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Evaluation of the Non Saponifiable Matter of Oils Extracted from Nine Varieties of Sunflower Seeds

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ABSTRACT: In this study the non-saponifiable matter of oils extracted from nine varieties of sunflower seed were subjected a series of chemical tests. The non-saponifiable matters were fractionated into a number of chemical classes of compounds namely sterols, 4 methyl sterols, triterpene alcohols, tocopherols and hydrocarbons. The two major and important classes of compounds consisting of sterols and tocopherols were evaluated qualitatively and quantitatively. The results indicated that α-tocopherol and β-sitosterol were the predominant tocopherol and sterol respectively. Zaria variety was evaluated as the superior variety in respect of tocopherol concentration and Lacumca variety in respect of sterol concentration.

Keywords: *Nonsaponifiable Matter, Sunflower, Sterol, Tocopherol.*

Introduction

Sunflower seed (Helianthus annuus) is a type of plant that belongs to Chicory type plant sunflower. This plant is a member of compound plant families, and it is a two-cotyledon and one-year-old plant. Helianthus plant has 67 varieties that exist in America.

H. annuus and H. Tuberousous species are grown as edible plants and some species are used as ornamental plants, while others are wild species. Sunflower seeds are found in white and gray stripes, brown, dark purple and black colors (Gerami, 1998).

Sunflower seed is one of the four major oil seeds planted all over the world in large quantities. Sunflower is a nourishing seed that contains 40% oil (Rajiv *et al*., 2009).

This crop contains 45 mg of α tocopherol in each 100 gr of oil, that has the highest level of α tocopherol in comparison with other varieties of vegetable oils. Thus in addition to good antioxidant properties, it contains a high level of vitamin E (Ghiassi Tarzi *et al*., 2006).

Up to August 2009, sunflower cultivation in Russia indicated a growth of over 0.15 million hectares a year. Ukraine, the second producer of sunflower, had the cultivation growth of 1.4 million hectares a year, up to October 1st, 2009. Argentina was introduced as the third main sunflower seed producer in 2009. These countries produced the total sum of 276000 and 349000 tons of sunflower seeds in August and July 2009, respectively (Bnunam, 2009).

From September 2008 to August 2009, the total export of sunflower by the three main countries of Ukraine, Russia and Argentina rose to 4.03 million tones that is equivalent to 41% growth (Bnunam, 2009). In 2006 to 2007, the amount of sunflower

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seed production in Iran was around 19000 tones and this figure increased to 30000 tons in 2008 and 2009, that indicates a turning point in production of sunflower in the country (No and Hamzeh, 2009).

Sunflower has an important role in oil production throughout the world and provides almost 12% of the world's oil production (Bernwieser and Sontag, 1992; Hui, 1996). The amount of oil in fully developed sunflower seed consists of triglyceride (97%) along with some phospholipid and glycolipid (Karimzadeh, 2001). The mixture of fatty acids affects the oil behavior and quality (Cattivelli *et al*., 1990). The oil quality is mainly determined by oleic, linoleic acids ratios (Bruckner and Frobery, 1987; William and English, 1978).

The free fatty acids of sunflower seed oil is used in production of soaps and sunflower wax might be employed as supplementary to animal feed (Guilllermo *et al*., 1999; Hui, 1996).

Negar Tabatabie *et al* (2008) analysed the heat resistance and oxidation of sunflower oil (Tabatabaei, N *et al*., 2008).

Another class of compound present in sunflower seed oil is the non-saponifiable matter that includes tocopherols and sterols, where tocopherols are used as potent antioxidants and sterols are utilized as raw material in order to produce stroeids in the pharmaceutical industry (Giti raven, 2001).

Studies concerned with sunflower seed oils revealed the presence of 0.75-1.5% nonsaponifiable matters (Rose *et al*., 2009). Hui reported the percentage of nonsaponifiable matters in raw sunflower oil to be almost 1.5% (Hui, 1996).

