The Analysis of Sterol Degradation Products to Detect Vegetable Fats in Chocolate

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ABSTRACT: A method for the detection of hydrocarbon sterol degradation products (sterenes) has been adapted for the analysis of noncocoa butter vegetable fats in chocolate. The method involves solvent extraction of the fat separation of the sterene fraction, and analysis of individual sterenes with mass spectrometric detection. The sterene composition of refined noncocoa vegetable fats was determined in samples of cocoa butter, and retail chocolates. The presence of known sterenes was confirmed for all of the refined vegetable fats and for a single sample of cocoa butter, the processing history of which was not known. Detection of vegetable fat added to chocolate at the 5% level was demonstrated. Sterenes were detected only in chocolates labeled as containing vegetable fats. This technique has potential use as a screening method for the detection, but not quantification, of refined vegetable fat in chocolate.
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KEY WORDS: Chocolate, cocoa butter, gas chromatography, mass spectroscopy, sterenes, sterols, vegetable fats.

The addition of up to 5% vegetable fat to chocolate is a well-established and accepted practice in some but not all member states of the European Union (EU). The EU is currently revising the Directive on Cocoa and Chocolate Products (1) and is trying to develop uniform standards for the use of vegetable fats in these products.

The complex nature of vegetable fats makes their detection and quantitative measurement in chocolate difficult. Quantification of noncocoa fats in chocolate over the last 20 yr has involved the analysis of fatty acids, triacylglycerol (TG) and sterols (2–7). Analysis of TG composition is the most reliable quantitative technique, especially when the full identification of the vegetable fat is known. Therefore, improved methods are required to identify the components of vegetable oils present in chocolate.

Methods have been developed for the detection of refined oils in virgin olive oil based on the analysis of unsaturated steroidal hydrocarbons (sterenes) formed during refining (8–10). Sterenes may be formed from free sterols and possibly from sterol esters. The normal (desmethyl) sterol composition of oilseed plants varies little between the commercially important species. β-Sitosterol, stigmasterol, and campesterol usually predominate; but in shea butter, triterpene alcohols (4,4′-dimethylsterols) form the bulk of the nonsaponifiable fraction (11). Shea butter also contains relatively high total levels (up to 6%) of sterols.

The fats most often used as cocoa butter alternatives are derived from palm, illipe, and shea. These fat mixtures, which are designed to be compatible with cocoa butter, must be bleached and deodorized before use. The effect of refining on sterols has been reviewed (12). Bleaching causes dehydration of normal sterols, those with a hydroxyl function at position 3 and a double bond at position 5, forming principally 3,5-dienes with smaller amounts of 4,6-dienes and a number of conformational isomers (13,14). Sterene formation has been widely reported for the desmethyl sterols. Although some degradation products of triterpene alcohols have been reported (15,16), these have not included sterene type compounds. This paper describes the quantitative application of sterene analysis to indicate the presence of refined vegetable fats in chocolate. It may be used as an initial screening method to identify the nature and level of fat addition.

EXPERIMENTAL PROCEDURES

Materials. Lipid fractionation was carried out using a chromatographic column of 1.5 cm i.d. by 50 cm length fitted with a polytetrafluoroethylene stopcock and containing 15 g silica gel 60, 70–230 mesh (Merck Co., Poole, United Kingdom). The column was topped with 0.5 cm anhydrous sodium sulfate. The eluting solvent was hexane.

Gas chromatography (GC) was carried out using a Carlo Erba 4160 gas chromatograph (Thermo Separations, Stone, United Kingdom) fitted with a 50 m × 0.32 mm column with a 0.2 μm polar stationary phase (J&W DBWax, Jones Chromatography, Mid-Glamorgan, United Kingdom; or CP Wax 52CB, Chrompack, London, United Kingdom) operated isothermally at 250°C. Some analyses were conducted using a J&W DB5 50 m × 0.32 mm column with a 0.2 μm polar stationary phase with the oven temperature programmed from 200 to 280°C at a rate of 7°C/min. The columns were interfaced directly into the source of a VG 12-250 quadrupole mass spectrometer (Thermo Separations).

Standards. Stigmasta-3,5,22-triene (92%), cholesta-3,5-diene, and a mixture of campesta-3,5-diene plus stigmasta-

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TABLE 1
Details of the Cocoa Butter Samples

Code	Notes	Code	Notes
CB1	No information	CB20	Mixture nondeodorized
CB2	Unrefined	CB21	Mixture deodorized
CB3	Deodorized	CB22	Mixture deodorized
CB4	Pressed	CB23	Mixture deodorized
CB5	Expeller	CB24	Mixture deodorized
CB6	Deodorized pressed	CB25	Mixture deodorized
CB7	Deodorized solvent extracted	CB26	Mixture deodorized
CB8	1992 crop Malaysia Tawu	CB27	Mixture nondeodorized
CB9	1991 crop Malaysia Tawu	CB28	Mixture deodorized
CB10	1994 crop Brazil Ilheus	CB29	Malaysia
CB11	1994 crop Ivory Coast Abidjan	CB30	West Africa
CB12	1993 crop Nigeria Apapa	CB31	Brazil
CB13	Deodorized	CB32	Sao Tome 1994
CB14	Deodorized	CB33	Indonesia 1994
CB15	Nondeodorized	CB34	Papua New Guinea 1994
CB16	Nondeodorized	CB35	Ecuador 1994
CB17	Nondeodorized	CB36	Ghana 1994
CB18	Mixture nondeodorized	CB37	Deodorized
CB19	Mixture nondeodorized	CB38	Nondeodorized

