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A Data Processing Method for the Determination of the Concentration of the Components of Unsaponifiable Matter in Vegetable Oils

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ABSTRACT

A simple method of data processing was developed for determination of the components of unsaponifiable matter in vegetable oils. Gas-liquid chromatography (GLC) was applied to the direct analysis of a sample of unsaponifiable matter as a whole, and to preparative thin-layer fractions of the same sample. The data obtained by both methods were manipulated to determine calculation factors for the different thin-layer chromatography (TLC) groups of the unsaponifiable matter, and these factors were used to quantify the individual components of each group. Identification of the components was achieved by comparison of R_f values (R_f) and retention times (R_t) and those of standard substances separated simultaneously by the same techniques. The identity of the components was confirmed by combined GLC–mass spectral analysis of the same preparative TLC fractions, and a comparison of R_t and m/e data with those of standard substances. The new method was applied to the determination of the concentration of squalene, α-tocopherol, γ-tocopherol, sesamine and sesamolene (together), obtusifoliol, gramisterol, citrostadienol, β-amyrin, cycloartenol, 24-methylenecycloartanol, cholesterol, brassicasterol, campesterol, stigmasterol, β-sitosterol, and Δ7-stigmasterol in different vegetable oils. The results obtained with this method, in mg/g of oil, are in line with accepted levels.

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INTRODUCTION

The composition of unsaponifiable matter of vegetable oils has recently been given greater importance as an additional criterion, complementary to fatty acid composition, for oil identification. Its complex nature necessitated the use of different techniques for complete separation and identification of the various components. The preparative separation, making use of chromatographic methods, is usually employed to separate oil unsaponifiables into the fractions of sterol, 4-methylsterol, triterpene alcohol, tocopherol, aliphatic hydrocarbons, and squalene. The components of each of these fractions may be further separated by gas-liquid chromatography (GLC) and identified by a suitable technique. Combined column chromatography–gas liquid chromatography (CC–GLC) has been the most widely used technique (Eisner & Fireston, 1963; Eisner *et al.*, 1966; Moura *et al.*, 1975; Severson *et al.*, 1978). Separation of preparative TLC fractions by GLC has also been employed for analysis of the unsaponifiable matter in other oils (Fedeli *et al.*, 1966; Fioriti *et al.*, 1971; Itoh *et al.*, 1973; Homberg & Beilfeld, 1982). Analytical techniques, in lieu of the preparative methods, have been demonstrated by Pyle *et al.* (1976) for quantitative determination of sterol, 4-methylsterol, and triterpene alcohols. They employed GLC to calculate the amounts of these groups as a whole, by direct separation of the unsaponifiable matter. The same sample was separated by combined TLC–GLC, and the percentage of each component of the three groups was calculated in the total sum of these groups assuming constant recoveries for all bands. Bastic and Jovanovic (1979) proposed a method for qualitative separation of the unsaponifiable matter, without previous fractionation, by capillary column GLC. They reported that most of the components detected by a combined TLC–GLC method could be satisfactorily detected by this direct capillary GLC method.

The objective of the present work was to develop a simple data processing method for accurate routine determination of the concentration of unsaponifiable components (mg/g of oil) in vegetable oils.

MATERIALS AND METHODS

Materials

Individual standard cholesterol, brassicasterol, campesterol, stigmasterol, β-sitosterol, and Δ7-stigmasterol were purchased from Applied Science Laboratories Inc., State College, Pennsylvania, USA. Individual quantitative solutions in diethyl ether and mixtures containing known weights of

these sterols were prepared. Pure α-tocopherol, γ-tocopherol, α-tocopherolacetate, and a mixture of alkanes were purchased from the same source. A mixture of triterpene alcohols (α- and β-amyrin, cycloartenol, and 24-methylenecycloartanol) and a mixture of normal chain saturated alcohols with carbon atom numbers from 22 to 36 were donated by the Scientific and Applied Research Center, University of Qatar. Squalene and pentacosanol were supplied by Unilever Research Laboratories, Vlaardingen, The Netherlands.

Pure oils of corn, soybean, sunflower, cottonseed, groundnut, coconut, palm, and palm kernel were donated by Unilever Research Laboratories. Pure crude olive and sesame oils were selected from the commercial brands available in the local Doha market. Refined rapeseed oil was obtained from J. Bibby Edible Oils Ltd, Liverpool, UK.

