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Qualitative and Quantitative Evaluation of Tocopherols and Phytosterols in Soybean Oil Distillate

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ABSTRACT: Soyabean might be considered as the most popular oil seed crop in the world and the extracted oil has gained it is popularity due to it is application in formulated food products as well as by itself as oil or hardened form as frying or cooking media. Soyabean oil has been subjected to various refining stages in particular deodorisation where different classes of compounds are collected in the received distillate. A considered quality of sterol and tocopherol fractions are collected in this fraction in the same order as are present in the crude oil. The collected fractions indicated that β–sitosterol among the class of sterols and γ and δ tocopherols among the class of tocopherols were the predominant compounds present. Therefore two mentioned classes of compounds individually or together might be considered valuable products to be used in the food formulations.

Keywords: *Nonsaponifiable Matter, Sterols, Tocopherols*.

Introduction

Soyabeans now dominate and probably will continue to dominate world protein and oil market in spite of the competition from other oil seeds. The reason for such dominance includes factors as favourable agronomic characteristics, reasonable return to the farmer and processor, high quality protein meal for animal feed, high quality protein concentrates and isolates in food formulation for human consumption and high qualify edible oil products. The popularity and success of soyabean is not only due to its oil content but also to its high yield of high quality protein meal. Soyabean oil contributes a major share of world markets where it is sold as salad, cooking oils, shortening and margarine. Soyabean oil has both advantages and disadvantages when compared to other vegetable oils. The oil might be hydrogenated to some extend to make it more suitable for certain products and more resistant to oxidative changes. The phosphatides are present in relatively large amounts and are removed by processing due to their unwanted presence in the oil that might cause problems during soyabean oil processing and further use and application, however these compounds are quite valuable as emulsifiers and other functional properties they process. Naturally occurring components namely tocopherols and sterols or sterol esters that might function as antioxidants are present in the nonsaponifiable

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matter of extracted crude oils. These compounds are removed partially during refining operations particularly in the deodorization process and are accumulated in the distillate. Therefore distillate might be considered as a rich source of naturally occurring substances that are valuable and might be employed in various food and pharmaceutical formulations and applications. Distillate is the side product of deodorisation and consists of tocopherols, tocotrienols, squalene, sterols, free fatty acids, aldehyds, alcohols, and ketones (Mendes *et al*., 2002). Since the distillate in this research work is concerned with soyabean oil, therefore the sterols and tocopherols of soyabean oil are present in the distillate. β-sitosterols, stigmasterol, campesterol and γ-tocopherol, δ-tocopherol and α-tocopherol, are the respective sterols and tocopherols present. Economical value of the distillate is related to its sterol and tocopherol contents (Fernandes and Cabral, 2007).

Batistella and Maciel (1998) used molecular distillation to recover tocopherols and sterols from the distillate and found that the separation was difficult due to the boiling range of these compounds present. In a study concerned with the free fatty acid and tocopherol contents of distillate from soyabean oil, Martins *et al.* (2004) were able to separate free fatty acids by molecular distillation and separate the acids and recover tocopherols considerably.

Ito *et al.* (2007) also investigated the molecular distillation of tocopherols from distillate and believed that molecular distillation has a high potential for tocopherols recovery.

Wan *et al.* (2008) by using column chromatography tried to separate tocopherols from soyabean oil distillate. Cyclohexane and ethanol were the solvents employed and their results indicated that tocopherols with high purity was recovered.

Yang *et al* (2010), studied the recovery of phytosterols from soyabean oil distillate by crystallisation.

The results indicated a good recovery with the purity of 92 to 97 percent. Since both sterols and tocopherols are considered important compounds to promote and improve the stabilising and functional properties of certain products, it is the aim of this research work to identity and quantify the concentrations of these products in the oil distillate particularly soyabean oil that is considered a rich source of tocopherols and plant sterols.

Materials and Methods

Soyabean oil distillates were obtained from Behshahr Edible Oil Industries, Tehran. stigmasterol, β–sitosterol (59% β–sitosterol, 41% campesterol) were purchased from Sigma of London DL, α, γ and δ-tocopherols were supplied by Roche Ltd.

- Isolation of nonsaponifiable matter

The nonsaponifiable matters of the oils and distillates were isolated by ethanolic alkalli saponification followed by the extraction of the nonsaponifiable matter with diethyl ether according to Ghavami *et al.* (2008).

- Fractionation of the nonsaponifiable matter

20-25mg of the nonsaponifiable matter was spotted along a line on a 20×20 cm thin layer chromatography (TLC) plate covered with 0.5 mm thickness of silica gel G type 60 (Merck). The plate was developed in hexane: ether (4:1) and sprayed with 0.01% rhodamin 6G in ethanol and observed under UV lamp. Different fractions were separated according to Ghavami *et al* (2008) and made ready for application to gas chromatography.

- Qualitative and quantitative determinations of tocopherols

Tocopherols were identified and quantified according to AOCS method number CE 8-89 using YOUNGLIN Acme

9000 HPLC equipped with Rstech Hector – Msilica column and flourecence detector (jascofp-4025) where the mobile phase was isopropane in hexane.

- *Identification and determination of sterols*

The sterol fraction was isolated and injected to an Agilent 6890 gas chromatography equipped with SE 54 capillary column and Flame Ionisation Detector using Helium as the carrier gas. The sterols were identified according to their Relative Retention Time (RRT) as compared to the standards and quantified using α-cholestan as the internal standard.

