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A PACKED COLUMN GAS LIQUID CHROMATOGRAPHIC METHOD FOR CHARACTERIZATION AND DETERMINATION OF UNSAPONIFIABLE MATTER IN OILS

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ABSTRACT

A packed column GLC method for the detection and determination of the components of unsaponifiable matter in oils is described. Squalene, δ −tocopherol and short chain hydrocarbons with up to 26 carbon atoms are determined by direct GLC analysis of the total unsaponifiable matter. Sterols are isolated from the unsaponifiable matter as digitonides and determined according to the standard method of the AOAC(1982), after a prior chromatographing of the fraction containing the remaining components, obtusifoliol, n-triacontane, △7-stigmasterol, cycloartenol and α-tocopherol are determined. Citrostadienol and 24-methylenecycloartanol are quantified by GLC analysis of the solution containing their acetate derivatives. Data obtained by the present method for detection and determination of unsaponifiable matter in a number of oil brands agree fairly well with their known composition.

INTRODUCTION

The unsaponifiable matter of vegetable oils is a complex material consisting of many components including sterols, 4-methylsterols, triterpene alcohols, hydrocarbons, aliphatic alcohols and tocopherol isomers. They possess characteristic composition in the different oils. Determination of the composition of unsaponifiable matter is interesting for identification of oils and assessment of their purity. The current methods for the quantitative analysis of the components of unsaponifiable matter are time consuming and utilizing combined techniques of TLC-GLC (Itoh *et. al.*, 1963) or the combined column chromatography −GLC (Eisner and Firestone, 1963; Eisner *et. al.*, 1966 and Moura *et. al.*, 1975). The development of a simplified procedure for the determination of the concentration of unsaponifiable components was the subject of several previous research reports (Pyle *et. al.*, 1976 and Abou-Hadeed *et. al.*, 1990). These studies, however, followed preparative chromatographic techniques employing both TLC and GLC.

In the present paper, a new method employing a simple packed column GLC technique is described for quantitative determination of the components of sterols, 4-methylsterols, triterpene alcohols, hydrocarbons and tocopherols in some oils.

MATERIALS AND METHODS

Materials:

Individual standard cholesterol, brassicasterol, campesterol, stigmasterol, β-sitosterol, △7-stigmasterol, α-tocopherol and γ-tocopherol were obtained from E. Merk, Darmstadt (F.R.G.) Digitonine, acetic anhydride, solvents and other chemicals were also obtained from the same source. The solvents were of chromatographic grade and used without distillation. A mixture of triterpene alcohols (α-amyrin, β-amyrin and cycloartenol) were donated by Scientific and Applied Research Center, University of Qatar. Oil samples were selected from the commercial brands available in the local market of Doha.

Methods:

Preparation of unsaponifiable matter:

The unsaponifiable matter was separated from the oil using the standard method of the AOAC(1982) and 2g of oil with an appropriate quantities of the reagents.

Separation of sterols as their digitonide derivative:

The dry residue was heated to 50°C and 10 ml of 1% (w/v) alcoholic digitonine solution was added, followed by 20 ml alcohol and 5 ml distilled water. The flask was heated to 50°C for 30 minutes with occasional shaking then kept at room temperature for 2 hours. The precipitate of sterol digitonide formed was filtered, successfully washed with 30 ml alcohol and 2 times with 25 ml ether, transferred quantitatively into a 50 ml conical flask and dissolved in n-hexane. The ethereal phase of the filtrate and washings mixture, containing the remaining components of the unsaponifiable matter (filtrate fraction), was separated from the aqueous layer, washed with distilled water, dried, evaporated to a volume of 4 ml and kept for GLC analysis.

Preparation of acetate derivatives:

Sterol digitonide precipitate and the components in the filtrate fractions of the other unsaponifiable group component mixtures were converted into their acetate derivatives by heating their dry residues with acetic anhydride according to the method of the IUPAC (1979).

Gas liquid chromatography

The assay was conducted on a Packard model 439 GLC equipped with a flame ionization detector and connected to a chrompac CR-3A integrator. The GLC was fitted with 2m x 2mm (id) glass column packed with 10% SE30 on chromosorb W and operated at temperature programme retaining 200°C for 3 minutes and raised to 270°C at 5°C/min. The detector and injector were kept at 320°C.