3,5-diene (97%) were obtained from Chiron AS (Heimdal, Norway) as 0.1 mg/mL solutions, the purity of each was checked by GC–flame ionization detection (FID) and GC–mass spectrometry (MS).

Samples. The majority of the samples of cocoa butter and noncocoa vegetable fats were provided by the Joint Research Centre (Ispra, Italy). Other samples of cocoa butter, vegetable fats, and chocolate components were provided by industry sources in the United Kingdom. Only limited information was available on the processing history of the samples. The cocoa butter samples are listed in Table 1, and details of the vegetable fat samples are listed in Table 2.

Chocolate bars, mostly without added fat sources such as nuts or cookies and mostly of United Kingdom manufacture, were purchased from retail outlets in Norwich and The Netherlands. Chocolate samples and details of their composition are listed in Table 3.

TABLE 2
Details of the Vegetable Fat Samples[a]

Code	Sample	Code	Sample
CB39	Shea butter	CB54	Vegetable fat
CB40	Illipe butter	CB55	Vegetable fat
CB41	Illipe butter (unrefined)	CB56	Vegetable fat
CB42	Illipe butter (refined)	CB57	iPOm
CB43	Shea butter (unrefined)	CB58	Illipe
CB44	Shea butter (refined)	CB59	Illipe fat
CB45	Chocolate crumb	CB60	Palm midfraction
CB46	Soy lecithin	CB61	CBE
CB47	CBE A	CB62	PMF
CB48	CBE B	CB63	Illipe
CB49	CBE C	CB64	POP
CB50	CBE D	CB65	CBE
CB51	Vegetable fat	CB66	CBE soft
CB52	Vegetable fat	CB67	CBR
CB53	Vegetable fat		

[a]Abbreviations: CBE, cocoa butter equivalent; iPOm, POP, vegetable fat fractions; PMF, palm oil midfraction; CBR, cocoa butter replacer.

Extraction. A subsample of approximately 25% of each chocolate bar sample was chilled in a refrigerator, grated to a powder, and mixed. A 2.5-g aliquot of the mixture (containing about 1 g of fat) was placed in a beaker. Internal standard (cholestadiene, 5 µg, in 0.05 mL isooctane) was added, and the chocolate was melted at 55°C for 5 min. Celite, 3 g at 55°C, was added and the mixture was mixed with a warm spatula. The mixture was placed in a Soxhlet thimble and extracted overnight with 150 mL dichloromethane. The extract was filtered through a cellulose filter paper, the solvent was evaporated and the residue was transferred to a fractionating column as described below. Cocoa butter and vegetable fat samples received no treatment prior to column chromatography.

Column chromatography. Cocoa butter and vegetable fat samples, 1 g, or the residue from the chocolate extraction, were transferred to the fractionating column with three 1-mL volumes of hexane. Elution was carried out with hexane at a flow rate of approximately 1 mL/min. The first 25-mL fraction, containing alkanes, was discarded, and the sterenes were collected in the next 40 mL. Hexane was evaporated from the sterene fraction and the residue transferred to a 1-mL vial with three 0.3-mL volumes of hexane. The solution was evaporated to dryness and redissolved in 0.1 mL isooctane prior to GC–MS.

GC–MS. Scanned spectra were obtained in electron ionization mode at 200°C with an electron current of 70 electron volts and a trap current of 200 microamps. Spectra were acquired over the mass range 35 to 600 daltons at a rate of 0.7 s/scan with an interscan delay of 0.5 ms. Selected ion monitoring was used for quantitative analyses. Molecular ions of cholestadiene (*m/z* 368), campestadiene (*m/z* 382), stigmastatriene (*m/z* 394), and stigmastadiene (*m/z* 396) were selected. In addition, ions corresponding to $[M - alkyl]^+$ and the common ring fragment ions at *m/z* 255 and *m/z* 275 were monitored to provide confirmation of peak identity. The molecular ion of campestatriene (*m/z* 380) and ions thought to be derived from nonpolar degradation products of triterpene alcohols (*m/z* 189, 218, 365, 393, and 408) were also monitored.

Measurement and validation. Sterenes were quantified on the basis of peak area measurements relative to the internal standard using the response in the molecular ion channel of each sterene. Calibration graphs were constructed and their slopes used to calculate the sterene content of the sample. Calibrations were linear up to 100 µg/mL sterene with correlation coefficients *r* typically 0.996 for each analyte. Peak identity confirmations were based on relative ion abundances for sample peaks compared with standards.

