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The Effects of Refining Operations on Quality and Quantity of Sterols in Canola, Soyabean and Sunflower Seed Oils

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ABSTRACT: Sterols due to their properties might be considered as valuable products to be employed in food, pharmaceutical and cosmetic industries. Vegetable oils, namely canola, soyabean and sunflower seed oils, contain considerable quantities of phytosterols, mainly β–sitosterol. The aim of this research is to determine the effect of refining operations, particularly deodorization on this valuable class of compounds. Three popular and common vegetable oils were sourced from local refineries and were subjected to neutralization, bleaching and deodorization procedures. The sterols were determined by saponification of the oils followed by extraction of the nonsaponifiable matter, and isolation of sterols by TLC and their final qualitative and quantitative determination by application to gas chromatography apparatus. The results indicated that sterols are lost in neutralization, bleaching and particularly deodorization stages to some extend and some might be collected in the distillate after deodorization. The distillate that is considered as a valuable source of sterols might be purified and used in various applications and industries.

Keywords: *GC, Sterol, Thin Layer Chromatography, Vegetable Oil.*

Introduction

Phytosterols or plant sterols are triterpenes similar to cholesterol, both in structure, given the four-ring steroid nucleus, the 3β-hydroxyl group and often a five or six double bond, as in function, given their role in the stabilization of the phospholipid bilayers in cell membranes. However, cholesterol has a side-chain composed of eight carbon atoms, whereas more common phytosterols have a side-chain composed of nine or ten carbon atoms, out of a total of twenty-eight or twenty-nine carbon atoms. The alkyl side chain may also contain a double bond (Figure 1). More than 100 types of phytosterols have been reported in plant species, which more of them consist of Sitosterol, Stigmasterol and Campesterol. Other relevant phytosterols that can be found in plants in minor amounts are Brassicasterol, Δ 5-Avenasterol, Sitostanol and Campestanol. Sterols in plants exist in the form of free alcohols, fatty-acid esters, steryl glycosides and acylated steryl glycosides (Fernandes & Cabral, 2007).

In edible oils, phytosterols are mainly present in free and esterified forms. Phytosterols play major roles in several areas, namely in pharmaceuticals (production of therapeutic steroids), nutrition (anti-cholesterol additives in functional foods, anti-cancer properties), and cosmetics (creams, lipstick)

