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Analytical Methods Section

Steryl Ester Analysis as an Aid to the Identification of Oils in Blends

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Sixty steryl esters have been synthesised in simple mixtures and used to optimise conditions for the separation of steryl esters by high performance liquid chromatography (HPLC) and gas chromatography (GC). Both techniques give good separations of many steryl esters, but the techniques complement each other since the elution sequence differs.

A steryl ester-rich fraction has been isolated from 14 oil samples by thin-layer chromatography and analysed by GC and HPLC. GC analysis is more sensitive and faster than HPLC, and also has the advantage that residual triacylglycerols are separated from the steryl esters. The pattern of chromatographic peaks is similar for different samples of the same oil, but varies widely for different oils. It is therefore concluded that GC analysis of the intact steryl ester fraction may be useful as a technique for identifying mixtures of oils.

INTRODUCTION

Edible oils are commonly blended in order to prepare suitable products, using raw materials which vary in cost and availability. In order to monitor trade in edible oils, it is important that good analytical procedures are available to detect and identify oils used in blends. Triacylglycerol or fatty acid methyl ester (FAME) analyses are limited in their ability to identify admixtures of oils, although they may be very useful in particular cases such as detecting and quantifying cocoa butter equivalents in cocoa butter (Padley & Timms, 1980) or detecting the presence of lauric fats in nonlauric oils. Other methods used to differentiate oils and identify mixtures include pyrolysis – GC (Nazer *et al.* 1985) and lipolysis – FAME analysis, which is used to detect adulteration of lard (Sonntag, 1982). However, a combination of FAME analysis and analysis of minor components appears to be necessary to quantify oils in blends in many cases. A combination of FAME, sterol and tocopherol analyses is adequate for some blends (Van Niekerk & Burger, 1985), and a method based on FAME, 4-methylsterols, triterpene alcohols, tocopherols and squalene analysis has also been developed (Abu-Hadeed & Kotb, 1988).

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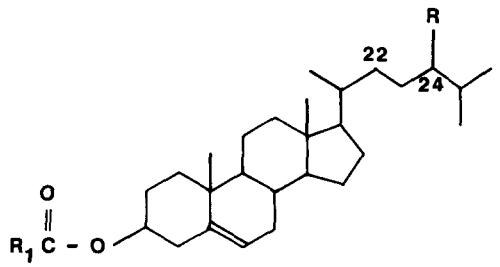
One problem with characterising oils by their minor components is that compounds may be removed to varying extents during the refining of oils. Neutralisation, bleaching and deodorisation reduce the level of tocopherols in processed soybean oil (Gutfinger & Letan, 1974), and the sterol content of oils is also reduced by refining.

Sterols may be lost during the bleaching stage (Johansson & Hoffman, 1979) and during the neutralisation and deodorisation stages (Gutfinger & Letan, 1974). The composition of the sterol fraction may also change during refining. Campesterol and stigmasterol are reduced to a greater extent than β-sitosterol during physical refining (Jawad, *et al.*, 1984). Clearly the magnitude of the losses of minor components depends to a large extent on the refining conditions used.

Steryl esters are lost to a much smaller extent than sterols during oil refining (Johansson & Hoffman, 1979) and these components may, therefore, be useful in identifying either crude or refined oils in blends. Refined oils may undergo interesterification at deodorisation temperatures. Steryl esters (Fig. 1) are usually analysed by saponification and analysis of the sterols and fatty acids present, but this procedure loses all information about the combination of the sterols with particular fatty acids.

Analysis of cholesteryl esters by gas chromatography (GC) has been performed on short packed columns (Ross *et al.*, 1984) and nonpolar capillary columns (Novotny *et al.*, 1972; Smith, 1982). Separation can be



R IS THE STEROL MOIETY SIDE CHAIN:

CHOLESTEROL	R = H
β-SITOSTEROL	R = C_2H_5
CAMPESTEROL	R = CH_3
STIGMASTEROL	R = C_2H_5, Δ^{22}
Δ^5- AVENASTEROL	R = $CH(CH_3)$, $\Delta^{24(28)}$
BRASSICASTEROL	R = CH_3, Δ^{22}

R_1COOH IS A FATTY ACID:

SATURATED ACIDS FROM 8:0 - 20:0

PLUS 18:1, 18:2, 18:3.

Fig. 1. Structures of steryl esters.

achieved mainly on the basis of carbon number on these columns, but little separation on the basis of unsaturation can be achieved. However, the use of a polar stationary phase allows the separation of cholesteryl esters varying in unsaturation (Smith, 1983). Phytosterol esters of plant oils and microbial oils have also been studied using a nonpolar capillary column (Evershed & Goad, 1987; Evershed *et al.*, 1987; Fenner & Parks, 1989), and the esters of three sterols have been separated on a polar SP-2330 capillary column (Kuksis *et al.*, 1986).