Limited validation experiments performed during previous work on olive oil containing 0.4 mg/kg stigmasta-3,5-diene showed the standard deviation to be typically 0.04. Average recoveries of sterenes added to vegetable fats at 5 mg/kg were 94% (range 81 to 107%, $n = 6$). Analysis of six replicate samples of a chocolate labeled as containing vegetable fat gave coefficients of variance for the sterene to in-

TABLE 3
Details of the Chocolate Samples

Code	Vegetable fat	Other noncocoa ingredients			Source	Note[a]
1	No	Lecithin	Milk	Vanillin	Continental	a
2	No	Lecithin		Vanilla	Continental	
3	No	Lecithin		Vanilla	Continental	
4	No	Lecithin	Milk	Flavoring	United Kingdom	
5	No	Lecithin		Vanilla	United Kingdom	b
6	No	Lecithin	Milk	Flavoring	Continental	c
7	No	Lecithin		Vanilla	Continental	
8	No	Lecithin		Vanilla	Continental	
9	No	Lecithin	Milk	Vanillin	United States	
10	No	Lecithin	Milk	Vanillin	Continental	d
11	No	Lecithin		Vanilla	Continental	
12	Yes	Lecithin		Vanilla	Continental	e
13	Yes	E442	Milk	Flavoring	United Kingdom	f
14	Yes	E442	Milk	Flavoring	United Kingdom	f
15	Yes	Lecithin			United Kingdom	
16	Yes	E476 + lecithin	Milk	Flavoring	United Kingdom	g
17	Yes	Lecithin	Milk	Flavoring	United Kingdom	
18	Yes	Lecithin	Milk	Flavoring	United Kingdom	
19	Yes	None	Milk	Vanillin	Continental	h

[a]a, Contained hazelnut paste; b, contained spice and fruit extracts; c, contained malt extract; d, contained butter concentrate and whey powder; e, contained unhydrogenated vegetable fat; f, E442 = ammonium phosphatides; g, E476 = polyglycerol polyricinoleate; h, contained hazelnuts, coconut oil, and cookie.

ternal standard peak area ratio within the range of 8 to 9% for each analyte.

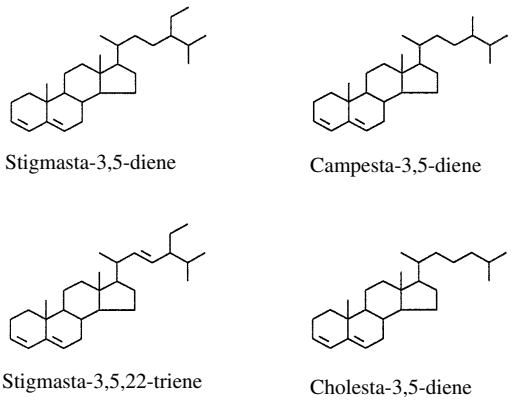
Recovery of sterenes from chocolate was studied by adding sterene standards to replicate molten portions of a chocolate which had been found previously to be free of detectable sterenes. The average recoveries of campesta-3,5-diene, stimasta-3,5,22-triene, and stigmasta-3,5-diene added to chocolate at 5 mg/kg were 93% (range 81 to 101%, $n = 6$). Limits of detection based on a signal-to-noise ratio of 3:1 for a 0.5 µg/mL standard corresponded to about 0.02 mg/kg in fat and to about 0.01 mg/kg in chocolate.

The detection and recovery of vegetable fats in chocolate at the 5% by weight level were studied by adding refined vegetable fats to replicate molten portions of a chocolate which had been found to be free of detectable sterenes. The vegetable fats used were a commercial cocoa butter equivalent (CBE) (CB47), a refined illipe butter (CB42), and a refined shea butter (CB44). The recoveries of sterenes added to chocolate in the form of illipe butter were campestadiene 67%, stigmastatriene 74%, and stigmastadiene 86%. The recoveries of sterenes added as commercial CBE were campestadiene 100%, stigmastatriene 100%, and stigmastadiene 108%. In the chocolate to which shea had been added, recovery of unidentified nonpolar compounds measured by comparison of peak area data with those obtained by analysis of the fat samples alone ranged from 86 to 140%. The presence of shea and illipe could easily be detected in this experiment.

The repeatability of the method was tested by performing replicate analyses (6) of a chocolate sample (sample 13) which contained about 3 mg/kg each of campesta-3,5-diene and stigmasta-3,5-diene. Coefficients of variance for the sterene-to-internal standard peak area ratios of three ions each of campestadiene and stigmastadiene ranged from 8 to 9%. Three analyses, performed on different occasions, of another chocolate sample (sample 9) which contained less than 0.1 mg/kg of each sterene gave coefficients of variance within this range. Determinations of stigmasta-3,5-diene in an olive oil reference sample containing approximately 0.16 mg/kg analyzed with each batch for quality control purposes gave a mean of 0.15 mg/kg with a standard deviation of 0.021.