Preparation of chromatographic standards:

Standard solutions of known concentration of the individual unsaponifiable components were prepared. Individual mixtures of sterols, 4-methylsterols, triterpene alcohols, tocopherols and hydrocarbons were also prepared by mixing together known weights of each group components. The chromatograph was calibrated using these mixtures by external standard method (Table 1, column 1). A portion of each standard mixture was individually treated with digitonine according to the method described above and the filtrate fractions were recovered and chromatographed individually. Calculation factor (CF) was determined for each component from the following relationship and recorded with its Rt value (Table 1, column 2).

$$CF = \frac{\text{known concentration of the component}}{\text{concentration evaluated by GLC}}$$

The sterol digitonide precipitate and the components in the filtrate fractions from the other group mixtures were then converted into acetate derivatives, chromatographed again individually and another similar Rt and CF values were measured (Table 1, columns 4 and 3, respectively). The actual concentration of each of the unsaponifiable components in an oil was determined by multiplying its peak area as determined by GLC times the calculation factor of the derivative of this component (Table 2).

RESULTS AND DISCUSSION

The Rt data of the unsaponifiable components in column 1 (Table 1) indicated that γ-tocopherol, squalene and the aliphatic hydrocarbons with even number of carbon atoms from 22 to 26 were eluted as single component peaks at Rt 17.50, 18.03, 8.14, 11.00, 14.28 min respectively. The concentrations of these components in oils were estimated simply by GLC analysis of the unsaponifiable matter as a total. The data of column (1) also showed that the Rt of obtusifoliol, gramisterol, β-amyrin and β-sitosterol were close to each other, and when the unsaponifiable matter in different vegetable oils were chromatographed as a total, one major peak only was

Table 1

Retention times and calculation factors of some components in the solutions of the different preparations of unsaponifiable matter.

Component name	Column 1		Column 2		Column 3		Column 4	
	Rt	CF*1	Rt	CF*2	Rt	CF*1	Rt	CF*2
n-Docosane	8.14	1.114	8.21	1.190				
n-Tetracosane	11.00	1.114	11.15	1.300				
n-Hexacosane	14.28	1.114	14.31	1.110				
Squalene	17.50	1.262	17.69	1.316				
n-Nonacosane	20.50	1.319	20.56	1.230				
n-Triacontane	21.09	1.450	21.25	1.552				
γ-Tocopherol	18.03	2.104	18.30	2.602	24.40	2.563		
α-Tocopherol	21.90	3.600	22.17	3.982	25.90	4.091		
Obtusifoliol	23.91	1.625	24.04	2.568				
Gramisterol	25.10	1.643	25.25	2.622				
Citrostadienol	28.77	1.617	29.06	2.560	34.42	3.540		
β-Amyrin	25.34	1.651	25.61	2.532				
Cycloartenol	26.66	1.651	26.94	2.532				
24-Methylene-cycloartanol	28.54	1.651	28.83	2.532	33.25	3.630		
Cholesterol	21.15	1.597					24.20	1.710
Brassicasterol	22.09	1.607					25.33	1.805
Campesterol	23.03	1.612					26.26	1.772
Stigmasterol	23.94	1.627					27.26	1.785
β-Sitosterol	25.16	1.602					29.12	1.730
Δ7-Stigmasterol	26.96	1.460	29.90	1.791				

Column 1: Free components. **Column 2:** Solution remained after separation of sterol digitonide. **Column 3:** Acetate derivatives of the unsaponifiable matter. **Column 4:** Sterol acetate. *1 Response factor X 10^{-6}. *2 Calculation factor X 10^{-6}.

Table 2

Estimation of the unsaponifiable components in a corn oil sample.

Peak No.	Rt	Component name	Column 1*¹ a	Column 1*¹ b	Column 2	Column 3	Column 4	mg/g of oil
1.	8.20	n-Docosane	6					0.007
2.	11.05	n-Tetracosane	8					0.009
3.	14.31	n-Hexacosane	16					0.018
4.	17.55	Squalene	254					0.320
5.	19.50	n-Nonacosane	23					0.030
6.	18.03	γ-Tocopherol	15					0.032
7.		Triacontane			24	26		0.037
	21.12		30	0.044				
8.		Cholesterol					3	0.005
9.		α-Tocopherol			3.5			0.014
	22.10		5	0.018				
10.		Brassicasterol						0.000
11.	23.16	Campesterol	1340	2.160			1225	2.170
12.	23.98	Stigmasterol	350	0.569			750	1.339
13.		Obtusifoliol			50			0.128
14.		β-Sitosterol					3021	5.226
	25.20		3436	5.511			3021	5.226
15.		Gramisterol						
					90			0.234
16.		β-Amyrin						

Contd. Table 2

Estimation of the unsaponifiable components in a corn oil sample.

