

Research Article



Detection of Vegetable Oils Adulteration Using Desmethylsterols Composition

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ABSTRACT

A set of samples was prepared by mixing virgin olive oil with different percentages of sunflower oil, soybean oils or cottonseed oil (5, 10, 20%). Another set of samples was prepared by mixing corn oil with different percentages of canola oil (5, 10, 20%). After two preparative steps including unsaponifiable matter determination and unsaponifiable matter fractionation by Thin Layer Chromatography (TLC), the desmethylsterols composition and content of all samples was determined in triplicate using Gas Chromatography-Flame Ionization Detector (GC-FID). The results have shown the possibility of using $\Delta 7$ stigmasterol and campesterol content to detect adulteration of virgin olive oil by sunflower oil or soybean oil, respectively. While it was impossible to detect the adulteration of virgin olive oil by cottonseed oil using desmethylsterols content even in percentages up to 15%. The results have also shown the possibility of using brassicasterol content to detect the adulteration of corn oil by canola oil.

Keywords: Adulteration, Desmethylsterols, GC-FID, Vegetable oils.

INTRODUCTION

Checking the purity of oils is of great importance since they can be illegally mixed with cheaper oils. Therefore, authenticity of oils is necessary from the stand points of both commercial value and health aspects, accordingly, efficient analytical methods are required for authentication of oils adulteration.

Therefore, different methods have been developed to detect the adulteration of vegetable oils. Some of these methods are based on the qualitative analysis of oils such as colour, triglycerides and fatty acids.¹

The content of selected phytosterols is widely accepted as one of the most important markers for the detection of adulterated vegetable oils. Phytosterols are structurally similar to cholesterol, they mainly exist in plants.^{2,3} Their chemical structure is illustrated in **Figure 1**. They are divided into three main groups: 4,4 dimethylsterols, 4 mono-methyl sterols and 4, desmethylsterols.

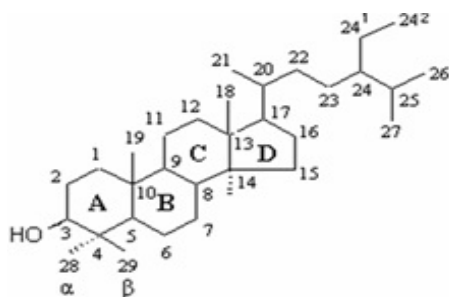


Figure 1: Desmethylsterol main structure.

Desmethylsterols are the most important group⁴ and its most abundant products are sitosterol, campesterol and stigmasterol.⁵ Those products are found in high percentages in most phytosterol sources like vegetable oils. Another sterols such as $\Delta 7$ -stigmastanol and

brassicasterol discriminate some vegetable oils and plant species.⁶

Desmethylsterols composition and content are used for the identification of vegetable oils, regardless of their presence in lipids as minor constituents.⁷ Hence, phytosterols and other unsaponifiable compounds in oils are often used as markers for the assessment of oil adulteration.⁸

All over the world, there has been a high incidence of adulteration in vegetable oils, mainly in olive oil and other oils of high commercial value such as corn oil.

Olive oil is adulterated in many ways because of its high price in comparison to other vegetable oils. The main vegetable oils used for its adulteration are cottonseed oil, sunflower oil and soybean oil which are cheaper and less valuable than olive oil.⁹

Numerous researchers have proposed various methods to determine adulteration resulting from the mixing of the olive oil with other vegetable oils.^{9,10} Firestone et al. (1985) proposed a method based mainly on the analysis of different olive oil constituents, such as sterols, triterpenes and saturated fatty acid in the 2-position of triglyceride.¹¹

In our work, the method proposed in previous researches for the detection of olive oil adulteration was applied to olive oil marketed in Syria and then used for the detection of corn oil adulteration by canola oil. Results can throw light on the possibility of using desmethylsterols composition for the detection of corn oil adulteration. This work also may be applied to mixtures of other vegetable oils, after changes in the data introduced to the model.



MATERIALS AND METHODS

Ethyl ether, chloroform, hexane, potassium hydroxide, anhydrous sodium sulphate and silica gel plates (20×20 cm) were purchased from Merck chemical company. Standard α -cholestanol and pyridine were obtained from Fluka chemical company. Silylation agents, trimethylsilylfluoroacetamide: trimethyl (99:1) and dichlorofluorosilane were obtained from Supelco and Sigma, respectively.

GC-FID [Focus GC Thermo corporation serial number 10602102 cod.12550060 (split/splitless) – FID].

