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Analysis of steryl esters in cocoa butter by on-line liquid chromatography–gas chromatography

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Abstract

On-line liquid chromatography–gas chromatography (LC–GC) has been applied to the analysis of steryl esters in cocoa butter. Separation of the steryl esters was achieved after on-line transfer to capillary GC. HPLC removes the large amount of triglycerides and pre-separates the components of interest, thus avoiding time-consuming sample preparation prior to GC analysis. The identities of the compounds were confirmed by GC–MS investigation of the collected HPLC fraction and by comparison of the mass spectra (chemical ionization using ammonia as ionization gas) to those of synthesized reference compounds. Using cholesteryl laurate as internal standard, steryl esters were quantified in commercial cocoa butter samples, the detection limit being 3 mg/kg and the quantification limit 10 mg/kg, respectively. Only slight differences in percentage distributions of steryl esters depending on the geographical origin of the material were observed. The patterns were shown to remain unchanged after deodorization. The method described might be a valuable tool for authenticity assessment of cocoa butter. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Steryl esters; Cocoa butter

1. Introduction

The investigation of minor lipid constituents plays an important role in the assessment of quality and authenticity of fats and oils [1–3]. Among the diverse classes of compounds, sterols proved to be suitable to determine the genuineness of vegetable oils [4,5]. Sterols are present as free alcohols as well as in esterified form [6–9] and analysis of the steryl esters has been proposed as a tool for the characterization of fats and oils [10,11].

The most routine approach to the analysis of steryl esters is based on isolation by thin layer chromatography (TLC) and subsequent gas chromatographic (GC) or liquid chromatographic separation (LC) [12]. The similar polarities of steryl esters and the abundant triglycerides require highly efficient separation by TLC and/or the application of steps such as column chromatography [13], low-temperature crystallization [7] or lipase-catalyzed hydrolysis of the triglycerides [14].

The separation of intact steryl esters by HPLC can

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be achieved by reversed-phase chromatography [9]. However, the application of this technique is limited by the lack of resolution of different steryl esters and the relatively poor detection limit [12]. The scope of the approach might be broadened by the use of LC–MS as described for the analysis of steryl esters in blood plasma [15]. An alternative overcoming some of the difficulties encountered with HPLC is the analysis of steryl esters by GC and GC–MS. However due to their relatively low volatility, the GC analysis of steryl esters calls for high temperature GC applications with demands on thermal stability of the separation columns and injection techniques used. Therefore, saponification is often applied prior to GC analysis followed by analysis of the sterols as trimethylsilyl derivatives and of the fatty acids as methyl esters [7]. This approach prevents the recognition of the individual steryl esters and thus valuable information is lost.

There have been approaches to combine HPLC and GC off-line for analysis of steryl esters and sterols [16,17]. An elegant and very efficient solution based on the use of HPLC as pre-separation step and GC for final resolution has been realized by on-line LC–GC [18]. The two methods developed for the simultaneous determination of sterols and steryl esters involve reaction of the sample with pivalic acid anhydride [19] or a silylating reagent [20] and subsequent on-line transfer of the HPLC fraction containing the derivatized hydroxy compounds and the steryl esters onto the GC column. This combination allows the direct GC analysis of the intact steryl esters and has been applied to the analysis of various fats and oils [21,22].

This paper reports the application of the LC–GC procedure involving silylation of the sample [20] to the analysis of steryl esters in cocoa butter. The sterol composition of cocoa butter has been investigated in detail [23]. Although esterified sterols have been shown to amount to 11.5% of total sterols [24], knowledge on identities and amounts of individual members of this class of compounds is limited. The only information available is an assignment of the gas chromatographic peak pattern obtained from a steryl ester rich fraction of cocoa butter to GC and HPLC retention data of synthesized references [10]. The objective of this study was to identify and quantify individual steryl esters in cocoa butter and thus to lay the analytical basis for potential use of these constituents in authenticity assessment.

2. Materials and methods

2.1. Chemicals

Cholesteryl laurate (98%), stigmasterol (96%), β-sitosterol (98%), campesterol (65%) and *tert*-butyl methyl ether for liquid chromatography (gradient grade) were purchased from Sigma (Buchs, Switzerland). Palmitoyl chloride, stearoyl chloride, oleoyl chloride and linoleoyl chloride (all at least 99% pure) were supplied by Nu-chek-Prep (Elysian, MN). *N*,*O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA + 1% TMCS), molecular sieve (4 Å) and 4-dimethyl-aminopyridine (DMAP) were obtained from Fluka (Buchs, Switzerland). Analytical grade pyridine, dichloromethane, chloroform, methanol, sodium hydrogencarbonate, sodium sulfate, hydrochloric acid (0.1 mol/l), acetonitrile and *n*-hexane for liquid chromatography (gradient grade) were purchased from Merck Eurolab (Dietikon, Switzerland).