RESULTS AND DISCUSSION

The structures of the sterene analytes and cholestadiene internal standard are shown in Scheme 1 and their mass spectra in Figure 1. The spectra match those published previously by Mennie *et al.* (14) and are characterized by intense molecular ions with fragment ions corresponding to the unsaturated ring system at *m/z* 255 and 275.



SCHEME 1

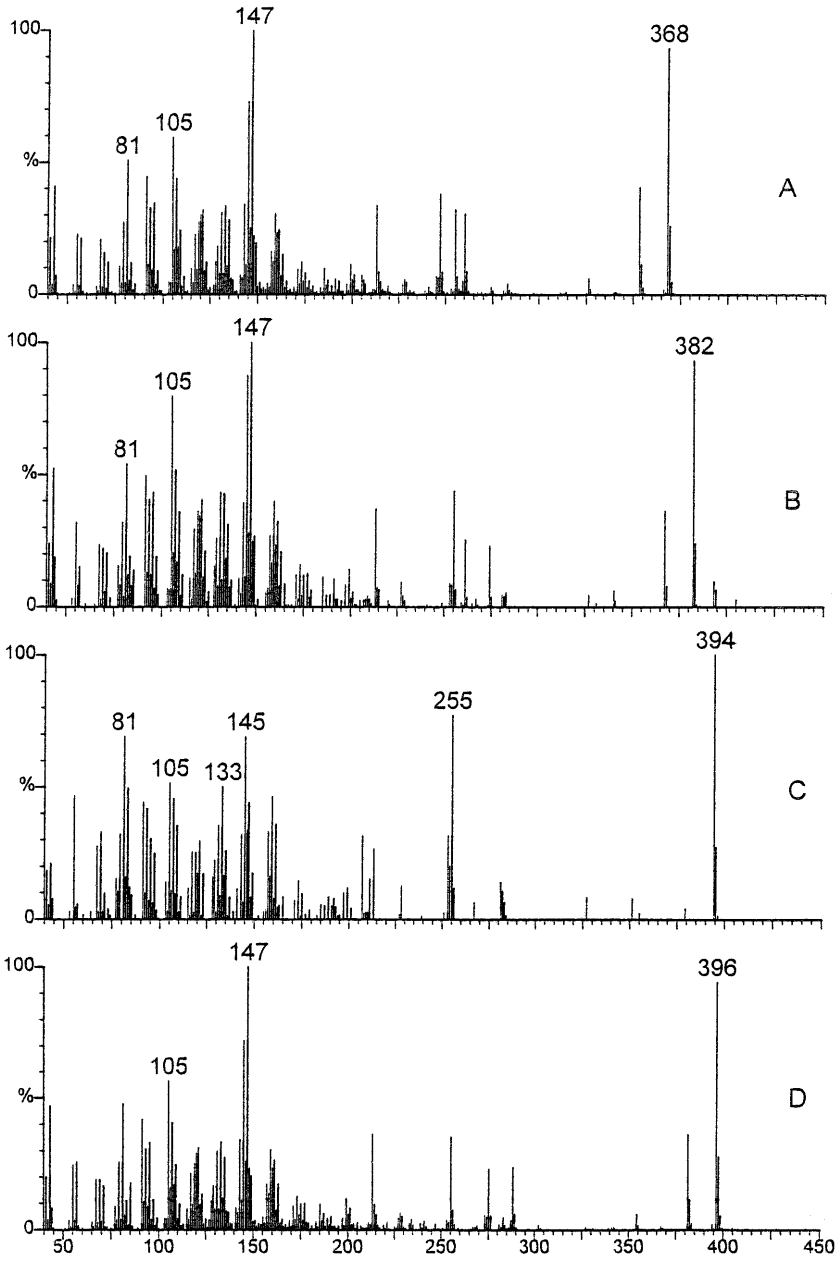


FIG. 1. Mass spectra of the major sterene analytes. (A) Cholesta-3,5-diene; (B) campesta-3,5-diene; (C) stigmasta-3,5,22-triene; (D) stigmasta-3,5-diene.

Qualitative analysis. GC–MS in full scan mode was used to identify sterenes in selected single samples of unrefined (CB2) and deodorized cocoa butters (CB3), a refined illipe butter (CB42), a refined shea butter (CB44), and a CBE sample (CB61). No sterenes were found in the unrefined and deodorized cocoa butters. Refined illipe butter was found to contain campesta-3,5-diene, stigmasta-3,5-diene, and stigmasta-3,5,22-triene. There was some evidence of campesta-3,5-diene and stigmasta-3,5-diene in the refined shea butter sample. The parent sterols of these sterenes have, however, not been reported in shea, and their presence might indicate addition of a nonshea fat to this sample.

The refined shea butter and CBE samples contained numerous compounds with mass spectra having a molecular ion at mass 408. This is 18 mass units lower than the molecular weight of many major triterpene alcohols and suggests that nonpolar dehydration products may be formed from these sterols. Details of these findings will be published elsewhere.