Methods

The methods were carried out in duplicate and the mean values were calculated (Table 4). The results differed within a 4% limit.

Preparation of sample

For saponification of oil samples the AOAC (1976) procedure was used with slight modification where 2 g of oil was saponified with 20 ml potassium hydroxide. The unsaponifiable matter was extracted three times with 30-ml portions of ether. The combined ether extract was washed with 15 ml of 0·5M aqueous potassium hydroxide solution, followed by three washings with 20 ml of distilled water until alkali-free. The ether extract was dried over anhydrous sodium sulphate and evaporated. The unsaponifiable residues were further dried, if necessary, by recrystallization from acetone, without any appreciable losses. The dry residues were dissolved in 3 ml of ether and kept for GLC analysis.

Chromatographic analysis

Capillary column GLC analysis of the unsaponifiable matter was carried out using a Pye Model 304 gas–liquid chromatograph, equipped with a flame ionization detector (FID) and connected to a computing integrator, model PU4810, for data processing. For packed column GLC analysis of the same samples of unsaponifiable matter, a Packard model 430, equipped with FID and a built-in computer for data processing and control of operating conditions, was used. Hydrogen was supplied from a hydrogen generator, model OPGU-500, and other gases (nitrogen and air) from cylinders.

The GLC integrator was calibrated according to its response for cholesterol, so that the peak areas were converted into mg/g of oil. The

available standards of known concentration were injected individually onto GLC (on-column injection), and their response factors, relative to that of cholesterol relative response factor (RRF) and retention times, relative to that of β-sitosterol (RR_t), were determined. Results of the RRF and (RR_t) are given in Table 1. The percentage recoveries of the components of unsaponifiable matter by the direct GLC analysis were estimated by processing known amounts of the standard components, following the same

TABLE 1

Relative Response Factors and Retention Times of the Main Components of Unsaponifiable Matter Relative to that of β-Sitosterol

R_t	RR_t	*Component*	*I*	*II*	*III*
Hydrocarbons					
2·19	0·13	*n*-Octadecane	6·39	4·5	0·70
3·05	0·18	*n*-Eicosane	4·80	3·00	0·62
3·75	0·34	*n*-Docosane	2·27	1·80	0·79
8·19	0·49	*n*-Tetracosane	7·55	6·00	0·79
12·22	0·73	Squalene	1·00	0·80	0·80
12·27	0·73	*n*-Octacosane	12·36	7·70	0·62
16·07	0·96	*n*-Dotriacontane	5·35	4·40	0·82
21·79	1·30	*n*-Hexatriacontane	3·00	2·60	0·87
Triterpene alcohols					
16·85	0·98	α-Amyrin	—	—	—
16·85	0·98	β-Amyrin	14·21	13·5	0·95
17·89	1·04	Cycloartenol	—	—	—
18·75	1·09	24-Methylene cycloartanol	—	—	—
Sterols					
14·59	0·88	Cholesterol	7·50	7·30	0·97
14·78	0·89	Brassicasterol	6·30	6·10	0·97
15·65	0·94	Campesterol	8·50	8·80	1·04
15·94	0·96	Stigmasterol	8·20	8·00	0·98
16·61	1·00	β-Sitosterol	5·50	5·20	0·95
Aliphatic alcohols					
13·10	0·70	*n*-Pentacosanol	2·48	2·60	1·05
14·30	0·76	*n*-Hexacosanol	—	—	—
15·60	0·83	*n*-Heptacosanol	—	—	—
16·28	0·87	*n*-Octacosanol	—	—	—
18·03	0·96	*n*-Nonacosanol	—	—	—
19·11	1·02	*n*-Triacontanol	—	—	—
23·42	1·25	*n*-Dotriacontanol	—	—	—

Columns I, II and III contain the amount of the standard substance calculated by GLC on the basis of cholesterol response factor, their actual known amount (in mg/g), and the relative response factors, respectively.

method as used for oil samples. The prepared standards were injected directly onto GLC and the percentage recovery of each compound was determined from the relation

$$\% \text{ recovery} = \frac{\text{estimated amount of compound}}{\text{known amount of compound}} \times 100$$

Direct GLC analysis

A dosage of 2 μl of the prepared sample of the whole unsaponifiable matter was injected directly on a 25 cm long × 0·32 mm i.d. fused silica capillary column coated with SE30. The column was operated with an oven temperature programme retained at 200°C for 3 min, then raised to 260°C at 3°C/min. The same sample of unsaponifiable matter was chromatographed on a 2 m × 4 mm i.d. glass column, packed with 3% SE30 on chromosorb W, operated with a temperature programme retained at 200°C for 3 min, then raised to 280°C at 7°C/min. In both analyses the detector and injector were kept at 320°C.