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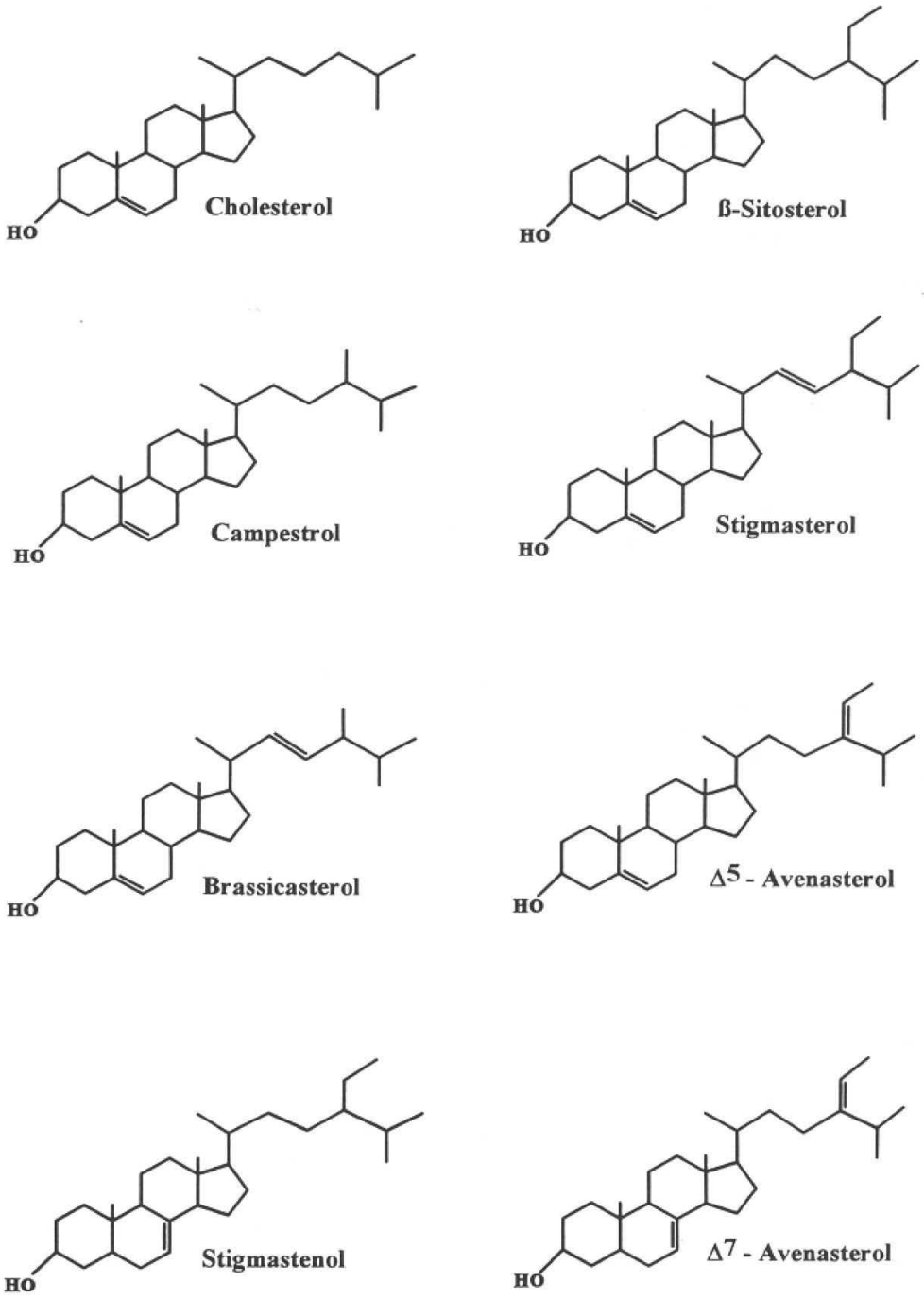

Fig. 1. Structure of some representative phytosterols (Dutta *et al*., 2007)

(Fernandes & Cabral, 2007). Sterols are known to have a wide range of biological activities and physical properties. Phytosterols, in particular, are valuable agricultural products for the health and nutrition industries. They are useful emulsifiers for cosmetic manufacturers and supply the majority of steroidal intermediates and precursors for the production of hormone pharmaceuticals. A number of plant sterol with specific structures are known to inhibit oxidative

deterioration of oils serving as potential anti -polymerization agents for frying oils. Hypocholesterolemia activities of some phytosterols (e.g., soy sterols, vegetable oil components and sitosterol) have been documented. The saturated analogues of phytosterols and their esters have been suggested as effective cholesterol-lowering agents offering cardiologic health benefits (Abidi, 2001). Research on adequate and efficient methodologies for phytosterols recovery from natural sources mostly wastes from vegetable oils and cellulose processing is limited. In recent years, efforts from industry resulted in a significant number of publications reporting better and improved methods for phytosterols recovery and its purification. Such an increase in demand relates to the growing market for phytosterols, particularly given the widespread dissemination of functional foods. (Fernandes & Cabral, 2007).

Each refining processing step has specific functions for removing certain minor components. Refining processes not only removes the unwanted substances but also vitamins, antioxidants and antioxidant precursors (e.g. tocopherols, sterols and EFA's) are removed, decreased or partially destroyed at the same time (El-Mallah *et al*., 2011).