Quantitative measurements. To provide data on sterene levels, we analyzed a total of 38 cocoa butter samples and 29 vegetable fats by selected ion monitoring. Of the cocoa butter samples, 13 were described as deodorized, 9 as nondeodorized or unrefined, and information concerning the processing history of the remainder was not provided. Sterenes were de-

tected in only one sample (CB29), the processing history of which was unknown. This sample contained stigmasta-3,5-diene at a level of 0.2 to 0.4 mg/kg (two determinations), the identity of which was confirmed by ion abundance ratio measurements. Small peaks at the retention times of campestadiene (*ca.* 0.03 mg/kg) and stigmastatriene (*ca.* 0.03 mg/kg) were also seen in this butter, but the identity of these could not be confirmed by ion abundance data.

Vegetable fat samples. Sterene levels measured in the vegetable fats are listed in Table 4. Campesta-3,5-diene, stigmasta-3,5,22-triene, and stigmasta-3,5-diene only were quantified. These sterenes were found in all of the samples with the exception of the shea butters CB39 and CB43. Sample CB39 had no processing description, and sample CB43 was labeled as unrefined.

Figure 2 shows a typical response for a vegetable fat (CB65) containing about 0.3 mg/kg total sterenes analyzed using the DB-5 column. The principal ions of the sterenes are essentially free from interference, and there are numerous responses for postulated nonpolar triterpene alcohol degradation products shown in the *m/z* 408 chromatogram.

TABLE 4
Sterene Levels (mg/kg) Measured in the Vegetable Fat Samples[a]

Code	Sample	CAMP	TRIENE	DIENE	Total
Fat mixtures					
CB60	Palm	6.10	1.81	19.25	27.16
CB57	Fat mixture	5.81	1.78	18.39	25.98
CB62	PMF	3.07	0.99	10.47	14.53
CB61	CBE	1.91	0.78	6.91	9.60
CB67	CBR	1.68	1.25	3.79	6.72
CB53	Fat mixture	0.95	0.31	2.26	3.52
CB49	CBE	0.94	0.25	1.51	2.70
CB51	Fat mixture	0.51	0.18	1.76	2.45
CB56	Choclin	0.42	0.19	1.77	2.38
CB47	CBE	0.69	0.17	1.50	2.36
CB64	Fat mixture	0.46	0.13	1.48	2.07
CB50	CBE	0.60	0.16	0.75	1.51
CB48	CBE	0.43	0.12	0.73	1.28
CB54	Fat mixture	0.33	0.13	0.81	1.27
CB55	Coberine	0.23	0.11	0.87	1.21
CB52	Fat mixture	0.28	0.12	0.79	1.19
CB66	CBE	0.09	0.04	0.27	0.40
CB65	CBE	0.06	0.03	0.20	0.29
Illipe, shea, and miscellaneous samples					
CB42	Illipe	4.54	1.17	20.10	25.81
CB58	Illipe	0.54	0.11	2.71	3.36
CB59	Illipe	0.51	0.10	2.64	3.25
CB63	Illipe	0.43	0.14	1.79	2.36
CB40	Illipe	—	—	0.11	0.11
CB41	Illipe	—	—	0.08	0.08
CB44	Shea	0.33	—	0.44	0.77
CB43	Shea	—	—	—	—
CB39	Shea	—	—	—	—
CB45	Chocolate crumb	—	—	0.02	0.02
CB46	Lecithin	1.20	0.09	3.11	4.40

[a]Abbreviations: CAMP, campesta-3,5-diene; TRIENE, stigmasta-3,5,22-triene; DIENE, stigmasta-3,5-diene. —, less than 0.01. For other abbreviations see Table 2.