Tocopherol concentrations in various sunflower seed oils have been reported throughout the world. The concentration is significantly affected by the geographical situation and regional weather condition of the area where the oil seeds are cultivated. Warner et al. studied 66 different varieties of sunflower seeds from different parts of America, and reported the total tocopherol content of 440-1520 ppm in typical sunflower seed oil and 509-741 ppm in sunflower seed oil with mediate oleic acid and 450-1120 ppm in sunflower seed oil with high oleic acid. Tocopherols have antioxidant effects in addition to having Vitamin E activity. α tocopherol has the most vitamin E property and Delta and γ tocopherols have the highest antioxidant activities. α tocopherol might be regarded as an indicator in sunflower seed oils where the concentration is quite high (Warner *et al*., 2003; Ghavami *et al*., 2008).

The amount of sterols in vegetable oil is highly important since they can be used as an indicator to identity the origin of oil and fat in question apart from their other characteristics. Warner et al. identified the amount of β-sitosterol in different sunflower seed oils (Warner *et al*., 2003).

In most vegetable oils, the sterol concentration constitutes an important percentage of the non-saponifiable matter and also the amount and the type of sterols are dependent upon the vegetable oil characteristics. In a study Rosa et al. identified the concentration β-sitosterol in sunflower seed oil and regarded it as an indicator (Rosa *et al*., 2009).

In a research on sunflower seed oil sterol, it was reported that β-sitosterol had the maximum concentration of sterol in the presence of campesterol, stigma sterol, Δ7-stigmasitenol and Δ7-avenasterol (Sharma *et al*., 1990).

Therefore the aim of this study is to evaluate the nonsaponifiable matter and the two major fractions namely the tocopherols and sterols in nine varieties of sunflower seed oils.

Materials and Methods

In order to conduct this research, the seeds were obtained from Karaj supply Seed Institute. The nine varieties of sunflower seeds consisted of Progress, Zaria,

Favourate, Azar Gol, Alstar, Armavirski, Youroflower, Lacumca and Master. Table 1 present the characteristics of these varieties.

Table 1. Some characteristics of new sunflower seed varieties

Varieties	Origion	Growth group
Zaria	Yugoslavia	Serotinal
Favourate	Russia	Medium growth
Azar Gol	Iran	Serotinal
Progress	Yugoslavia	Serotinal
Alstar	Russia	Medium growth
Armavirski	Russia	Serotinal
Lacumca	Russia	Medium growth
Master	Rumanian	Medium growth

Source: Karaj Supply seed Institute

The oil was extracted using soxhlet procedure and the nonsaponifiable matter was isolated by alcoholic potassium hydroxide saponification followed by the extraction with ether according to the method described by Ghavami *et al.* (2008). The nonsaponifiable matter was fractionated on a thin layer chromatography plate covered with 0.5 mm thickness of silica Gel G type 60 into a numbers of chemical classes of compounds using hexane: ether (9:1) as developing solvents and finally spraying the plates with 0.01% rhodamin 6G in ethanol to observe the plate under U.V according to *Ghavami et al.* (2008). The sterols and tocopherols were qualitatively and quantitatively identified according to the methods described by *Ghavami et al.* (2008).

The MSTAT-C software was used to analyse the results. A quite randomized plan was used for this object and for the analysis of the statistical results. As other statistical plans, analysis of variance (ANOVA) was employed and the Duncan test in 5 percent possibility was used to clarify the significant differences.

Results and Discussion

Non soaponifiable materials are composed of groups of chemical compounds existing in oils and fats that are not saponifiabled with alcoholic potassium hydroxide; yet they are dissolved in typical solvents, oils and fats. Since these materials contain valuable compounds such as sterols and tocopherols, they might stabilise the oil against oxidation. According to Table 2, the most and the least amounts of nonsoaponifiable matters are present in Azar Gol (1.64%) and Armavirski varieties (1.08%), and there is a significant statistical difference in 1% level among all varieties.