Combined TLC–GLC analysis

Thin-layer glass plates (20 × 20 cm) precoated with a 0·5 mm layer of silica gel were purchased from Merck Darmstadt, FRG. The same sample of unsaponifiable matter, which was chromatographed as a whole on GLC, was evaporated to a volume of 0·3 ml and applied on a TLC plate, using a 100 μl micropipette, in the form of two lines 7 cm each and 1 cm apart. Standard cholesterol, α-tocopherol, squalene, and hydrocarbons were applied on both ends of the plate. Development was carried out twice using chloroform and the plate was dried by a flow of air, sprayed with rhodamine-B colour reagent and viewed under UV light at λ_{max} 366 nm. Six main zones, corresponding to the main groups of hydrocarbon (and squalene), tocopherol isomers, triterpene alcohol, 4-methylsterol, sterol, and an unidentified zone, were visible in most of the oils, in the order of decreasing R_f value. The TLC preparative fractions were individually injected onto GLC at the same conditions as used for the direct GLC analysis (TLC–GLC). Identification of peaks was achieved by comparing R_f and RR_t of each component and those of standards. No standards were available for Δ5-avenasterol, δ-tocopherol or citrostadienol. Identification of δ-tocopherol and citrostadienol was achieved by combined chromatographic mass spectral analysis of the 4-methylsterol and triterpene alcohol fractions of TLC (Table 2). Δ5-avenasterol was identified by comparison with the work of Itoh (1973).

Mass spectral analysis

Confirmation of identities was carried out by GLC–mass spectral analysis of

TABLE 2

RR_t Data and Mass Spectral Data of the Different Components of the Unsaponifiable Matter

Component	*GLC data* RR_t	*Mass spectral data m/e*								
Obtusifoliol	0·98	426	411	327						
Gramisterol	1·02	412	397	328	299	285				
Citrostadienol	1·09	426	411	328	285					
δ-Tocopherol	0·78	402	177	137						
β-Amyrin	1·02	426	411	393	365	339	315	286	273	255
Cycloartenol	1·04	426	410	392	364	339	315	286	273	255
24-Methylene cycloartanol	1·09	440	300	285						
n-Tetracosane	0·50	338	323	309	295	281	267	253	→	54
n-Pentacosane	0·55	352	337	323	309	———	———	———	→	54
n-Hexacosane	0·62	366	295	281	267	———	———	———	→	54
n-Heptacosane	0·68	380	365	351	→	281	———	———	→	54
n-Octacosane	0·72	394	379	365	→	281	———	———	→	54
Squalene	0·72	410	376	341	299	273	231	217	205	203
n-Tetracosanol	0·64	336	308	280	252	238	———	———	→	56

Arrows indicate a steady decrease from upper to lower value.

the same preparative fractions of TLC (Table 2). The instrument used for mass spectral analysis was a Finnigan 4000 system, equipped with a data general Nova 3 computer. The column, installed in a Carlo Erba 4160 and equipped with a split and a cold on-column injector, was directly connected to the ion source via 1 m HTS deactivated fused silica tubing attached to the glass column with a polyamide seal.

Quantification method

Assuming that the total unsaponifiable matter was fractionated by TLC into a number of zones (j) corresponding to the groups of hydrocarbon, etc., and if each of these zones was separated by GLC into a number of components (n components), then, in general, the component number n of the zone number j was denoted by $A(j,n)$ and its concentration $C(j,n)$, was estimated by the following equation:

$$C(j,n) = [C(j,n)]_{\text{TLC-GLC}} \times R(J) \times (\text{rel. res. fct})_{A(j,n)} \qquad (1)$$

Where, $[C(j,n)]_{\text{TLC-GLC}}$ is the percentage of the component $A(j,n)$ determined by TLC–GLC analysis. $R(J)$ is the calculation factor of the TLC group J and was determined from the relationship

$$R(J) = \frac{[C(j)]_{\text{GLC}}}{[C(J)]_{\text{TLC-GLC}}} \qquad (2)$$

where, $C(J)$ is the concentration in mg/g of a compound, $A(J)$, chosen from the components determined in the TLC zone (J). $A(J)$ should be completely separable by the direct GLC of the whole unsaponifiable matter. The subscript GLC and TLC–GLC indicate the technique used for the determination. The concentrations of squalene, citrostadienol, cycloartenol, γ-tocopherol, and campesterol were assigned for $C(1)$ through $C(5)$, respectively, as demonstrated later.