Peak No.	Rt	Component name	Peak areas					mg/g of oil
			Column 1*[1]		Column 2	Column 3	Column 4	
			a	b				
17.		Cycloartenol			120			0.303
	26.81		290	0.459				
18.		Δ7-Stigmasterol			90			0.161
19.		24-Methylene-cycloartanol				37		0.131
	28.62		150	0.245		28		0.101
20.		Citrostadienol				28		0.101

Column 1: Free components. **Column 2:** Solution remained after separation of sterol digitonide. **Column 3:** Acetate derivatives of the unsaponifiable matter. **Column 4:** Sterol acetate. **Column 5:** Concentration of the unsaponifiable components as mg/g of oil.

[1] a: The peak areas of the composite peak as measured by GLC b: The sum of the quantities of the overlapping components.

eluted at Rt 25.2 min (chromatogramme 1 and Table 2). On further TLC-GLC analysis of these oils it was confirmed that this peak was consisting of the same four components peaks.

The difference between the Rt of cholesterol and that of n-triacontane (21.15 and 21.09 min, respectively) was small. The concentration of the latter component was relatively higher in sunflower oil than the other vegetable oils (Table 3) and obscuring the minor peak of cholesterol at Rt 21.12 min. Brassicasterol and α-tocopherol eluted at Rt 22.09 and 21.9 min, respectively when injected individually (Table 1) and when the unsaponifiable matter of a vegetable oil containing the 2 components was chromatographed (chromatogramme 1) they appear as one peak only at Rt 22.1 min (Table 2). In sunflower, soybean, cottonseed and corn oils, the levels of brassicasterol is negligibly small compared with the level of α-tocopherol while in rapeseed oil brassicasterol predominates (Abou-Hadeed and Kotb, 1988). △7-Stigmasterol eluted at Rt 26.96 min which was close to the Rt of cycloartenol (26.66 min). The peaks of the 2 components in the total unsaponifiable matter chromatogramme were overlapping at Rt 26.81 min (Table 2). △7-Stigmasterol concentration is significantly high in sunflower oil (Abou-Hadeed, 1987) while, in the contrary, olive, sunflower and corn oils contain higher concentration levels of cycloartenol. The retention times of 24-methylenecycloartanol and citrostadienol (Table 1, column 1) were also close to each other (28.54 and 28.77 min, respectively) and they appear as one peak at Rt 28.62 min in the total unsaponifiable matter chromatogramme (Table 2).

When the standard mixtures of group components of the unsaponifiable matter were treated individually with digitonine according to the method described above and the filtrate from each mixture chromatographed (Table 1, column 2) it was found that the filtrate from the sterol mixture was containing △7-stigmasterol only which eluted at Rt 29.9 min. The other sterols formed insoluble digitonide and were separated from the solution by filtration. The major difference between the Rt values of △7-stigmasterol in the chromatogrammes of the original sterol mixture and its filtrate fraction (2.94 min) indicated the formation of soluble △7-stigmasterol digitonine. On the other hand the small differences between the Rt values of the components of 4-methylsterols and triterpene alcohols (Table 1, columns 1 and 2) revealed that these components remained unreacted with digitonine. When the filtrate fraction obtained after treating the unsaponifiable matter of an oil with digitonine was chromatographed (chromatogramme 2), the peak of △7-stigmasterol was, contrary to its peaks in chromatogramme (1), eluted separately from that of cycloartenol and both components were evaluated individually. All sterols, except △7-stigmasterol digitonide, disappeared from chromatogramme (2) demasking the minor components which were covered by them in chromatogramme (1). Thus n-triacontane, α-tocopherol and obtusifoliol appeared in chromatogramme (2) as single component peaks at 21.25, 22.17 and