Phytosterols are partly removed during refining, and the magnitude of phytosterols loss largely depends on the refining conditions applied. Accordingly, several factors might contribute to phytosterols loss, including adsorption, partitioning and oxidation. Dehydration also occurs, which results in the formation of stearadienes (El-Mallah *et al*., 2011). During neutralization, in chemical refining, large parts of the phytosterols (9-21%) are transferred by liquid-liquid partitioning to the soapstock (Gutfinger & Letan, 1974; Sciancalepore, 1981; Karaali, 1985). In addition, bleaching and especially high- temperature steam refining or deodorization remove a portion of the sterols (Karabulut *et al*., 2005). Actually, the deodorized distillate is a good source of tocopherols and phytosterols (Gutfinger & Letan, 1974; Verleyen *et al*., 2001).

This study has aimed to investigate the effect of the chemical refining steps namely, neutralization and degumming, bleaching and deodorization on the change and the relation in the oils examined.

Materials and Methods

Canola, soyabean and sunflower seed oils were obtained from Behshahr and Oila refineries. The oils were subjected to alcoholic potassium hydroxide saponification followed by extraction of nonsaponifiable matter with diethyl ether, according to Ghavami *et al*. (2008).

The nonsaponifiable matter was fractionated on thin-layer chromatography in order to separate different classes of compounds as described by Ghavami *et al*. (2008).

The separated sterol fraction was injected to a Young Lin 6500 GC equipped with SE54 capillary column and flame ionization detector. Betulin was used as an internal standard to quantify each sterol. Each sterol was identified as the Relative Retention Time (RRT) of each peak obtained were compared to the standard.

Results and Discussion

Figures 2, 3 and 4 present the fractionation of canola, soyabean and sunflower seed oils nonsaponifiable matters on TLC. As shown and indicated the sterols fraction is the major zone and accounts for almost fifty percent of the total nonsaponifiable matter. Other fractions based on non-polarity are 4-methyl sterols, triterpene alcohols, ɣ - tocopherol, δ-tocopherol, α- tocopherol and the least nonpolar compounds; the hydrocarbons that are located according to relative factors in respective increasing order.