Vegetable oil samples

Virgin olive oil samples were obtained from different regions of Syria. Cottonseed oil, sunflower oil, soybean oil, corn oil, canola oil were purchased from local markets. A set of vegetable oil mixtures was prepared according to **Table. 1**.

Table 1: Mixtures of olive and corn oils with other vegetable oils.

		Cottonseed oil	Sunflower oil	Soybean oil	Canola oil
Olive oil	95%	5%	5%	5%	—
	90%	10%	10%	10%	—
	85%	15%	15%	15%	—
Corn oil	95%	—	—	—	5%
	90%	—	—	—	10%
	85%	—	—	—	15%

Sample preparation

1. Determination of unsaponifiable matter: Oil samples were heated for 35 min at 90°C with 50 ml KOH₂N using α -cholestanol as an internal standard.

After saponification, 150 ml of water was added and the unsaponifiable phase was extracted 3 times with 70 ml diethyl ether. The combined diethyl ether fractions were washed 3 times with 50 ml water and dried over anhydrous sodium sulphate. The residue obtained after evaporation was dissolved with chloroform to get 5% solution.^{12,13}

2. Fractionation of the unsaponifiable matter: 250 μ l of unsaponifiable matter solution was placed as a line along the silica gel plates which are previously impregnated with 0.2 N ethanolic potassium hydroxide. Elution was performed using a mixture of hexane: diethyl ether(65:35).

The corresponding bands were developed by spraying with a solution of 0.2% dichlorofluorescein in ethanol. The borders of the desmethylsterols were marked, scratched and extracted with hot chloroform then evaporated to dryness at 70°C water bath.¹²⁻¹⁴

3. GC: The residue obtained after evaporation was derivatized using a silylation agent solution of bis-

trimethylsilyl trifluoroacetamide containing 1% trimethyl chlorosilane. Then the sample was injected in GC-FID.

GC was performed using Zerbon ZB5 capillary column (30m×0.32mm×0.25 μ m) and helium as the carrier gas with a flow rate of 0.9ml/min and split ratio 80:1. The injector temperature was 295°C and the oven temperature was 265°C. Detection was made by flame ionization with the detector temperature set at 305°C.^{7,13,15}

The desmethylsterols content was calculated according to AUC of α -cholestanol used as an internal standard (**Figure. 2**).

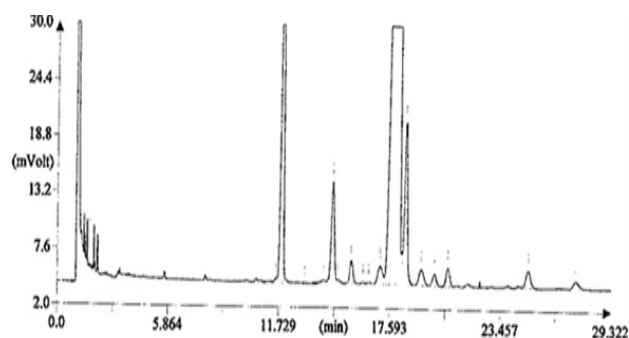


Figure 2: GC chromatogram of Desmethylsterol from virgin olive oil.

RESULTS

As shown in **Table. 2** the desmethylsterols composition of studied oils meet the reported data by Moreda et al, 2004., Reina et al 1999, Thrope et al 1972.^{14,16,17}

Table 2: Desmethylsterols composition (%) of some vegetable oils.

	Sunflower	soya	cotton	Canola	Olive	Corn
Cholesterol	0.11	0.28	0.31	0.26	0.38	0.3
Brassicasterol	0.03	0.11	0.01	9.37	0.04	0.03
24 Methelen cholesterol	0.15	0.16	0.07	0	0	0
Campesterol	8.23	21.53	8.27	34.15	2.37	20.49
Campestanol	8.26	17.52	0.14	0.85	0.27	0.94
Stigmasterol	0.24	0.72	1.81	0.32	1.52	6.56
$\Delta 7$ Campesterol	3.06	1.11	0.21	1.2	0.09	0.38
$\Delta 5.23$ Stigmastadienol	1.19	0.64	0.24	0.85	0.08	0.42
Clerosterol	0.39	0.85	1.11	0	1.02	0.82
β Sitosterol	57.05	51.37	82.49	48.49	85.01	63.63
Sitostanol	2.89	1.18	0	2.89	0.86	2.35
$\Delta 5$ Avenasterol	0.1	0.47	2.55	0.08	5.04	2.72
$\Delta 5.24$ Stigmastadienol	2.29	1.01	1	1	0.92	0.68
$\Delta 7$ Stigmastenol	12.42	1.77	1.14	0.32	0.88	0.27
$\Delta 7$ Avenasterol	3.95	1.27	0.65	0.22	1.08	0.44

Results reported in **Table. 3** represent the desmethylsterols composition of virgin olive oil, sunflower oil and their different mixtures.