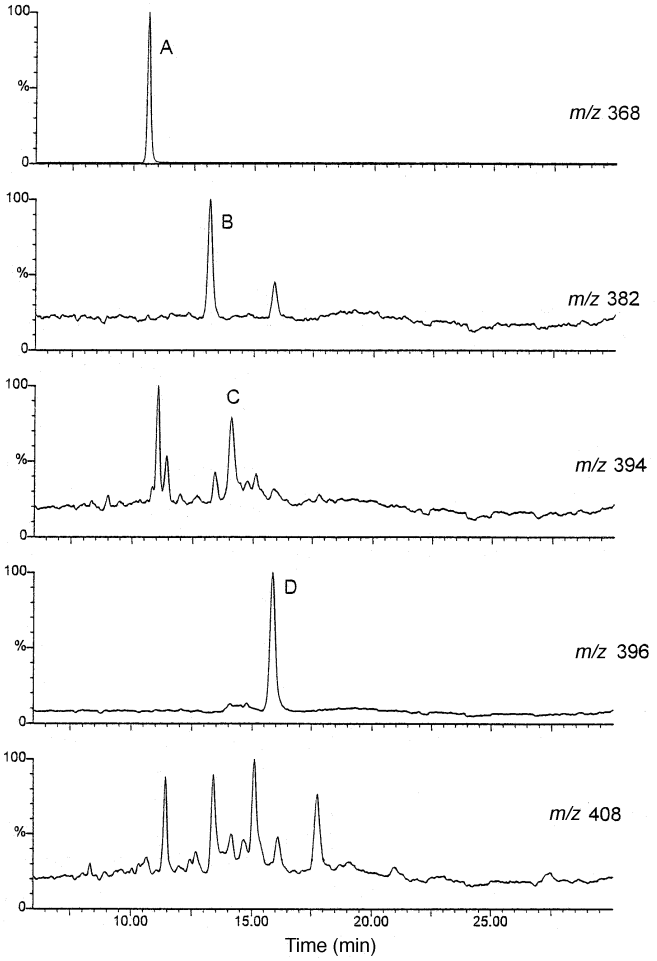


FIG. 2. Selected ion chromatograms for a vegetable fat. (A) Internal standard; (B) campesta-3,5-diene; (C) stigmasta-3,5,22-triene; (D) stigmasta-3,5-diene; lower trace, unidentified compounds believed to be nonpolar degradation products of triterpene alcohols.

Total quantified sterene levels varied from about 1 to 20 mg/kg and were markedly high in the two palm oil samples CB60 and CB62. Excluding shea and illipe samples, the ratios of campesta-3,5-diene and stigmasta-3,5,22-triene to stigmasta-3,5-diene did not vary greatly. The mean campesta-3,5-diene to stigmasta-3,5-diene ratio was 0.4, with a range of 0.3 to 0.7; the mean stigmasta-3,5,22-triene to stigmasta-3,5-diene ratio was 2.8, with a range of 1.3 to 4.2; $n = 18$. For illipe samples the ratio of stigmasta-3,5,22-triene to stigmasta-3,5-diene was distinctly different with a mean of 4.7, and a range of 4.1 to 5.2; $n = 4$. This difference might be of value in identifying the presence of illipe in vegetable fat and chocolate. Relative ion abundance data confirm the identities of the sterene peaks although for campesta-3,5-diene doubt must be expressed regarding the purity of the peaks in samples CB56, CB66, and CB67.

Of the six illipe samples, one (CB41) that was presented as unrefined contained a low level of stigmasta-3,5-diene (0.08 mg/kg). A second sample (CB42) was described as refined and contained a high level of stigmasta-3,5-diene (20 mg/kg). The four remaining samples had no processing

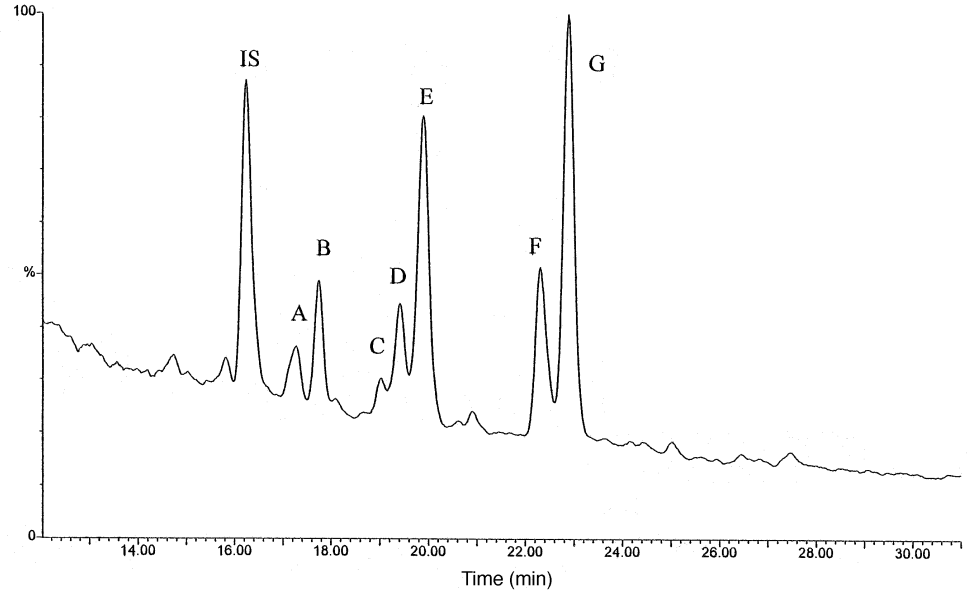


FIG. 3. Total ion current chromatogram of chocolate sample 13. Peaks A–G are identified in Figure 4. IS, internal standard.

description; two, CB58 and CB59, contained about 2.7 mg/kg stigmasta-3,5-diene, the third (CB63) 1.79 mg/kg, and the fourth (CB40) 0.11 mg/kg.

The presence of refined rapeseed oil in mixtures can be detected by measuring the response for the molecular ion of campesta-3,5,22-triene, the sterene derived from brassicasterol, which is unique to Brassicas. A response for this ion was observed in samples CB53 and CB54 and the peak identity confirmed by comparing the retention time and ion abundance ratios with those obtained from the analysis of a refined rapeseed oil.