- *Statistical analysis*

SPSS software and Duncan multiple test was employed to analyse the results and compare the mean values respectively.

Results and Discussion

Table1 presents the sterol and tocopherol contents of soyabean oil before and after deodorisation that was carried out at 260°C under vacuum. The determinations were carried out for two different batches of oils at different periods of processing. The sterol compositions of both batches of soyabean oils have almost stayed the same meaning that distillations of these compounds have been carried out in equal quantities irrespective of different chemical structure. The reduction in total concentrations of sterols have varied for both batches but do not show a significant difference. Here in all the cases β-sitosterol is the predominant sterol followed by campesterol and stigmasterol. Small concentrations of other sterols namely Δ^7-avenasterol, Δ^5-avenasterol, cholesterol, Δ^7-stigmasterol and brassicasterol have been detected. Soyabean oil is a rich source of γ and δ-tocopherols with small conceutration of α-tocopherol. Although tocopherols are good sources of vitamin E, they are excellent sources of antioxidants and their activity to retard the oxidation chain reactions is concentration dependent. The potential activity is at low concentration where γ and δ tocopherols can be considered superior to α although both exhibit variable superiority to the other at different concentrations. Tocopherols are affected by deodorization process and a considerable quantity is removed during this stage of refining.

Table 1. Sterol and tocopherol analysis of soyabean oil before and after deodorization

Name sample	Sample 1		Sample 2	
	Before deodorization	After deodorization	Before deodorization	After deodorization
Cholesterol (% of total sterol)	0.93	1.40	0.15	0.15
Brassicasterol (% of total sterol)	0.12	0.35	ND	ND
Campesterol (% of total sterol)	20.23	19.16	21.27	19.42
Stigmasterol (% of total sterol)	19.16	18.51	19.61	18.88
β –Sitosterol (% of total sterol)	50.48	51.72	51.64	53.13
Δ5- Avenasterol (% of total sterol)	2.41	2.25	2.89	3.31
Δ7-Stigmasterol (% of total sterol)	0.35	1.02	ND	1.75
Δ7- Avenasterol (% of total sterol)	2.91	4.12	3.71	2.20
Others (% of total sterol)	4.34	2.87	0.88	1.31
Total Sterols (mg/kg)	**1956.19**	**1939.13**	**2357.95**	**2277.86**
Alpha tocopherol (ppm)	19.42	18.18	27.68	18.62
Beta tocopherol (ppm)	ND	ND	ND	ND
Gamma tocopherol (ppm)	782.08	599.17	792.89	582.87
Delta tocopherol (ppm)	380.40	272.11	358.35	242.64
Alpha tocotrienol (ppm)	ND	ND	ND	ND
Delta tocotrienol (ppm)	ND	ND	ND	ND
Gamma tocotrienol (ppm)	ND	ND	ND	ND
Total Tocopherols (ppm)	**1181.90**	**889.46**	**1178.92**	**844.13**

ND: Not determined

Table 2. Sterol and tocopherol analysis of soyabean oil distillate

Name sample	Sample 1	Sample 2
	Distillate 1 of total sterol	Distillate 2 of total sterol
Cholesterol (% of total sterol)	0.02	0.07
Brassicasterol (% of total sterol)	ND	0.13
Campesterol (% of total sterol)	25.66	25.67
Stigmasterol (% of total sterol)	23.81	23.27
β –Sitosterol (% of total sterol)	46.58	46.73
Δ5-Avenasterol (% of total sterol)	1.41	2.12
Δ7- Stigmasterol (% of total sterol)	0.17	0.21
Δ7-Avenasterol (% of total sterol)	1.32	0.51
Others (% of total sterol)	1.05	1.36
Total Sterols (mg/kg)	**4873.13**	**5622.24**
Alpha tocopherol (ppm)	3834.59	6637.62
Beta tocopherol (ppm)	ND	ND
Gamma tocopherol (ppm)	23562.25	30440.52
Delta tocopherol (ppm)	23792.68	28290.50
Alpha tocotrienol (ppm)	ND	ND
Delta tocotrienol (ppm)	ND	ND
Gamma tocotrienol (ppm)	ND	ND
Total Tocopherols (ppm)	**51189.52**	**65368.64**

ND: Not determined

Table 2 presents the composition of collected distillates at different periods concerned with sterols and tocopherols. The composition of distillate apart from the tocopherols and sterols that are the subjects of this study might include 4-methylsterols, triterpene alcohols, triterpene diols, free fatty acids, squalene, aldehydes, ketones and other compounds and fractions that were distilled during the course of refining operations. Collected distillate is a valuable source of both sterols and tocopherols. However there are significant differences between the two obtained distillate in term of quantities of tocopherols and sterols collected. The reason for such difference depends on the volumes and quantities of different batches of oils processed. Separation and purification of these two fractions from other compounds present and their return to food cycle in combination and individually might enhance the stability and quality of the finished products. Therefore distillate might be regarded as a valuable fraction that might be employed in food formulation in order to promote the quality of the food products.

Conclusion

The results of this study indicated that the distillate obtained during deodorisation of soyabean oil can be considered as a valuable source of phytosterols and tocopherols.

Other fractions namely free fatty acids, squalene and some others are present that might be attractive to food processors or other industries. Therefore methods of separation and purification of these compounds present must be studied to obtain an economical and purified product of high quality.

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