Reversed-phase high performance liquid chromatography (HPLC) has been employed successfully to separate mixtures of cholesteryl esters (Duncan *et al.*, 1979; Carroll & Rudel, 1981; Chu & Schroepfer, 1988) and has also been applied to phytosteryl esters (Billheimer *et al.*, 1983; Evershed & Goad, 1987; Kuksis *et al.*, 1986). It has been found that separation is achieved according to carbon number and degree of unsaturation by this procedure. Sensitivity of detection is poor if a UV detector is used and 10 to 50 μg of each component is required with this method of detection (Carroll & Rudel, 1981).

This paper describes the chromatographic analysis of steryl esters by GC and HPLC and the application of these procedures to the analysis of a nonglyceride lipid fraction of oils, which mainly comprises steryl esters.

MATERIALS AND METHODS

Δ^5-Avenasterol was isolated from green algae, *Ulva lactuca*, and brassicasterol was isolated from rapeseed oil (Griffith, 1989). Stigmasterol, cholesterol, a mixture of β-sitosterol and campesterol, oleic acid, linoleic acid, linolenic acid, capric acid, caprylic acid, lauric acid, myristic acid, palmitic acid, stearic acid and arachidic acid were purchased from Sigma Chemical Co.

Simple mixtures of saturated steryl esters were synthesised from each sterol and a mixture of all even numbered saturated fatty acids from 8:0 to 20:0 via the fatty acid chloride formed with thionyl chloride (Kuksis & Beveridge, 1960). Each sterol was also esterified with a mixture of oleic acid (18:1), linoleic acid (18:2) and linolenic acid (18:3) via the fatty acid chloride formed with oxalyl chloride (Kuksis & Beveridge, 1960). The mixture of β-sitosterol and campesterol was used for these syntheses without prior separation of the sterols.

The oils analysed were all commercial samples except for the following samples, which were solvent extracted with hexane in the laboratory: cocoa butter (Ivory Coast beans) rapeseed oil–2 (New Zealand, south island seeds); rapeseed oil–3 (New Zealand, north island seeds), and sunflower oil–2 (French seeds).

The corn oil was a produce of the UK, purchased from Asda Stores. Extra virgin olive oil was 'Filippo Berio', packed and bottled in Lucca, Italy. Grapeseed oil was purchased from Asda Stores, produce of Italy. Groundnut oil was 'Epicure', produce of more than one country. Olive oil was 'Filippo Berio', packed and bottled in Lucca, Italy. Palm oil was crude oil supplied by Anglia Oils Ltd. Rapeseed oil–1 was 'Golden Fields', produce of the UK. Safflower oil was 'Epicure' produce of more than one country. Soyabean oil and sunflower oil–1 were purchased from Asda Stores.

Isolation of steryl esters

The steryl ester fraction was isolated by thin-layer chromatography (TLC) on 20 cm × 20 cm Silica gel G 60 plates (0·75 mm thick).

A sample of oil (1 g) containing cholesteryl palmitate (2·0 mg) as an internal standard, dissolved in a minimum amount of chloroform, was carefully applied to five plates in a narrow band. The solvent mixture for development was hexane–diethyl ether–acetic acid (80:20:2). The developed plates were sprayed with 0·2% (m/v) solution of 2', 7'–dichlorofluorescein in ethanol. The bands were located under UV light; lipids showed up as yellow/ green bands on a purple background. The bands corresponding to the steryl ester fraction were then carefully scraped off the plates, combined and placed in a column. The steryl esters were eluted with 5% diethyl ether in hexane containing 0·01% TBHQ (*t*–butylhydroquinone). The fraction was then dried over anhydrous sodium sulphate, and the solvent was removed under vacuum.

For samples where analytical TLC indicated excessive contamination of the steryl ester fraction with triacylglycerols, an additional purification step involving

sorbent extraction (Kaluzny *et al.*, 1985), using a 500 mg Bond Elut aminopropyl column, was included.

GC analysis of steryl ester fraction

The GC analysis was performed on a Perkin-Elmer 8500 chromatograph fitted with a cold on-column injector and flame ionisation detector. The analytical column was a 25 m × 0·25 mm i.d. WCOT capillary column containing a 0·1 μm thick, bonded tri-glyceride analysis phase (TAP) (50% phenyl, 50% methyl polysiloxane) supplied by Chrompack. The carrier gas was hydrogen (2·5 ml min^{-1}) with nitrogen as make-up gas (40 ml min^{-1}). The oven temperature programme was 120°C (0·2 min), 30°C min^{-1} to 250°C, 5°C min^{-1} to 355°C, and isothermal at 355°C. The detector temperature was 400°C. A Hewlett-Packard model 3390A integrator was used for quantification.