As shown in Table 2, the highest concentration of total tocopherols is present in Zaria (838.1 ppm) and the least corresponds to Azar Gol variety (140.9 ppm), and there are significant statistical differences among all the varieties. According to Table 2, the highest amount of α tocopherol is associated with Zaria variety (792 ppm), and the lowest is related to Azar Gol variety (133.9 ppm). γ and β tocopherols constitute a small and minute fraction of the total tocopherols.

α-Tocopherol protects the unsaturated oil against oxidation and although the potency is not as much as γ and δ-tocopherols but in term of vitamin E activity it has more potential.

The highest quantity of sterol was present in Lacumca variety (4425 ppm), and the least amount was related to Armavirski variety (2124 ppm). As shown in Table 3, cholesterol is almost absent in all the varieties examined. β -sitosterol constituted the highest concentration of sterols present.

Campesterol and Stigmasterol are present and contribute a considerable fraction to this class of compounds. However it is the rare sterols namely Δ5-avenasterolis and Δ7-stigmasterol that have not only a considerable share but also are quite important to be used as a tool to identify the origin of the oils or fats. Sometime in the past it was important to identify the fatty acid composition of oils in order to

understand its origin but now due to some adulterations and frauds fatty acid determinations cannot be used as the only tool. Therefore qualitative and quantitative determinations of sterols and possibly their ratio could be used to identify firmly the origin of the oil in question. Tocopherols might also be considered and serve this purpose as an alternative method to some point.

The highest concentrations of Δ7-stigmasterol and Δ5-avenasterolis were present in Alstar and Favourate varieties respectively.

Conclusion

Oils extracted from different varieties of sunflower seeds were analysed in term of their non-saponifiable matter contents, tocopherols and sterols, both qualitatively and quantitatively. It was concluded that the oils with differences in their sterol and tocopherol contents and compositions are rich sources of vitamin E and apart from the

Table 2. Concentrations of the nonsaponifiable and tocopherols in different varieties of sunflower seed oil

Samples	Non saponifiable (%)	Total tocopherol (ppm)	α-tocopherol (ppm)	γ and β - tocopherol (ppm)
Progress	1.52	247.4	237.1	10.3
Zaria	1.46	838.1	792.0	46.0
Favourate	1.56	238.2	227.2	11.0
Azar Gol	1.64	140.9	133.9	7.0
Alstar	1.38	168.6	168.6	-
Lacumca	1.38	462.1	423.7	23.9
Armavirski	1.08	312.5	297.9	29.1
Youroflower	1.18	818.8	748.6	49.8
Master	1.13	565.5	512.1	28.9

Table 3. Concentrations of sterols (as % of total sterol) in different varieties of sunflower seed oils

Sterols	Lacumka	Youroflower	Armavirski	Alstar	Azar Gol	Favourate	Zaria	Progress	Master
β-SitoSterol	64.0	69.2	66.1	55.7	61.1	60.6	60.2	62.1	62.5
Campesterol	10.0	10.3	11.2	9.8	12.9	9.7	11.0	12.1	10.9
Cholesterol	0.2	0.31	0.2	0.3	0.3	0.5	0.2	0.3	0.2
Stigmasterol	9.8	9.0	9.4	10.1	9.4	8.4	9.2	8.6	8.9
Δ5-Avenasterol	5.5	3.2	3.1	4.4	2.9	6.1	5.4	4.1	4.0
Δ7-Stigmasterol	5.6	3.0	2.8	10.4	8.8	9.7	8.1	6.7	8.5
Δ7-Avenasterolis	0.8	0.2	0.3	3.9	1.9	3.4	3.1	1.8	0.6
Othre sterols	1.2	1.9	1.2	5.7	2.7	1.5	2.7	4.3	1.2

presence of β-sitosterol that is the major sterol contains a considerable quantity of Δ7-stigmasterol that might be used as a marker in the identification of the oil.

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