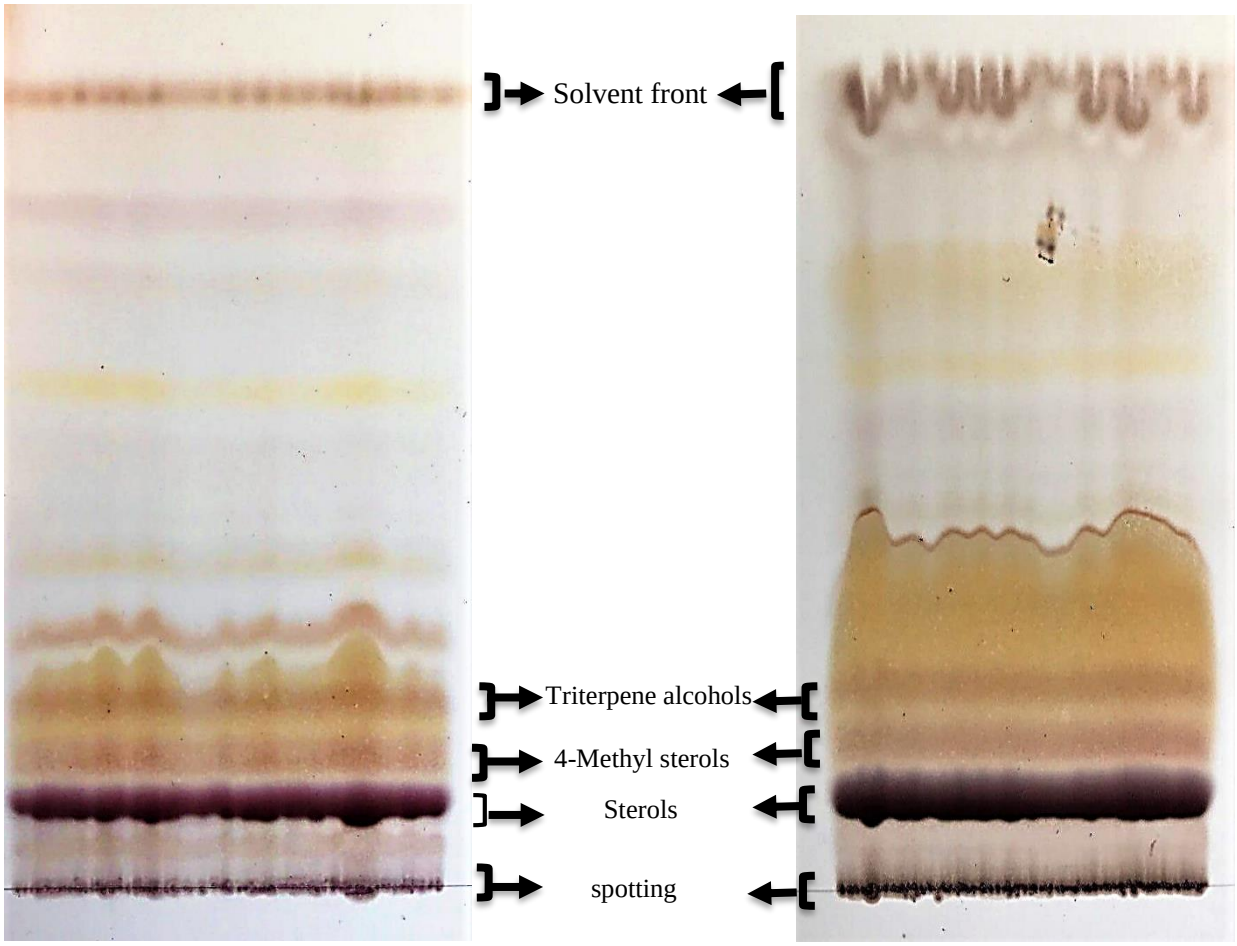

Fig. 2. Nonsaponifiacable matter of crude canola oil

Fig. 3. Nonsaponifiacable matter of crude soyabean oil

Table 1 and Figure 5 present the sterol composition and total sterols of canola oil regarding different stages of processing. As indicated, the composition of sterol fraction has stayed the same, but the quantity present is reduced in neutralization stage due to lower friction and removed by soap formed. During bleaching stage, since acid- activated earth, has been employed as bleaching earth the sterols are changed to steroidal hydrocarbons as they lose water at bleaching temperature that is about 110℃. During deodorization stage, sterols are distillated and collected in the distillate. This fraction is quite valuable; however, it is present with other compounds namely tocopherols, aldehyde, ketones, peroxides, free fatty acids and others.

Table 2 and Figure 6 present the sterol composition of soyabean oil and the loss of sterols during refining operations. As pointed out previously, the percent composition of each sterol has almost stayed the same but losses have occurred during all the refining stages as described previously. The collected distillate due to having a collection of tocopherols and sterols are considered quite valuable.

Table 3 and Figure 7 present the effect of refining procedures on sunflower seed oil. It is quite clear that β-sitosterol is the major sterol in all the oils examined. However, variations in campesterol and stigmasterol have been shown in all the three oils examined. But the composition of each oil has almost remained the same during all the refining operations, although a large quantity has been lost during refining process. Here

as well as other oils examined the distillate that is recoverable contains quantities of tocopherols and other compounds that might be separated and purified and used in other industries, namely food, cosmetic and pharmaceutical industries.