HPLC

Reversed-phase HPLC analysis of steryl ester mixtures was performed at room temperature using a Spectra Physics SP 8800 ternary HPLC pump, and an Applied Chromatography Systems mass detector with the evaporation temperature set at 55°C. Samples were injected in ethyl acetate via a 5 μl Rheodyne loop injector. The column comprised a 1 cm guard column and two 10 cm × 3 mm i.d. Spherisorb ODS-2 (particle size 5 μm) cartridges supplied by Chrompack. The solvent used was a linear gradient of 55% acetonitrile–water (9:1) and 45% ethyl acetate changing to 20% acetonitrile–water (9:1) and 80% ethyl acetate in 80 min. The flow rate was 0·4 ml min^{-1}. A Hewlett–Packard model 3396A integrator was used for the quantification.

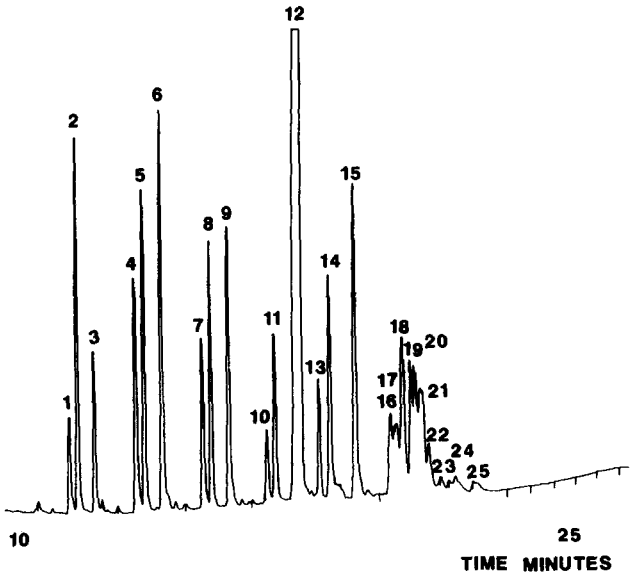


Fig. 2. GC analysis of 31 steryl esters prepared from β-sitosterol, campesterol and stigmasterol, with cholesteryl palmitate as internal standard.

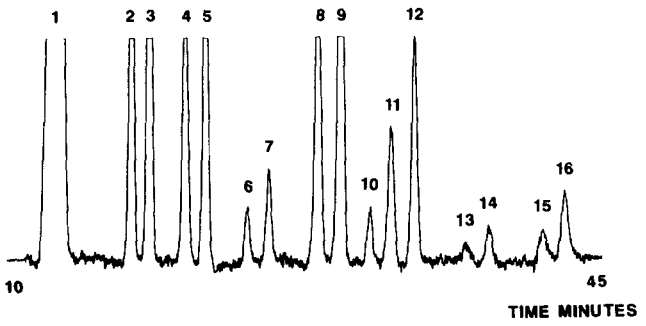
RESULTS AND DISCUSSION

GC analysis on the 50% phenyl, 50% methyl polysiloxane capillary column allowed many steryl esters to be separated, but coelution occurred in some cases. Thus, the analysis of a mixture of 31 steryl esters pre-

Table 1. Relative retention time values for the steryl esters analysed by GC on a 50% methyl, 50% phenylpolysiloxane capillary column

	Sterol moiety					
Fatty acid moiety	Cholesterol	Brassicsterol	Campesterol	Stigmasterol	β-sitosterol	Δ^5-avenasterol
8:0	0·61	0·64	0·66	0·67	0·70	0·73
10:0	0·71	0·74	0·76	0·77	0·80	0·83
12:0	0·81	0·84	0·86	0·87	0·90	0·92
14:0	0·90	0·93	0·96	0·97	1·00	1·01
16:0	1·00	1·02	1·05	1·06	1·08	1·10
18:0	1·09[a]	1·11[a]	1·13[a]	1·15	1·17	1·18
18:1	1·09[a]	1·11[a]	1·13[a]	1·16	1·19	1·19
18:2	1·10	1·12	1·15	1·17	1·20	1·20
18:3	1·12	1·14	1·16	1·18	1·22	1·21
20:0	1·17	1·19	1·22	1·23	1·25	1·25

[a] Steryl esters of 18:0 and 18:1 were separable.