2.2. Materials

Cocoa butter samples of four different origins (South America, Asia, Africa, African/Asian mix) were obtained from commercial suppliers (Nord Cacao, Gravelines, France; Archer Daniels Midland, Koog Aan De Zaan, The Netherlands; Barry Callebaut, Lebbeke-Wieze, Belgium; SACO, Abidjan, Côte d'Ivoire; Gerkens Cacao, Wormer, The Netherlands; Aarhus Olie, Aarhus, Denmark).

2.3. Sample preparation

Cocoa butter samples were liquified by heating in an oven (40°C). About 100 mg oil were weighed into an 11-ml vial containing 50 μl of a cholesteryl laurate solution (1 mg/ml) used as internal standard (IS). After adding 100 μl of the silylating agent (BSTFA + 1% TMCS) and 20 μl of pyridine as catalyst, the mixture was homogenized by vortexing. The solution was heated for 20 min at 80°C using a heating block. After heating, 8 ml of *n*-hexane were

added and the mixture was gently shaken. This solution was used for analysis by LC–GC.

2.4. Instrumentation

2.4.1. On-line LC–GC

The analysis was performed using a fully automated on-line LC–GC instrument (Dualchrom 3000, C.E. Instruments, ThermoQuest, Rodano, Italy). The HPLC part of the instrument included two 20-ml syringe pumps (Phoenix 20), one used as the master pump for eluent delivery and one used as slave pump for backflushing the column system. Detection was performed with a variable wavelength detector (Micro UVIS 20, Linear Instruments, Reno, NV). The separation column was thermostated using a column oven (Jetstream Plus, Advanced Separation Technologies Inc., USA). The GC was equipped with a fully automated interface valve system. A loop-type interface with a solvent vapour exit was used for the transfer of the fraction of interest from LC. A flame ionization detector was used for detection. All components were controlled by the Dualchrom software. Data acquisition was performed by the Chromcard Software (C.E. Instruments).

2.4.2. GC–MS

GC–MS analyses were performed on a Finnigan SSQ 7000 mass spectrometer (Finnigan MAT GmbH, Bremen, Germany) working in positive chemical ionization mode at 150 eV with ammonia as ionization gas. Samples were introduced via a gas chromatograph (Hewlett-Packard 5890, Agilent, Geneva, Switzerland) equipped with a HP-7673 autosampler using the following conditions: cold on-column injection, fused-silica capillary column DB-5ht (J&W Sci, MSP Friedli, Koeniz, Switzerland) 15 m×0.25 mm I.D., film thickness 0.1 μm. The carrier gas used was helium (186 kPa). The temperature program was 60°C (1 min), then programmed with 30°C/min up to 270°C (5 min), then 10°C/min up to 340°C (1 min). The transfer line and the source were heated to 300°C and 180°C, respectively. The analysis of steryl esters was carried out in full scan mode at unit resolution from 100 to 800 Da (scan time: 0.5 s, inter-scan delay: 0.1 s).

2.5. LC–GC conditions

HPLC pre-separation of the fraction containing the silylated sterols and the steryl esters from the di- and triglycerides was performed with a cyano bonded silica column Zorbax-SB, 150×2.1 mm, 80 Å pore size, 5 μm particle size (Agilent, Geneva, Switzerland) using a mixture of 2% of dichloromethane and 0.1% of acetonitrile in *n*-hexane as eluent. The separation column was kept at 30°C, the flow-rate was set to 200 μl/min and UV detection was at 215 nm. Backflush of the column was performed with *tert*-butyl methyl ether. The latter was delivered by the slave pump at a flow-rate of 200 μl/min during backflush and at 10 μl/min during stand-by. The backflush was started after 10 min and returned to stand-by 5 min later.

GC separation was performed on a 15 m×0.25 mm I.D. fused-silica capillary coated with a film thickness of 0.1 μm (DB-5ht), connected in series with 3 m×0.25 mm I.D. of an uncoated phenyldimethyl silylated fused-silica capillary and a coated pre-column (2 m×0.25 mm I.D.) having the same coating as the analytical column. An early solvent vapour exit (0.2 m×0.25 mm I.D. fused-silica) was installed between the coated pre-column and the separation column via a Y-piece press-fit connector (BGB Analytik, Anwil, Switzerland). Hydrogen was used as carrier gas at a flow-rate of 2.5 ml/min, measured at 120°C. Carrier gas inlet pressure behind the flow regulator was set to 250 kPa.