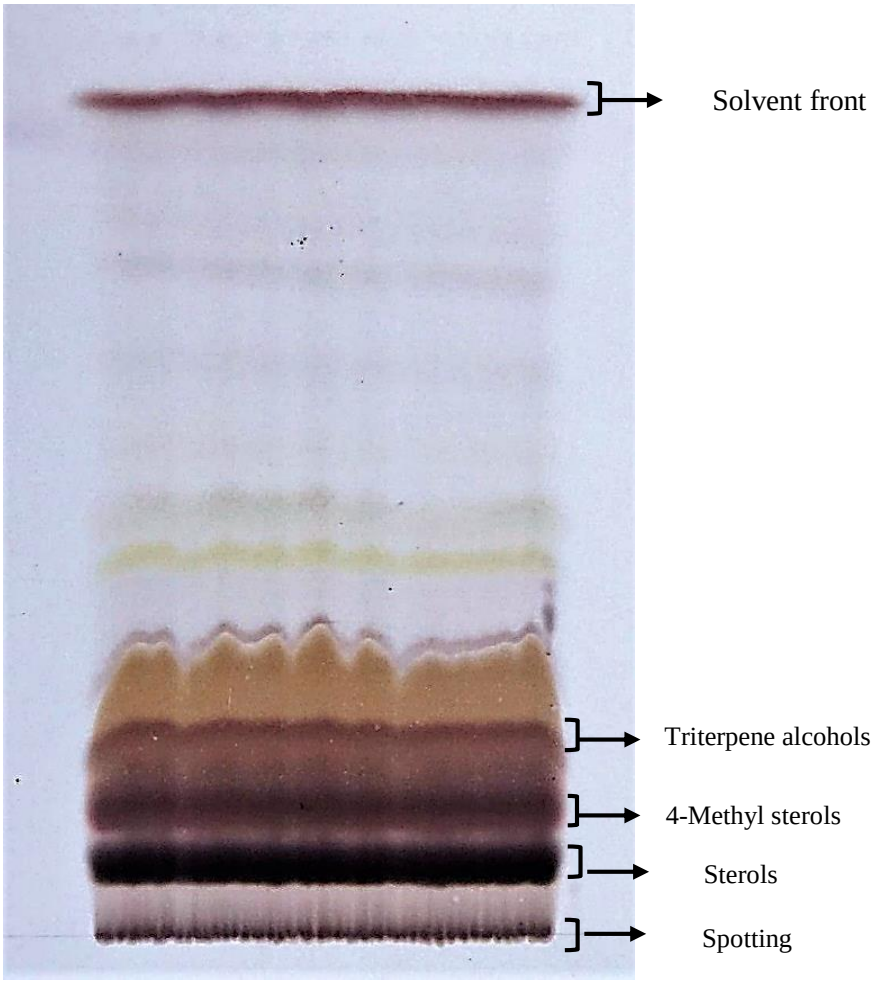

Fig. 4. Nonsaponifiacable matter of crude sunflower seed oil

Table 1.Sterol composition of canola oil

Sterol composition	Crude oil		Neutralized		Bleached		Deodorized	
	% of total sterol	(mg/kg)	% of total sterol	(mg/kg)	% of total sterol	(mg/kg)	% of total sterol	(mg/kg)
Cholesterol	ND	ND	ND	ND	ND	ND	ND	ND
Brassicasterol	8.96	770.00	9.38	663.21	9.21	593.03	8.97	482.19
Campesterol	33.46	2870.08	32.77	2317.04	33.95	2185.61	33.73	1809.98
Stigmasterol	0.80	68.47	0.68	48.22	0.69	44.37	0.45	23.91
Betasitosterol	52.99	4545.46	53.56	3787.38	52.35	3370.68	53.01	2844.39
Δ-5-Avenasterol	2.98	255.18	2.89	204.19	3.10	199.50	2.86	153.64
Δ-7-Stigmastanol	0.54	45.88	0.51	35.80	0.42	27.10	0.69	37.21
Δ-7-Avenasterol	0.27	23.07	0.22	15.41	0.29	18.33	0.27	14.53
Total sterol of oil (mg/kg)	8578.15		7071.25		6438.62		5365.84	