1	Ch 2:0	9	S 14:0
2	C 8:0	10	Ch 16:0
3	S 8:0	11	C 16:0
4	C 10:0	12	S 16:0
5	S 10:0	13	C 18:0
6	C 12:0	14	S 18:0
7	S 12:0	15	C 20:0
8	S 14:0	16	S 20:0

Fig. 3. HPLC analysis of saturated steryl esters of β-sitosterol and campesterol.

pared from β-sitosterol, campesterol and stigmasterol, together with the internal standard cholesterol palmitate, gave 25 peaks (Fig. 2). The steryl esters prepared from cholesterol, brassicasterol, campesterol, stigmasterol, β-sitosterol and Δ^5-avenasterol and fatty acids 8:0–20:0, 18:1, 18:2 and 18:3 eluted with relative retention times of 0·61 to 1·25 (Table 1).

Inspection of the elution sequence shows that steryl esters are eluted in the sequence of increasing number of carbon atoms and carbon-carbon double bonds in general, although a double bond in the sterol side chain at C-22 reduced the retention time, causing stigmasteryl 18:0 to elute before β-sitosteryl 18:0.

The elution sequence on the GC column differs from that on the reversed-phase HPLC column. In the latter technique, increasing numbers of carbon atoms caused an increase in retention time, but an increase in the number of carbon–carbon double bonds caused a reduction in retention time (Table 2.) The magnitude of the effect of a double bond varies with position, with reductions in the order fatty acid double bond >C–24>C–22 being evident since retention times increase in the sequence β-sitosteryl 18:1<Δ^5-avenasteryl 18:0<stigmasteryl 18:0. In general, HPLC analysis gave a good separation of steryl esters, as shown for the saturated esters of β-sitosterol and campesterol (Fig. 3).

Table 3. Capillary GC of standard mixtures of cholesteryl esters to assess the detector response

Sample	$x^{a,b}$	$6^{a,c}$	$6/x \times 100^d$
Cholesteryl 12:0	0·97	0·013	1·34
Cholesteryl 14:0	0·99	0·011	1·11
Cholesteryl 16:0	1·03	0·008	0·80
Cholesteryl 18:0	1·13	0·121	10·69
Cholesteryl 18:1	1·09	0·049	4·45
Cholesteryl 18:2	1·40	0·215	15·38
Cholesteryl 18:3	1·51	0·131	8·68

[a] 10 determinations performed.
[b] Mean response factor relative to cholesteryl acetate = 1.
[c] Standard deviation.
[d] Coefficient of variation.

The response factors of cholesteryl esters relative to cholesteryl acetate were investigated in both the GC (Table 3) and HPLC analytical systems (Table 4). Good reproducibility was achieved in the quantification of the steryl esters, and the relative response factors were in the range 0·97–1·51 for GC analysis and 1·06–1·70 for HPLC analysis. The minimum level of detection was less than 1 ng for GC analysis and 2 to 5 μg for HPLC analysis. The latter value for analysis with a mass detector compares with the range 10–50 μg quoted as a detection limit for HPLC analysis with a UV detector (Carroll & Rudell, 1981).

The steryl ester fraction of 14 oil samples was separated by TLC, and analysed by GC and HPLC. Both GC and HPLC analysis provided a useful separation of steryl esters (see Figs 4 and 5), but GC analysis was preferred due to greater sensitivity and speed, together

Table 2. Relative retention times for the steryl esters analysed by reversed phase HPLC on a C-18 column

Fatty acid moiety	Sterol moiety					
	Cholesterol	Brassicasterol	Campesterol	Stigmasterol	β-sitosterol	Δ^5-avenasterol
8:0	0·48	0·48	0·55	0·53	0·58	0·48
10:0	0·58	0·57	0·65	0·63	0·69	0·58
12:0	0·69	0·69	0·77	0·76	0·81	0·69
14:0	0·86	0·82	0·90	0·89	0·94	0·81
16:0	1·00	0·96	1·03	1·03	1·08	0·94
18:0	1·14	1·10	1·18	1·17	1·22	1·08
18:1	0·95	0·92	1·00	0·99	1·04	0·91
18:2	0·80	0·77	0·84	0·83	0·88	0·75
18:3	0·67	0·66	0·77	0·72	0·76	0·66
20:0	1·30	1·22	1·32	1·31	1·37	1·22

Table 4. Reversed phase HPLC of standard mixtures of cholesteryl esters to assess the detector response