RESULTS AND DISCUSSION

The wide practice of mixing the expensive oils, e.g. corn oil, with cheaper ones necessitated the development of a simple method for routine checking of the commercial brands of these oils. Compositional analysis of unsaponifiable matter, as complimentary to fatty acid analysis, provided useful data for identification of adulterants, especially when fatty acid composition of the original oil and the adulterants are similar. The application of the conventional method of chromatographic analysis to the quantitative determination of the components of unsaponifiable matter is not sufficiently accurate because of the possible errors associated with the gravimetric determination of the amount of fractions, especially those present in trace amounts (e.g. 4-methylsterol, triterpene alcohol, etc.). The low recovery of the components analyzed by the method of TLC–GLC is an additional problem when this method is used for quantitative determination, while the larger amounts of solvent required for quantitative elution of the fractions separated by column chromatography is an obvious disadvantage in routine analysis. The efficiency of the direct packed column GLC method for separating the unsaponifiable matter without prefractionation by other methods was studied in this work by comparing the chromatograms obtained by this method with those obtained by combined TLC–GLC analysis of the same sample. With the direct GLC method, maximum interferences were observed between the components of sterol, 4-methylsterol, triterpene alcohol, and alkanes with more than 31 carbon atoms. However, these interferences were reduced when a capillary column was used. Table 3 summarizes the efficiency of separation of unsaponifiable matter by packed column GLC, capillary column GLC, and a combined TLC–GLC method.

A method employing both direct GLC and combined TLC–GLC techniques is proposed, in this paper, for the quantitative determination of the components of unsaponifiable matter. The method is based on the determination of a calculation factor for each TLC fraction by mathematical manipulation of the data obtained from the analysis of the same sample

TABLE 3

Comparison Between the Efficiencies of the Techniques of Direct Capillary GLC, Direct Packed Column GLC, and Combined TLC–GLC in Separating the Unsaponifiable Matter

I—Capillary column GLC			*II—Packed column GLC*		
Direct separation RR_t[a]	*Combined TLC–GLC separation*		*Direct separation* RR_t[a]	*Combined TLC–GLC separation*	
	RR_t[a]	*Component*		RR_t[a]	*Component*
0·543	0·543	Squalene	0·720	0·720	Squalene
0·612	0·612	δ-Tocopherol	0·780	0·780	δ-Tocopherol
0·707	0·707	γ-Tocopherol	0·840	0·840	γ-Tocopherol
0·735	0·735	Cholesterol	0·870	0·870	Cholesterol
0·776	0·775	α-Tocopherol	0·890	0·890	α-Tocopherol
0·869	0·869	Brassicasterol	0·900	0·900	Brassicasterol
0·854	0·854	Campesterol	0·940	0·940	Campesterol
0·890	0·890	Stigmasterol	0·960	0·960	Stigmasterol
0·941	0·941	Obtusifoliol		0·980	Obtusifoliol
1·000	1·000	β-Sitosterol	1·000	1·000	β-Sitosterol
	1·010	Δ5-Avenasterol		1·010	Δ^5-Avenasterol
	1·015	Gramisterol		1·020	Gramisterol
1·032	1·032	β-Amyrin		1·020	β-Amyrin
1·067	1·067	Δ-Stigmasterol	1·040	1·040	Δ^7-Stigmasterol
1·083	1·083	Cycloartenol		1·040	Cycloartenol
1·184	1·184	24-Methylene cycloartanol	1·090	1·090	24-Methylene cycloartanol
1·204	1·204	Citrostadienol		1·100	Citrostadienol

[a] Retention times relative to that of β-sitosterol.

by the two chromatographic methods. The applicability of this method was tested by a sample of unsaponifiable matter extracted from soybean oil. The sample was separated by the direct GLC method; the amount of the chromatogram peaks, calculated after the GLC run, are given in column I of Table 4. The same sample was separated by TLC into five main zones, corresponding to the groups of hydrocarbons ($j = 1$), tocopherol isomers ($j = 2$), triterpene alcohol ($j = 3$), 4-methylsterol and aliphatic alcohols ($j = 4$), and sterols ($j = 5$). The fractions from $j = 1$ to 5 were separated individually by GLC into their components, and their percentage compositions are listed in columns from two through five of Table 4, respectively.