24.04 min, respectively and their concentrations were estimated from their peak areas (Table 2). The peaks of 24-methylenecycloartanol and citrostadienol (Table 2) were still overlapping at R_t 28.95 min in chromatogramme (2). However, when these components were converted into their acetate derivatives and chromatographed (chromatogramme 3), they appeared as 2 separate peaks at Rt 33.25 and 34.42 min respectively (Table 1, Column 3) and measured individually from their peak areas. The peaks of β-amyrin and gramisterol were also overlapped at Rt 25.40 min in chromatogramme (2) but they remained overlapped even after converting them into their acetate derivatives (chromatogramme 3). Meanwhile the peaks of △7-stigmasterol acetate and cycloartenol acetate overlapped again and appeared as one peak in chromatogramme (3).

The precipitate from the sterol mixture containing cholesterol, brassicasterol, campesterol, stigmasterol and β-sitosterol was easily assayed by GLC as acetate derivative according to the standard method of AOAC (1982).

The data obtained by applying this method to the determination of the unsaponifiable matter in number of vegetable oils are listed in columns (A) of Table (3). The composition of each oil unsaponifiable matter, obtained by another method (Abou-Hadeed, 1990) are depicted in columns (B) of the same Table. Generally, there was a good agreement between the data obtained for each oil sample by the present method and those previously reported.

In conclusion, the GLC method described in the present work is simple and fast. Sample preparation and manipulation offers parallel routine determinations of large numbers of sampls. At present this method is employed in a survey study of the purity of the commercial brands of the vegetable oils available in the markets of the Arabian countries of the Gulf.

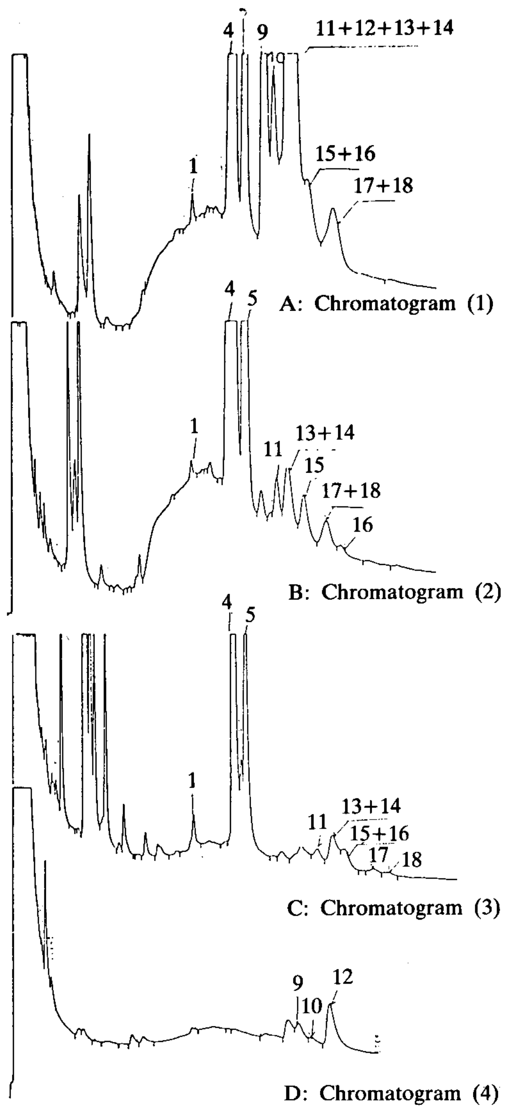

Fig. 1: Packed column gas liquid chromatographic analysis of the unsaponifiable matter of a sesame oil sample.
Chromatogramme (1): The total unsaponifiable matter; Chromatogramme (2): The unsaponifiable matter of the components in the filtrate fraction obtained after treatment with digitonine according to the paper's method. Chromatogramms (4 and 3): Acetate derivatives of sterols and the components in the filtrate fraction of the unsaponifiable matter. For peak identification see Table (2).

Table 3

The concentration of the components of unsaponifiable matter in a number of vegetable oils, determined by the method of present work (A) and by a TLC-GLC method (B).