Application to chocolate samples. Qualitative analysis. Full scanned spectra were obtained for a chocolate sample (sample 13) analyzed using the DB-Wax column with the aim of identifying sterenes and any potential interferences. The total ion current chromatogram from this analysis is shown in Figure 3. Spectra of selected major peaks are shown in Figure 4. The chromatogram was dominated by sterene peaks. Major peak G was identified as stigmasta-3,5-diene. Isomers of stigmastadiene were also present as peaks C and F. The early-eluting isomer C had a base peak at *m/z* 381 and a prominent fragment at *m/z* 241 but no ions at *m/z* 275 or 288. Work is in progress to identify this compound which has not previously been reported. The spectrum of isomer F matched published spectra for the 4,6-diene (14).

Campestadiene isomers were present at peaks D (4,6-diene) and E (3,5-diene). The spectra of peaks A and B had a molecular ion at *m/z* 380, and both matched published spectra of campesta-3,5,22-triene, which is derived from brassicasterol and is considered indicative of the presence of rapeseed oil; this oil might have been present as part of an emulsifier added to the chocolate during production.

Quantitative measurements. Selected ion chromatograms

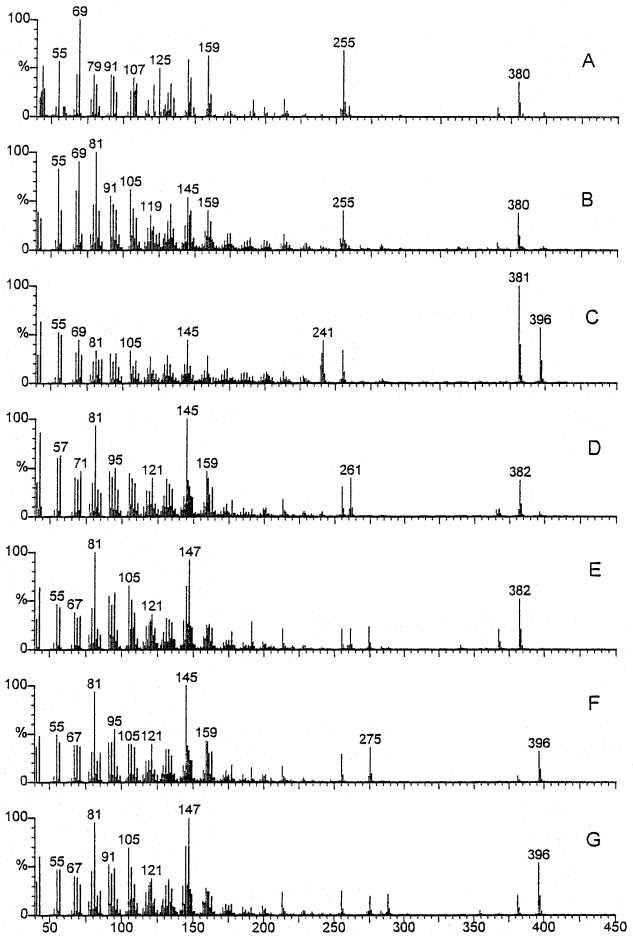


FIG. 4. Spectra of the major peaks of chocolate sample 13. Peaks A and B = campestatriene; peaks C, F, and G = stigmastadiene; peaks D and E = campestadiene.

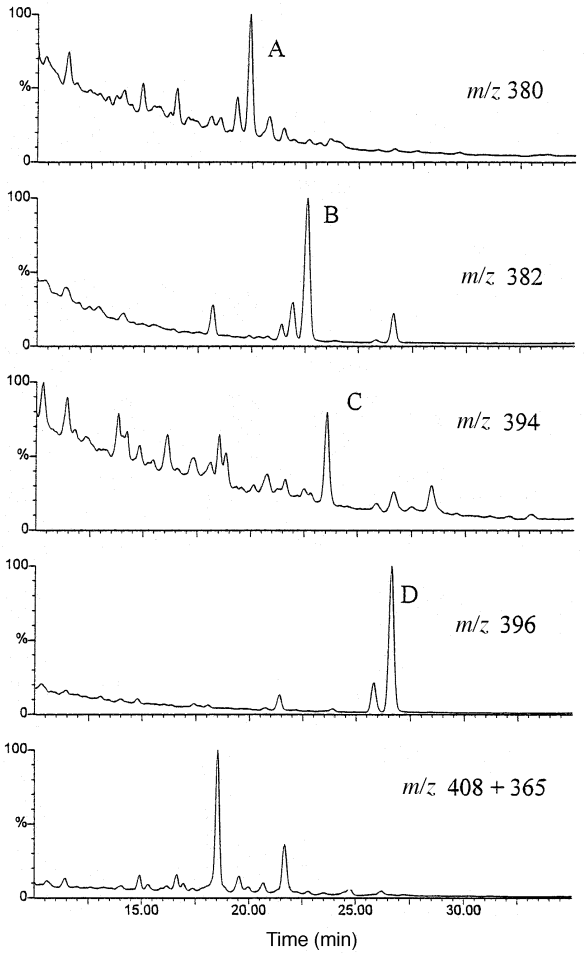


FIG. 5. Chromatograms of a typical chocolate sample containing about 1 mg/kg sterenes. (A) Campestatriene; (B) campesta-3,5-diene; (C) stigmasta-3,5,22-triene; (D) stigmasta-3,5-diene; lower trace, unidentified compounds believed to be nonpolar degradation products of triterpene alcohols.

of a typical chocolate sample containing about 1 mg/kg sterenes are shown in Figure 5. This shows signals for campestatriene, campestadiene, stigmastatriene, and stigmastadiene, and in trace are signals suggesting the presence of nonpolar triterpene compounds of molecular weight 408.