Comparison between the number of peaks and their RR_t of the direct GLC chromatogram and those of the TLC–GLC chromatograms proved that some peaks of the direct GLC method were composed of a number of overlapping compounds related to different groups of the unsaponifiable

TABLE 4

Quantitative Determination of the Components of Unsaponifiable Matter in Soybean Oil

Direct GLC separation of the total unsaponifiable matter			*Combined TLC–GLC separation of the components of unsaponifiable matter*						
Peak[a] number	RR_t	*Column 1 mg/g*	*Peak Number*	RR_t	*Columns*				
					2	*3*	*4*	*5*	*6*
0	0·725	0·106	0	0·725	37·000				0·086
1	0·780	0·207	1	0·780		40·000			0·212
				0·785	5·000				0·011
			2	0·840			91·000		0·561
2	0·840	0·456	H_{11}[b]	0·830	25·000				0·045
			A_7	0·845		2·300			0·012
			3	0·870				0·670	0·025
3	0·870	0·114	H_{12}[b]	0·870	25·000				0·059
			4	0·890			9·000		0·095
4	0·890	0·160	A_8	0·890		3·500			0·019
				0·895	8·000				0·018
5			5	0·900				1·330	0·051
6	0·940	0·754	6	0·940				20·000	0·760
				0·940		3·600			0·019
7	0·960	0·549	7	0·960				14·4	0·547
			8	0·960		8·900			0·047
I	1·000	2·360	9	1·000				63·600	2·416
			10	1·015					
			11	1·020		15·100			0·080
			12	1·020			39·300		0·063
14+									
15	1·040	0·073	13	1·040					
			14	1·040			42·200		0·068
15+									
16	1·090	0·170	15	1·090			18·500		0·030
			16	1·100		26·600			0·140

Column 1 contains the amounts (in mg/g) of the components of unsaponifiable matter separated by GLC without pre-fractionation by TLC.

Columns 2, 3 and 5 contain the composition (%) of hydrocarbon, aliphatic alcohols 4-methylsterol, and sterol.

Column 4 contains both tocopherols (in the upper part) and triterpene alcohols (in the lower part). The two parts are separated by the hatched square.

Column 6 contains the amounts of the components of unsaponifiable matter in mg/g.

[a] Peak identification: 0, squalene; 1,δ-tocopherol; 2,γ-tocopherol; 3, cholesterol; 4α-tocopherol; 5, brassicasterol; 6, campesterol; 7, stigmasterol; 8, obtusifoliol; 9, β-sitosterol; 10,45-avenasterol; 11, gramisterol; 12, β-amyrin; 13, Δ7-stigmasterol; 14, cycloartenol; 15, 24-methylenecycloartanol; and 16, citrostadienol. A_7 and A_8 denote aliphatic alcohols with 7 and 8 carbon atoms.

[b] H_{11} and H_{12} denote normal alkanes with 29 and 30 carbon atoms respectively.

matter; e.g. the peak at $RR_t = 1$ of the direct GLC chromatogram represented overlapped peaks of β-sitosterol (main constituent), obtusifoliol, β-amyrin, and gramisterol. Other components of the unsaponifiable matter were completely separable by the direct GLC method—squalene, at RR_t 0·72, stigmasterol, at RR_t 0·96, and cycloartenol, at RR_t 1·04 (Table 4). The amounts of these components, calculated after the direct GLC run, were assigned for the values of $C(1)_{GLC}$, $C(3)_{GLC}$, and $C(5)_{GLC}$, respectively, and the percentages in their groups, calculated by TLC–GLC method, were substituted for $C(1)_{TLC\text{-}GLC}$, $C(3)_{TLC\text{-}GLC}$, and $C(5)_{TLC\text{-}GLC}$, respectively. The calculation factors were determined by substituting in eqn (2) as follows:

$$\text{calculation factor of hydrocarbons, } R(1) = \frac{0{\cdot}106}{37{\cdot}0} = 0{\cdot}0029$$

$$\text{calculation factor of triterpene alcohols, } R(3) = \frac{0{\cdot}073}{42{\cdot}2} = 0{\cdot}0017$$

$$\text{calculation factor of sterols, } R(5) = \frac{0{\cdot}549}{14{\cdot}4} = 0{\cdot}038$$