Sample	$x^{a,b}$	$6^{a,c}$	$6/x \times 100^{d}$
Cholesteryl 12:0	1·06	0·060	5·88
Cholesteryl 14:0	1·08	0·085	7·88
Cholesteryl 16:0	1·12	0·074	6·63
Cholesteryl 18:0	1·41	0·101	7·20
Cholesteryl 18:1	1·45	0·189	13·09
Cholesteryl 18:2	1·40	0·085	6·10
Cholesteryl 18:3	1·70	0·142	8·36

[a] 12 determinations performed.
[b] Mean response factor relative to cholesteryl acetate = 1.
[c] Standard deviation.
[d] Coefficient of variation.

with the fact that any residual triacylglycerols in the isolated steryl ester fraction eluted after the steryl esters and did not interfere with the section of the chromatogram of interest. Thus, the triacylglycerols of rapeseed oil began to elute after 24·5 min in the GC analysis, while the steryl esters eluted within 23·5 min (Fig. 4). In contrast, the HPLC chromatograms of the steryl esters and triacylglycerols both show many peaks eluting between 10 and 45 min (Figs 5 and 6).

In cases where triacylglycerol contamination of the steryl ester fraction was excessive, an additional sorbent extraction step was included. The peaks detected

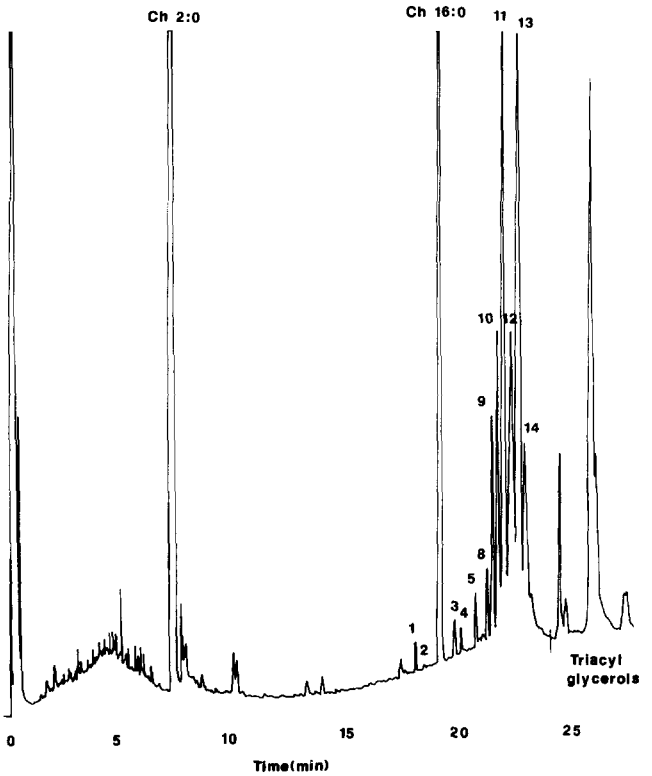


Fig. 4. GC analysis of the steryl ester fraction of rapeseed oil including some triacylglycerols (cholesteryl acetate and cholesteryl palmitate added as internal standards).

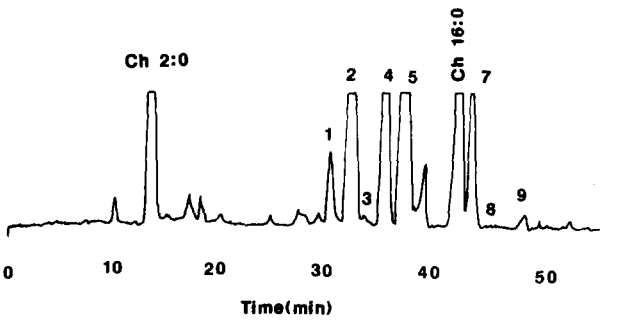


Fig. 5. HPLC analysis of the steryl ester fraction of rapeseed oil.

in the steryl ester fractions of the oils are shown in Table 5, and the major peaks present are summarised in Fig. 7. Retention times relative to cholesteryl palmitate (RRT values) are quoted because these values are much more reproducible than absolute retention times. It is clear that different samples of the same oil are similar to each other, but there is a large difference in the pattern of peaks from the different oils. Thus, palm oil is clearly different from all other oils in having significant peaks at RRT values of 0·90, 0·92, 1·01, 1·02, 1·07 and 1·16; cocoa butter has major peaks at RRT values of 1·05 and 1·08; corn oil is distinguished by its high content of the steryl ester eluting with an RRT value of 1·22 (55·6% of the steryl ester fraction compared with a maximum of 16·7% for the other oils analysed); olive oil is distinguished from all oils except sunflower oil by its pattern of four peaks in the RRT range 1·17–1·22; and rapeseed oil is distinguished by its large peak at RRT 1·14–1·15.