The levels of the quantified sterenes (campesta-3,5-diene, stigmasta-3,5,22-triene, and stigmasta-3,5-diene) measured in the chocolate samples are listed in Table 5.

Of the 11 chocolates with no added vegetable fat, nine had total sterene levels in the range <0.01 to 0.09 mg/kg (mean 0.02 mg/kg), with only two samples higher at 0.12 mg/kg. For the eight chocolates stated to contain vegetable fats, the total sterene levels in seven of them ranged from 0.58 to 6.10 mg/kg (mean 1.33 mg/kg). The single sample which had less than 0.5 mg/kg (sample 12) contained unhydrogenated fat which might have been unrefined. Sample 1 was labeled as containing hazelnut paste (but not added vegetable fat), sample 19 (with added vegetable fat) was labeled as containing additional ingredients including cookie and hazelnuts. In these two cases the presence of these ingredients did not prevent differentiation of the two sample types.

TABLE 5
Sterene Levels (mg/kg) Measured in the Chocolate Samples[a]

Code	CAMP	TRIENE	DIENE	Total
Without fat				
1	—	—	0.02	0.02
2	—	—	0.01	0.01
3	—	—	—	
4	0.03	0.01	0.08	0.12
5	0.02	0.01	0.06	0.09
6	—	—	0.01	0.01
7	—	—	0.01	0.01
8	-	0.01	0.04	0.05
9	0.03	0.02	0.07	0.12
10	—	—	—	—
11	—	—	—	—
With fat				
12[b]	—	—	—	—
13	2.55	0.04	3.53	6.12
14	0.42	0.05	0.75	1.22
15	0.23	0.02	0.35	0.60
16	0.11	0.08	0.39	0.58
17	0.26	0.13	1.08	1.47
18	0.20	0.09	0.46	0.75
19	0.35	0.05	0.82	1.22

[a]—, less than 0.01 mg/kg.
[b]Contained unhydrogenated fat. For abbreviations, see Table 4.

Samples 13 and 14 contained a substantial proportion of a stigmastadiene isomer, at a retention time relative to cholestadiene of 1.19, in which the 381:396 ratio was 1.9:1; this might have been derived from a component of the ammonium phosphatide emulsifier, E442, which was unique to these samples. Stigmasta-3,5-diene had a relative retention time of 1.48 and a 381:396 ratio of 0.43:1. Samples 15–19 contained significant amounts of an isomeric stigmastadiene at a relative retention time of 1.33, and several samples contained isomeric campestadienes. Samples 13–15 contained evidence of rapeseed oil, detected as campesta-3,5,22-triene derived from brassicasterol; the presence of this oil might also be related to the presence of emulsifier.

The method has been demonstrated to detect sterenes derived from sitosterol, stigmasterol, and campesterol and to be suitable for the detection of the presence of refined fats in chocolate. The sterene content of the vegetable fats has been found to vary widely, but minimal levels were about 0.2 mg/kg for the major sterene stigmastadiene. Quantification of the level of refined vegetable fat addition is, however, not possible because the degree of sterene formation varies with changes in the conditions of the bleaching process. The method has the potential to identify differences in chocolate composition attributable to the presence or absence of added vegetable fat.

Interference from other ingredients potentially containing refined vegetable fats, such as nuts, cookies and reprocessed chocolate, might cause difficulties although it was not found to be a problem here. The ubiquitous presence of lecithin,

which contains up to 40% soybean oil with sitosterol and stigmasterol (17), was also tolerated.

This work has revealed the presence in vegetable fat and chocolate of nonpolar compounds believed to be degradation products of triterpene alcohols. These have not been reported previously and could in the future be of some significance in the analysis of oil and cocoa butter adulteration. The technique developed has potential for use as a screening method for the detection of added refined vegetable fat in chocolate. Significant levels of the major sterenes stigmasta-3,5-diene, campesta-3,5-diene, and stigmasta-3,5,22-triene were detected in a wide range of noncocoa vegetable fats intended for use in chocolate, these sterenes being absent from the unrefined products. Although details of refining processes applied to the noncocoa fats were available for only four samples, the method was able to distinguish 23 noncocoa fats as clearly being refined from 38 unbleached cocoa butters and two unrefined noncocoa fats. The method successfully differentiated seven chocolate samples labeled as containing vegetable fat from 11 chocolate samples which did not list vegetable fat as an ingredient.

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