The concentrations of the individual components of hydrocarbons ($j = 1$) are obtained by substituting the corresponding values of $[C(1,n)]_{TLC\text{-}GLC}$ (column 2 of Table 4) and $(RRF)_{cn(1)}$ (from Table 1) in the equation

$$C(1,n) = [C(1,n)]_{TLC\text{-}GLC} \times (RRF)_{cn(1)} \times 0{\cdot}0029 \tag{3}$$

Therefore, the concentration of squalene $[(C(1,1)]$ is given by

$$\text{squalene} = 37 \times 0{\cdot}80 \times 0{\cdot}0029 = 0{\cdot}086\ \text{mg/g}$$
$$n\text{-nonacosane} = 25 \times 0{\cdot}62 \times 0{\cdot}0029 = 0{\cdot}045\ \text{mg/g}$$

Similarly, the concentrations of the other components of hydrocarbons were calculated (column 6 of Table 4). The components of triterpene alcohols were obtained by substituting the corresponding value of $[C(3,n)]_{TLC\text{-}GLC}$ (lower part of column 4, Table 4) in the following relationship:

$$[C(3,n)] = [C(3,n)]_{TLC\text{-}GLC} \times 0{\cdot}95 \times 0{\cdot}0017 \tag{4}$$

and the values obtained for β-amyrin (0·063 mg/g), cycloartenol (0·068 mg/g), and 24-methylenecycloartanol (0·030 mg/g) are listed in column 6 of Table 4. Sterols ($j = 5$) were estimated from the relationship

$$C(5,n) = [C(5,n)]_{TLC\text{-}GLC} \times 1 \times 0{\cdot}038 \tag{5}$$

Therefore, brassicasterol, cholesterol, campesterol, stigmasterol, β-sitosterol

(n from 1 to 5) were obtained by substitution with the corresponding values from column 5 in eqn (5), and the results were cited in column 6 of the same table.

Tocopherol isomers and 4-methylsterol peaks overlapped with peaks of other group components and, therefore, their calculation factors were determined after the other three group components had been estimated. Calculation factor of the group of 4-methylsterols ($j = 4$) was obtained by substituting the concentration of citrostadienol for $C(4)_{GLC}$ in eqn (2). The concentration of citrostadienol is obtained by subtracting the concentration of 24-methylenecycloartanol (column 6 of Table 4) from the amount of peak at RR_t 1·09 of direct GLC (Column 1). Thus

$$R(4) = \frac{0{\cdot}17 - 0{\cdot}03}{26{\cdot}6} = 0{\cdot}0053$$

and the concentrations of obtusifoliol, gramisterol, and citrostadienol were estimated by substituting the respective value of $[C(4,n)]_{TLC\text{-}GLC}$ (column 5) in eqn (6):

$$C(4,n) = [C(4,n)]_{TLC\text{-}GLC} \times 1 \times 0{\cdot}0053 \tag{6}$$

The same processing method was followed to determine $R(2)$ of tocopherol isomers based on the amount of peak at RR_t 0·84, which represented γ-tocopherol, and peaks of two other overlapping components (n-nonacosane, 0·045 mg/g, and n-heptacosanol, 0·012 mg/g).

Therefore,

$$R(2) = \frac{0{\cdot}456 - (0{\cdot}045 + 0{\cdot}012)}{91} = 0{\cdot}0044$$

Consequently, α-tocopherol concentration $= 9 \times 2{\cdot}44 \times 0{\cdot}0044 = 0{\cdot}097$ mg/g, and γ-tocopherol $= 91 \times 1{\cdot}4 \times 0{\cdot}0044 = 0{\cdot}561$ mg/g. Application of this method to the analysis of eleven different vegetable oils resulted in a set of data for sterol, 4-methylsterol and aliphatic alcohol, triterpene alcohol, tocopherol, and hydrocarbon compositions of these pure oils. The unit of concentration (mg/g oil) used to express the results of this method provided useful data for the determination of oil purity. The units of concentration of the component of unsaponifiable matter in the total oil (mg/g oil) was found to be more significant in this respect than the percentage of each component in the whole respective group. The method of the present work is especially helpful with the components which exist in trace amounts in some refined oils. It also enables accurate estimation of the readily decomposed tocopherol isomers.

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