Peak No.	Component	Concentration mg/g of oil									
		I		II		III		IV		V	
		(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)	(A)	(B)
1.	n-Docosane	0.019	0.021	0.003	0.000	0.009	0.011	0.000	0.000	0.012	0.007
2.	n-Tetracosane	0.029	0.014	0.011	0.010	0.011	0.007	0.000	0.000	0.000	0.000
3.	n-Hexacosane	0.038	0.021	0.008	0.005	0.028	0.025	0.000	0.000	0.000	0.000
4.	Squalene	0.576	0.330	0.345	0.330	0.071	0.126	0.170	0.156	0.114	0.320
5.	n-Triacontane	0.230	0.255	0.031	0.026	0.008	0.006	0.330	0.305	0.000	0.000
6.	γ-Tocopherol	0.269	0.273	0.547	0.615	0.943	1.066	0.100	0.088	0.000	0.000
	Sesamine	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.770	1.598
	sesamoline	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.652	0.650
8.	Cholesterol	0.005	0.003	0.000	0.000	0.014	0.002	0.011	0.009	0.006	0.004
9.	α-Tocopherol	0.112	0.143	0.100	0.086	0.226	0.209	0.293	0.226	0.000	0.000
10.	Brassicasterol	0.000	0.002	0.002	0.003	0.051	0.027	0.059	0.030	0.190	0.205
11.	Campesterol	1.678	1.858	1.304	1.198	0.716	0.770	0.625	0.588	2.170	1.896
12.	Stigmasterol	0.579	0.571	0.613	0.413	0.575	0.559	0.699	0.551	0.344	0.430
13.	Obtusifoliol	0.035	0.036	0.048	0.038	0.053	0.017	0.146	0.198	0.153	0.160
14.	β-Sitosterol	6.06	6.213	4.450	4.144	2.056	2.545	2.059	1.947	3.500	3.677
15.	Gramisterol + β-Amyrin	0.037	0.044	0.151	0.126	0.145	0.058	0.108	0.125	0.152	0.078
17.	Cycloartenol	0.303	0.237	0.118	0.192	0.095	0.092	0.320	0.288	0.166	0.120
18.	Δ7-Stigmasterol	0.015	0.022	0.048	0.040	0.036	0.029	0.423	0.416	0.000	0.000
19.	24-Methylene-cycloartanol	0.181	0.207	0.343	0.280	0.045	0.053	0.185	0.167	0.089	0.094
20.	Citrostadienol	0.044	0.076	0.154	0.121	0.062	0.068	0.249	0.275	0.097	0.083

Column I: Crude corn oil. **Column II:** Refined corn oil. **Column III:** Crude soybean oil. **Column IV:** Refined sunflower oil. **Column V:** Commercial sesame oil.

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تحليل المواد غير القابلة للتصبن في الزيوت بإستخدام الكروماتجراف الغازي ذو عمود محشو

أشرف محمد فريد أبو حديد

يصف هذا البحث طريقة جديدة لتحليل المواد غير القابلة للتصبن في الزيوت النباتية بطريقة مبسطة مستخدماً الكروماتوجراف الغازي المزود بعمود محشو .

تعتمد الطريقة على تقدير السكوالين والتوكوفيرول وبعض الهيدروكربونات ذات عدد ذرات كربون أقل من ٢٦ عن طريق حقن المواد غير القابلة للتصبن مباشرة في الكروماتوجراف ، يلي ذلك فصل الستيرولات من محلول المواد غير القابلة للتصبن بواسطة تحويلها إلى راسب ديجيتونات وترشيحها ثم تحليل المواد المتبقية في المحلول كروماتوجرافيا مرة أخرى لتقدير ألفا - توكوفيرول وأوبتوسيفوليول وسيكلوأرتينول والتراياكونتين . يتم تقدير مادتي السيتوستادينول و ٢٤ ميثيلينسيكلوارتانول بواسطة تحويلهما إلى مشتق الاسيتات ثم تحليلهما كروماتوجرافيا .

أما الستيرولات فتقدر بالتحليل الكروماتوجرافي لمشتق الاسيتات لها حسب طر AOAC المعروفة .

عند مقارنة نتائج تحليل عدد من الزيوت بإستخدام هذه الطريقة مع نتائج أحد الطرق المعروفة الأخرى - وجد أن هذه الطريقة تتصف بالدقة فضلاً عن سهولة إجرائها .