Comparison of the two olive oil samples or the three rapeseed oil samples indicates one problem in this work. The different samples show major peaks which are likely to correspond to the same steryl ester, but elute at RRT values differing by 0·01. Thus, the peak in olive oil-1 at RRT 1·16 is likely to be the same as the peak in olive oil-2 at RRT 1·17. The peak at RRT

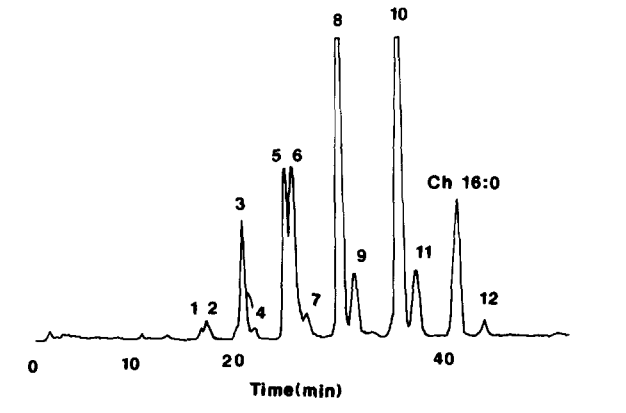


Fig. 6. HPLC analysis of the triacylglycerols of rapeseed oil.

Table 5. GC Chromatographic data for vegetable oils (area %)

RRT	Cocoa butter	Corn	Olive (1)	Olive (2)	Grapeseed	Groundnut	Palm	Rapeseed (1)	Rapeseed (2)	Rapeseed (3)	Safflower	Soybean	Sunflower (1)	Sunflower (2)
0·90	—	—	—	—	—	—	8·9	—	—	—	—	—	—	—
0·92	—	—	—	—	—	—	5·5	—	Tr	Tr	—	—	—	—
0·93	—	—	—	—	—	—	—	—	0·1	—	—	—	—	—
0·94	—	—	—	—	—	—	—	1·2	—	—	—	—	—	—
0·95	—	1·1	—	—	—	—	—	—	—	1·1	—	5·3	0·7	0·8
0·96	—	0·5	—	—	—	—	—	—	—	—	—	—	1·9	0·5
1·00	—	—	—	—	—	—	—	1·1	—	—	—	—	—	—
1·01	—	—	—	—	—	—	38·9	—	1·0	0·5	—	—	—	—
1·02	—	—	—	—	—	—	10·9	—	1·7	1·0	—	11·8	11·4	—
1·03	—	0·8	—	—	—	—	—	2·3	0·9	—	—	—	—	0·9
1·04	—	—	—	—	—	2·1	—	2·3	2·1	—	0·2	—	—	—
1·05	10·5	1·5	—	—	0·4	—	—	—	2·0	1·6	0·1	—	—	0·2
1·06	—	0·7	—	—	0·4	0·9	—	—	—	—	—	—	2·4	—
1·07	—	0·6	—	—	0·2	—	21·7	—	3·3	3·1	—	10·9	7·4	2·9
1·08	27·7	—	—	15·3	11·0	—	—	4·0	0·9	0·1	2·7	—	—	0·9
1·09	—	4·2	4·9	—	0·2	8·3	—	—	1·5	1·2	—	—	—	—
1·10	—	—	13·6	—	0·4	—	—	3·7	3·1	3·4	0·5	2·1	3·3	1·2
1·11	—	0·7	—	7·7	3·3	1·6	—	—	5·3	4·6	—	—	—	0·6
1·12	—	0·9	4·4	—	—	1·3	—	4·5	6·7	7·1	1·8	7·5	—	0·6
1·13	—	—	—	5·8	3·0	0·9	—	8·3	—	—	2·4	13·3	4·2	0·7
1·14	7·9	1·3	—	—	—	0·9	3·8	—	22·6	22·4	—	—	4·3	3·3
1·15	10·9	3·9	—	—	6·4	6·1	—	20·3	0·5	0·4	11·2	10·6	5·4	5·6
1·16	—	—	33·9	—	4·7	10·8	7·8	13·1	8·6	9·6	4·9	—	16·7	12·7
1·17	7·4	17·6	—	32·7	13·9	0·3	—	—	—	—	7·9	30·9	—	—
1·18	33·7	—	10·3	—	42·3	—	—	23·1	24·5	28·6	—	—	15·8	—
1·19	—	9·7	14·9	13·5	—	19·6	—	16·2	15·9	11·9	49·3	—	—	—
1·20	—	—	—	8·3	2·2	35·2	—	—	—	Tr	—	7·6	7·1	10·2
1·21	—	—	12·1	—	—	—	—	—	—	0·1	0·2	—	8·3	14·4
1·22	—	55·6	—	16·7	11·5	—	2·5	—	—	—	15·7	—	—	—
1·23	—	0·5	6·0	—	—	—	—	—	0·1	0·1	—	—	5·5	9·8
1·24	2·0	0·2	—	—	—	—	—	—	—	—	—	—	—	5·7
>1·24	—	0·3	—	—	—	12·0	—	—	0·1	0·1	—	—	4·7	5·8