ND = not determined

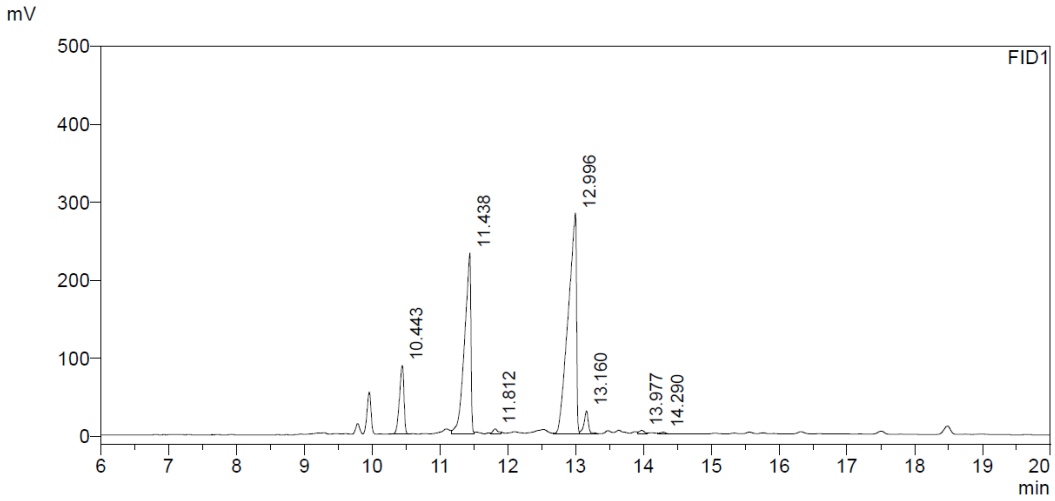

Fig. 5. Sterol composition of crude canola

Table 2. Sterol composition of soyabean oil

Sterol composition	Crude oil		Neutralized		Bleached		Deodorized	
	% of total sterol	(mg/kg)	% of total sterol	(mg/kg)	% of total sterol	(mg/kg)	% of total sterol	(mg/kg)
Cholesterol	0.76	38.19	0.51	27.31	0.52	19.62	0.64	16.92
Brassicasterol	ND	0.00	ND	0.00	ND	0.00	ND	0.00
Campesterol	20.20	1005.22	18.50	782.84	19.74	747.33	20.16	535.44
Stigmasterol	19.27	967.73	17.34	733.78	18.70	707.86	18.64	494.99
Betasitosterol	49.36	2478.23	53.68	2271.66	51.38	1944.83	52.62	1397.70
Δ-5-Avenasterol	4.29	215.47	3.71	157.17	4.17	157.78	3.14	83.37
Δ-7-Stigmastanol	4.49	225.37	4.37	184.70	4.23	160.18	3.52	93.53
Δ-7-Avenasterol	1.81	90.85	1.90	80.29	1.27	47.96	1.29	34.21
Total sterol of oil (mg/kg)	5021.07		4231.81		3785.56		2656.16	