As noticed, the percentage of campesterol in virgin olive oil sample was 2.37 and it increased to 3.08, 3.69 and 4.77 when mixed with 5, 10 and 15% of sunflower oil, respectively. While the percentage of stigmasterol increased from 1.53 to 2.19, 2.81 and 4.09 and $\Delta 5.23$ stigmastadienol raised from 0.08 to 0.32, 0.58 and 1.21 when mixed with 5, 10 and 15% of sunflower oil, respectively.

The pure virgin olive oil sample contained 0.88 of $\Delta 7$ stigmastenol and this percentage increased to 2.16, 3.34 and 4.78 when mixed with 5, 10 and 15% of sunflower oil, respectively.

Results reported in **Table 4** represent the desmethylsterols composition of virgin olive oil, cottonseed oil and their different mixtures.

It was remarked that the percentages of campesterol increased to 3.08, 3.9 and 4.41 when mixed with 5, 10 and 15% of cottonseed oil, respectively while it was 2.37 in the pure sample of virgin olive oil.

Table 5 shows the desmethylsterol composition of virgin olive oil, soybean oil and their different mixtures. It was noticed that campesterol percentage in olive oil increased to 5.15, 6.1 and 9.31, and percentage of stigmasterol increased to 3.8, 5.17 and 8.63 when mixed with 5, 10 and 15% of soybean oil, respectively.

Table 6 illustrates the desmethylsterol composition of corn oil, canola oil and their different mixtures. It was noticed that brassicasterol percentage was amounted to 0.03 in pure corn oil and increased to 0.5, 0.8 and 1.48 when mixed with 5, 10 and 15% of canola oil, respectively.

Table 3: Desmethylsterols composition (%) of olive oil, sunflower oil and their different mixtures.

	Olive	Sunflower	mix 5%	mix 10%	mix 20%
Cholesterol	0.38	0.11	0.33	0.22	0.17
Brassicasterol	0.04	0.03	0.02	0.06	0.01
24 Methelencholesterol	0	0.15	0.11	0.15	0.18
Campesterol	2.37	8.23	3.08	3.69	4.77
Campestanol	0.27	8.26	0.18	0.18	0.22
Stigmasterol	1.52	0.24	2.19	2.81	4.09
$\Delta 7$ Campesterol	0.09	3.06	0.1	0.14	0.26
$\Delta 5.23$ Stigmastadienol	0.08	1.19	0.32	0.58	1.21
Clerosterol	1.02	0.39	0.98	0.97	1.16
β Sitosterol	85.01	57.05	82.36	77.74	73.07
Sitostanol	0.86	2.89	0.75	0.7	0.6
$\Delta 5$ Avenasterol	5.04	0.1	5.01	4.9	4.87
$\Delta 5.24$ Stigmastadienol	0.92	2.29	0.89	1.03	1.28
$\Delta 7$ Stigmastenol	0.88	12.42	2.16	3.84	5.4
$\Delta 7$ Avenasterol	1.08	3.95	1.42	1.9	2.72

Table 4: Desmethylsterols composition (%) of olive oil, cottonseed oil and their different mixtures.

	Olive	cottonseed	mix 5%	mix 10%	mix 15%
Cholesterol	0.38	0.31	0.26	0.21	0.3
Brassicasterol	0.04	0.01	0.01	0.01	0.02
24 Methelencholesterol	0	0.07	0	0.02	0.03
Campesterol	2.37	8.27	3.08	3.9	4.41
Campestanol	0.27	0.14	0.2	0.2	0.32
Stigmasterol	1.52	1.81	1.52	1.54	1.65
$\Delta 7$ Campesterol	0.09	0.21	0.05	0.07	0.1
$\Delta 5.23$ Stigmastadienol	0.08	0.24	0.05	0.05	0.21
Clerosterol	1.02	1.11	1	1.02	1.17
β Sitosterol	85.01	82.49	85.18	84.27	82.66
Sitostanol	0.86	0	0.76	0.65	0.4
$\Delta 5$ Avenasterol	5.04	2.55	5.01	4.98	4.4
$\Delta 5.24$ Stigmastadienol	0.92	1	0.87	0.76	0.95
$\Delta 7$ Stigmastenol	0.88	1.14	0.89	0.83	1.02
$\Delta 7$ Avenasterol	1.08	0.65	1	0.95	0.65



Table 5: Desmethylsterols composition (%) of olive oil, soybean oil and their different mixtures.

