Tocopherols
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Table 4: Sterol composition (%) and content (mg/kg) in safflower seed oils grown in Iran.

Sterol	Padideh	Zendehrood	KF72	Bacum92	LSD
Cholesterol	0.08[c*]	0.63[a]	0.17[b]	0.2[b]	0.06
Campesterol	14.17[a]	10.98[c]	11.25[b]	9.45[d]	0.08
Stigmasterol	6.44[a]	4.78[d]	6.28[b]	5.26[c]	0.12
β-Sitosterol	50.57[b]	49.90[c]	53.51[a]	49.16[d]	0.18
5-Avenasterol	1.59[c]	3.24[b]	3.29[b]	4.54[a]	0.08
Δ7-Stigmasterol	17.65[d]	18.37[c]	19.03[b]	20.19[a]	0.16
Δ7-Avenasterol	3.50[c]	5.25[b]	3.22[d]	5.97[a]	0.12
Other sterols	5.98[b]	6.78[a]	3.24[d]	5.23[c]	0.12
Total sterols (mg/Kg)	2976.25[a]	2791.79[b]	1248/34[d]	2154.22[c]	2.72

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

The oil obtained from the seeds of four safflower varieties being relatively a rich source of various important functional nutrients such as essentials PUFAs and the lipid-soluble bioactive constituents (tocols and sterols) that appear to have a very positive effect on human health. From a nutritional point of view, the safflower seed oil has unique quality and may possess many potential health benefits because of its high level of linoleic acid, phytosterol (β-sitosterol) and α–tocopherol, the biologically active isomer of vitamin E, and safflower oil was also found to be effective in reducing the risk of coronary heart disease by lowering plasma total and bad LDL cholesterol levels. Thus safflower oil can be considered as a functional food additive and nutraceuticals and can be serves as the base for a number of medicines. In recent years, there has been a surge of interest in safflower seed oils because they represent an important base for the health and nutrition industry.

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Refrences

1. Abidi, S.L., 2001. Chromatographic analysis of plant sterols in foods and vegetable oils. J. Chromatogr. A. 935, 173-201.
2. AOCS, 1993. Official methods and recommended practices of the American Oil Chemists' Society, AOCS Press, Champaign, U.S.A.
3. Breck, G.S., S.C. Bahatia, 2008. Handbook of Industrial Oil and Fat Products, first Ed. CBS Publishers.
4. Camas, N., C. Cirak, E. Esendal, 2007. Seed yield, oil content and fatty acid composition of some safflower (*Carthamus tinctorius* L.) grown in Northern Turkey condition. J Fac Agri. 22: 98-104.
5. Cosge, B., B. Gurbuz, M. Kiralan, 2007. Oil content and fatty acid composition of some (*Carthamus tinctorius* L.) varieties sown in spring and winter. Int J Nat Eng Sci., 1: 11-15.
6. Dordas, C.A., C. Sioulas, 2009. Dry matter and nitrogen accumulation, partitioning, and retranslocation in safflower (*Carthamus tinctorius* L.) as affected by nitrogen fertilization. Field Crops Res., 110: 35-43.
7. FAO, 2010. http://www.faostat.fao.org.
8. Gecgel, U., M. Demirci, E. Esendal, M. Tasan, 2007. Fatty acid composition of the oil from developing seeds of different varieties of safflower (*Carthamus tinctorius* L.). J Am Oil Chem Soc., 84: 47-54.
9. Ham, W. and R.H. Hamilton, 2000. Edible Oil Processing. CRC Press, 281.
10. Han, X., L. Cheng, R. Zhang, J. Bi, 2009. Extraction of safflower seed oil by supercritical CO_2. J Food Eng., 92: 370-376.
11. Itoh, T., T. Tamura, T. Matsumoto, 1973. Sterol Composition of 19 Vegetable Oils. J Am Oil Chem Soc. 50: 122-125.
12. Oomah, D.B., S. Ladet, V.D. Godfrey, j. Liang, B. Giarard, 2000. Characteristics of raspberry (*Rubus idaeus* L.) seed oil. J. Food Chem., 69: 187-193.

13. Ortega-García, J., N. Gámez-Meza, J.A. Noriega-Rodriguez, O. Dennis-Quiñonez, H.S. García-Galindo, J.O. Angulo-Guerrero, L.A. Medina-Juárez, 2006. Refining of high oleic safflower oil: Effect on the sterols and tocopherols content. Eur Food Res Technol., 223: 775-779.
14. Pahlavani, M.H., 2005. Some technological and morphological characteristics of safflower (*Carthamus tinctorius* L.) from Iran. Asian J Plant Sci., 4: 234-237.
15. Paquot, C., A. Haufnne, 1992. International Union of Pure and Applied Chemistry (IUPAC) Standard Methods for Analysis of Oils, Fats and Derivatives, Method 2.432. Determination of tocopherols by HPLC, 7th edn. Blackwell. Oxford.
16. Sabzalian, M.R., G. Saeidi, A. Mirloh, 2008. Oil content and fatty acid composition in seeds of three safflower species. J Am Oil Chem, Soc., 85: 717-721.
17. Salvador, M.D., F. Aranda, S. G?mez-Alonso, G. Fregapane, 2001. Cornicabra virgine olive oil: a study of five crops seasons. C omposition, quality and oxidative stability. Food Chem., 74: 267-274.