ND = not determined

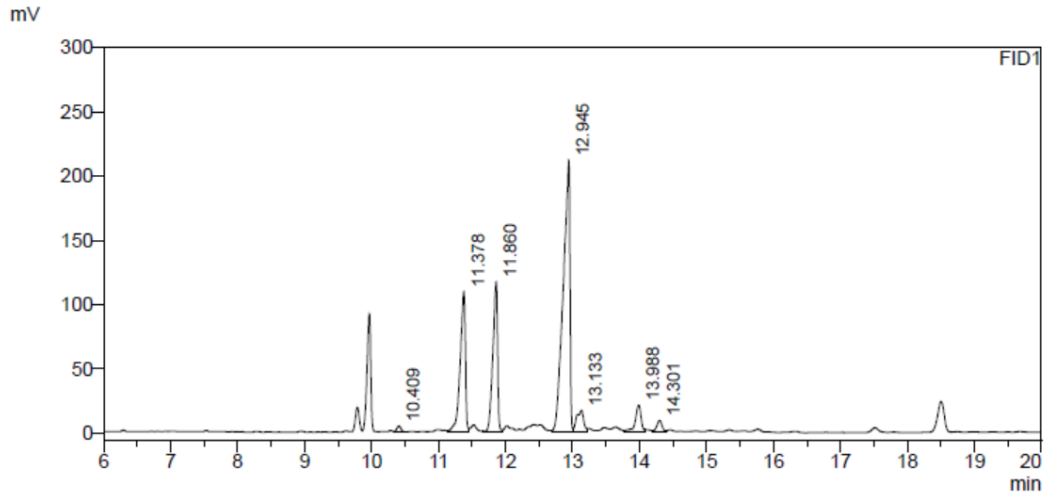

Fig. 6. Sterol composition of crude soyabean oil

Table 3. Sterol composition of sunflower seed oil

Sterol composition	Crude oil		Neutralized		Bleached		Deodorized	
	% of total sterol	(mg/kg)	% of total sterol	(mg/kg)	% of total sterol	(mg/kg)	% of total sterol	(mg/kg)
Cholesterol	0.13	6.49	0.35	15.27	0.42	15.05	0.26	8.05
Brassicasterol	ND	ND	ND	ND	ND	ND	ND	ND
Campesterol	9.27	450.56	8.83	388.02	8.78	313.58	8.80	268.53
Stigmasterol	8.80	427.79	8.66	380.56	8.50	303.55	8.66	264.22
Betasitosterol	58.11	2824.35	59.42	2610.85	60.33	2153.69	62.75	1913.98
Δ-5-Avenasterol	4.75	230.75	4.71	207.14	2.49	88.98	2.87	87.59
Δ-7-Stigmastanol	14.52	705.55	13.17	578.46	15.43	550.85	12.77	389.54
Δ-7-Avenasterol	4.41	214.54	4.86	213.45	4.04	144.30	3.87	118.12
Total sterol of oil (mg/kg)	4860.03		4393.75		3570.01		3050.03	

ND = not determined

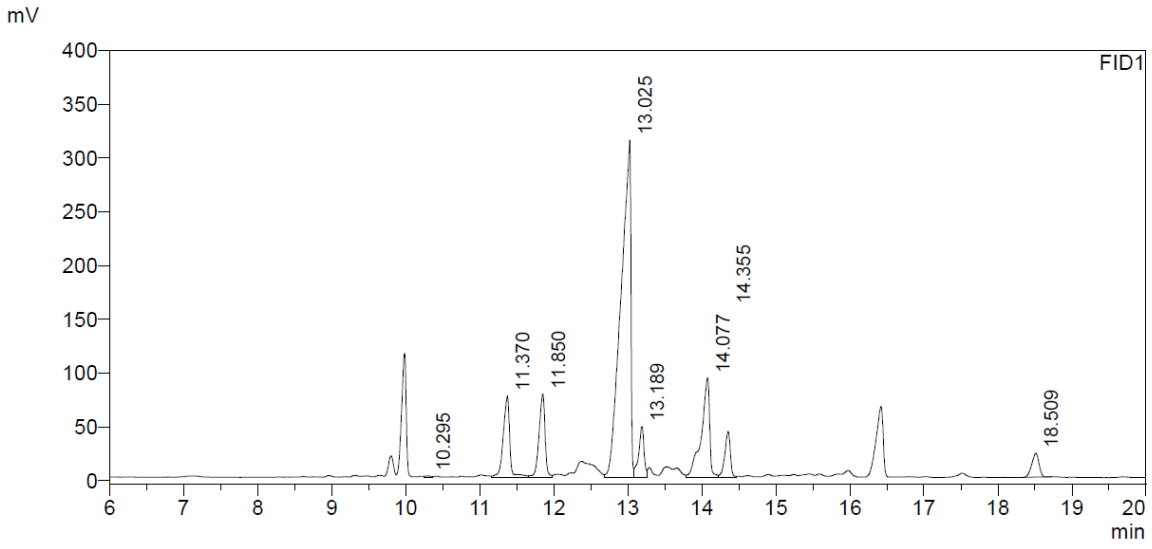

Fig. 7. Sterol composition of crude sunflower seed oil

Conclusion

The results have shown that during neutralization, bleaching and deodorization the loss of sterols has occurred. The recoveries after neutralization and bleaching stages might be complicated due to structural changes or losses with soap fraction. However, the fraction collected in distillate is considered a valuable source and might be fractionated to different components and later purified and employed in various food industries.

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