	Olive	Soybean	mix 5%	mix 10%	mix 20%
Cholesterol	0.38	0.28	0.62	0.33	0.48
Brassicasterol	0.04	0.11	0.03	0.02	0.04
24 Methelencholesterol	0	0.16	0.12	0.14	0.08
Campesterol	2.37	21.53	5.15	6.1	9.31
Campestanol	0.27	0.47	0.25	0.25	0.31
Stigmasterol	1.52	17.52	3.8	5.17	8.63
$\Delta 7$ Campesterol	0.09	0.72	0.05	0.13	0.43
$\Delta 5.23$ Stigmastadienol	0.08	1.11	0.1	0.19	0.42
Clerosterol	1.02	0.64	0.83	0.82	0.82
β Sitosterol	85.01	51.37	80.65	78.21	70.56
Sitostanol	0.86	0.85	0.91	0.98	1.17
$\Delta 5$ Avenasterol	5.04	1.18	4.56	4.5	3.73
$\Delta 5.24$ Stigmastadienol	0.92	1.01	0.71	0.75	0.77
$\Delta 7$ Stigmastenol	0.88	1.77	1.11	1.28	1.94
$\Delta 7$ Avenasterol	1.08	1.27	1.12	1.28	1.32

Table 6: Desmethylsterols composition (%) of corn oil, canola oil and their different mixtures.

	corn	Canola	mix 5%	mix 10%	mix 20%
Cholesterol	0.3	0.26		0.22	0.13
Brassicasterol	0.03	9.37	0.5	0.8	1.48
24 Methelencholesterol	0	0	0	0	0
Campesterol	20.49	34.15	21.04	21.36	22.51
Campestanol	0.94	0.08	0.92	0.86	0.77
Stigmasterol	6.56	0.85	6.26	5.89	5.59
$\Delta 7$ Campesterol	0.38	0.32	0.37	0.37	0.35
$\Delta 5.23$ Stigmastadienol	0.42	1.2	0.57	0.48	0.46
Clerosterol	0.82	0.85	0.82	0.78	0.79
β Sitosterol	63.63	48.49	62.89	62.35	61.82
Sitostanol	2.35	0	2.26	2.24	1.87
$\Delta 5$ Avenasterol	2.72	2.89	2.81	2.85	2.75
$\Delta 5.24$ Stigmastadienol	0.68	1	0.7	0.73	0.77
$\Delta 7$ Stigmastenol	0.27	0.32	0.27	0.27	0.28
$\Delta 7$ Avenasterol	0.44	0.22	0.41	0.4	0.4

DISCUSSION

Campesterol content of virgin olive oil mixture with cottonseed oil was more about 1.37 to 1.89 times compared with its content in pure virgin olive oil. A slight decrease of stigmasterol, sitostanol and $\Delta 5$ -avenasterol content has been noticed which can be due to their low percentages in cottonseed oil.

The most important changes in desmethylsterol content in olive oil mixed with sunflower oil appeared in $\Delta 7$ -stigmastenol and $\Delta 5, 23$ stigmastadienol which increased 2.45 to 6.13 times, and 4 to 15.4 times respectively. Whereas the campesterol and stigmasterol content increased only about 1.29 to 2.01 times and about 1.44 to 2.7 times, respectively. A decrease in β -sitosterol content was noticed in olive oil and sunflower oil mixture due to its low percentage in sunflower oil.

Stigmasterol and campesterol increased 2.5 to 5.68 times and 2.17 to 3.93 times in different mixtures of virgin olive oil and soybean oil. Even though stigmasterol content's variation cannot be used to detect olive oil adulteration because the Codex Alimentarius (2003) determined the stigmasterol content to be less than campesterol without a specific value.^{18,19}

Brassicasterol content of corn oil mixture with canola oil was more about 16.6 to 49.33 times compared with its content in pure corn oil.

CONCLUSION

$\Delta 7$ -stigmastenol and campesterol content can be used to investigate olive oil adulteration by sunflower oil or soybean oil because of the high increase in their content compared to their values in pure olive oil specified in



Codex Alimentarius (2003). Corn oil adulteration by canola oil can be detected using the variation in content of brassicasterol even in 5 and 10 % mixtures which has not been mentioned before.

Whereas desmethyl sterols content cannot be used to investigate the presence of cottonseed oil in olive oil even in mixtures up to 15% because there are no significant changes that are able to detect.

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