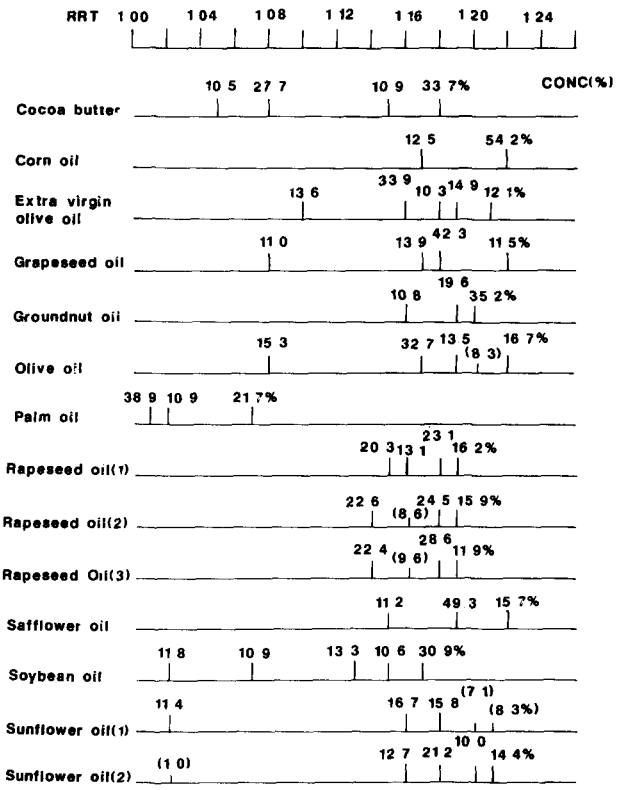


Fig. 7. The major peaks (>10%) present in the GC chromatograms of the steryl ester fraction of vegetable oils.

1·15 of rapeseed oil-1 is likely to be the same as the peaks at RRT 1·14 in rapeseed oil samples 2 and 3. In order to check the reproducibility of the RRT values of an individual steryl ester, a mixture of cholesteryl esters was analysed 10 times (Table 6).

The coefficient of variation was low in all cases with a maximum of 0·65%, indicating that RRT values were usually reproducible to ±0·01. However, any method of distinguishing oils on the basis of GC analysis of the steryl ester fraction must allow for variations in the RRT values between different analyses. Variations in RRT may increase up to ±0·03 as the column ages.

The small differences between steryl ester RRT values make it difficult to identify the components representing each peak unequivocally. Further work is being done in this area using GC–MS (mass spectrometry) analysis.

Some differences between the chromatograms of different samples of the same oil such as the sunflower samples, which contain a peak at RRT 1·02 at levels of 11·4 or 1%, may indicate that interesterification during deodorisation may cause differences between oils deodorised under different conditions.

The work reported in this paper indicates that there are major differences in the steryl ester fractions of different oils and, therefore, these differences may represent a basis for identifying admixtures of oils. The complexity of the steryl ester fraction suggests that pattern recognition techniques are likely to be required.

Table 6. Reproducibility of GC relative retention times (RRT)[a]

Steryl ester	Mean RRT	Standard deviation	Coefficient of variation (%)
Cholesteryl laurate	0·81	$5{\cdot}27 \times 10^{-3}$	0·65
Cholesteryl myristate	0·90	$5{\cdot}16 \times 10^{-3}$	0·57
Cholesteryl stearate	1·09	$5{\cdot}16 \times 10^{-3}$	0·47
Cholesteryl oleate	1·10	$4{\cdot}22 \times 10^{-3}$	0·38
Cholesteryl linoleate	1·12	$6{\cdot}75 \times 10^{-3}$	0·60
Cholesteryl linolenate	1·13	0	0

[a] 10 determinations.

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REFERENCES

Abu-Hadeed, A. M. & Kotb, A. R. (1988). A method for the quantitative determination of individual oils in a blend. *J. Am. Oil Chem. Soc.*, **65**, 1922–6.

Billheimer, J. J., Avart, S. & Milani, B. (1983). Separation of steryl esters by reversed-phase liquid chromatography. *J. Lipid Res.*, **24**, 1646–51.

Carroll, R. M. & Rudel, L. L. (1981). Evaluation of high-performance liquid chromatography method for the isolation and quantitation of cholesterol and cholesteryl esters. *J. Lipid Res.*, **22**, 359–63.

Chu, A., Schroepfer, G. J., Jr. (1988). Inhibitors of sterol synthesis. Reverse phase HPLC for the separation of cholesterol, cholest-8(14)-en-3*b*-ol-15-one and their fatty acid esters. *J. Lipid Res.*, **29**, 235-9.

Duncan, I. W., Culbreth, P. H. & Burtis, C. A. (1979). Determination of free, total and esterified cholesterol by high performance liquid chromatography. *J. Chromatogr.*, **162**, 281–92.

Evershed, R. P. & Goad, L. J. (1987). Capillary gas chromatography/mass spectrometry of cholesteryl esters with negative ammonia chemical ionization. *Biomed Environ. Mass Spectrom.*, **14**, 131–40.

Evershed, R. P., Male, V. L. & Goad, L. J. (1987). Strategy for the analysis of steryl esters from plant and animal tissue. *J. Chromatogr.*, **400**, 187–205.

Fenner, G. P. & Parks L. W. (1989). Gas chromatographic analysis of intact steryl esters in wild type *Saccharomyces cerevisiae* and in an ester accumulating mutant. *Lipids*, **24**, 625–9.

Griffith, R. E. (1989). Identification of mixtures of edible oils by the analysis of steryl esters. PhD Thesis, University of Reading, UK.

Gutfinger, T. & Letan, A. (1974). Quantitative changes in some unsaponifiable components of soya bean oil due to

refining. *J. Sci. Food Agri.*, **25**, 1143–7.
Jawad, I. M., Kochhar, S. P. & Hudson, B. J. F. (1984). The physical refining of edible oils. 2. Effect on unsaponifiable components. *Lebensm.-Wiss. u.-Technol.*, **17**, 155–9.
Johansson, A. & Hoffman, I. (1979). The effect of processing on the content and composition of free sterols and sterol esters in soybean oil. *J. Am. Oil Chem. Soc.*, **56**, 886–9.
Kaluzny, M. A., Duncan, L. A., Merritt, M. V. & Epps, D. E. (1985). Rapid separation of lipid classes in high yield and purity using bonded phase columns. *J. Lipid Res.*, **26**, 135–40.
Kuksis, A. & Beveridge, J. M. R. (1960). Preparation and certain physical properties of some plant steryl esters. *J. Org. Chem.*, **25**, 1209–19.
Kuksis, A. Myher, J. J. Marai, L., Little, J. A., McArthur, R. G. & Roncari, D. A. K. (1986). Fatty acid composition of individual plasma steryl esters in phytosterolaemia and xanthomatosis. *Lipids*, **21**, 371–7.
Nazer, J. M. A., Young, C. T. & Giesbrecht, F. G. (1985). Pyrolysis–GC analysis as an identification method of fats and oils. *J. Food Sci.*, **50**, 1095–1100.
Novotny, M., Segura, R. & Zlatkis, A. (1972). High temperature gas-chromatographic separations using glass capillary columns and carborane stationary phases. *Anal. Chem.* **44**, 9–13.
Padley, F. B. & Timms, R. E. (1980). The determination of cocoa butter equivalents in chocolate. *J. Am. Oil Chem. Soc.*, **57**, 286–93.
Ross, P. E. Kouroumalis, E., Clarke, A., Hopwood, D. & Bouchier, I. A. D. (1984). Cholesteryl esters in human gall bladder bile and mucosa. *Clin. Chim. Acta*, **144**, 145–54.
Smith, N. B. (1982). Determination of cholesteryl esters and cholesteryl and epicholesteryl silyl ethers by capillary gas chromatography. *Lipids*, **17**, 464–8.
Smith, N. B. (1983). Gas–liquid chromatography of cholesteryl esters on non-polar and polar capillary columns following on column injection. *J. Chromatogr.*, **254**, 195–202.
Sonntag, N. O. V. (1982). *Bailey's Industrial Oil and Fat Products*, Vol. 2, ed. D. Swern. John Wiley, New York, p. 481.
Van Niekerk, P. J. & Burger, A. E. C. (1985). The estimation of the composition of edible oil mixtures. *J. Am. Oil Chem. Soc.*, **62**, 531–8.