The transfer of the non-retained apolar fraction started 3 min after injection of 5 μl of the analytical sample and occurred by concurrent eluent evaporation at 120°C. With a delay of 40 s on the reduction of the inlet pressure by 40 kPa at the end of the transfer, the solvent vapor exit was switched to a restrictor (1 m×0.05 mm I.D. fused-silica), leaving a small purge flow during analysis.

After holding the transfer temperature of 120°C for 5 min, the column temperature was programmed to 260°C at 30°C/min, and then to 340°C at 15°C/min, which was held for 15 min. The flame ionization detector (FID) was set to 360°C.

2.6. Synthesis of steryl esters

In accordance with the procedure described by

Höfle et al. [25], 1 ml of a DMAP solution (10 mg/ml in anhydrous pyridine) was added to a solution of 150 mg of the fatty acid chlorides (palmitoyl chloride, stearoyl chloride, oleoyl chloride, linoleoyl chloride) in 1 ml *n*-hexane. The mixture was vortexed until the solution became clear. About 200 mg of the sterol were added together with 30 ml of chloroform and the mixture was stirred for 2 h at room temperature in the absence of light. Upon stirring, the initially turbid mixture became clear, otherwise an additional ml of dichloromethane was added dropwise. The solution was washed three times with hydrochloric acid (0.1 mol/l) and the aqueous phase containing the excess of the *N*-acylpyridinium salt was discarded. To neutralize the excess of acid, the solution was washed with a sodium hydrogencarbonate solution. The organic phase was dried with sodium sulfate and the solvent evaporated using a rotary evaporator. For the separation of non-reacted sterols, solid-phase extraction (SPE) on a reversed-phase silica gel column (Chromabond C18ec, 45 μm, 500 mg, Macherey–Nagel, Oensingen, Switzerland) was applied. The cartridges were conditioned with about 4 ml of methanol. The solid residue was transferred from the flask onto the cartridge, the flask was rinsed with about 0.1 ml chloroform. Elution was carried out with 8 ml methanol (fraction was discarded) and 8 ml of *n*-hexane/*tert*-butyl methyl ether (9/1, v/v). The steryl esters (purities >80%) were obtained after removal of the solvent of the latter fraction with a stream of nitrogen.

2.7. Linearity

Linearity of response in the concentration range of interest was checked using synthesized stigmasteryl palmitate as reference substance and cholesteryl laurate as internal standard. Different concentration levels of stigmasteryl palmitate (1.1–13.6 μg/ml) and a constant amount of cholesteryl laurate (10.5 μg/ml) were analyzed in triplicate by LC–GC.

2.8. Quantification of steryl esters

The amounts of steryl esters were determined by comparing the GC peak areas to those obtained for the internal standard cholesteryl laurate (378 μg/g cocoa butter). For the calculation, a relative response factor of 1.0 was used for all steryl ester homologues.

2.9. Recovery

The recovery was evaluated by spiking cocoa butter with a known amount of stigmasteryl palmitate. Two portions of a cocoa butter sample were mixed with a solution containing the internal standard cholesteryl laurate as described above. One of the portions was spiked with a stigmasteryl palmitate solution in *n*-hexane, resulting in an additional amount of 252 μg/g cocoa butter. The recovery was calculated on the basis of the GC peak areas obtained for the internal standard and for stigmasteryl palmitate in the spiked and the unspiked portion.

3. Results and discussion

3.1. Analysis of steryl esters

The identities of the synthesized steryl esters (palmitate, stearate, oleate and linoleate of campesterol, stigmasterol and β-sitosterol) were confirmed by GC–MS using positive chemical ionization with ammonia as ionization gas. The mass spectra of sitosteryl palmitate and sitosteryl stearate are shown as typical examples in Fig. 1. All spectra are characterized by intense $[M+NH_4]^+$ adduct ions, in general base peaks of the spectra, and two fragment ions corresponding to the loss of the fatty acid moiety ($[M+NH_4-RCO]^+$) and of one water molecule ($[M+NH_4-RCO-H_2O]^+$). It should be noticed that positive chemical ionization with ammonia as ionization gas is the only ionization method able to generate intense ions indicative of the molecular mass of the steryl esters. Neither electron impact nor positive chemical ionization (with methane) or negative chemical ionization (with ammonia or methane) were found to produce molecular or protonated molecular ions, respectively. These observations are in agreement with data published by Lusby et al. [26] and Evershed et al. [27]. Moreover, positive chemical ionization with ammonia as ionization gas allows to determine the sterol moiety of the steryl ester as the two distinctive fragments [M+

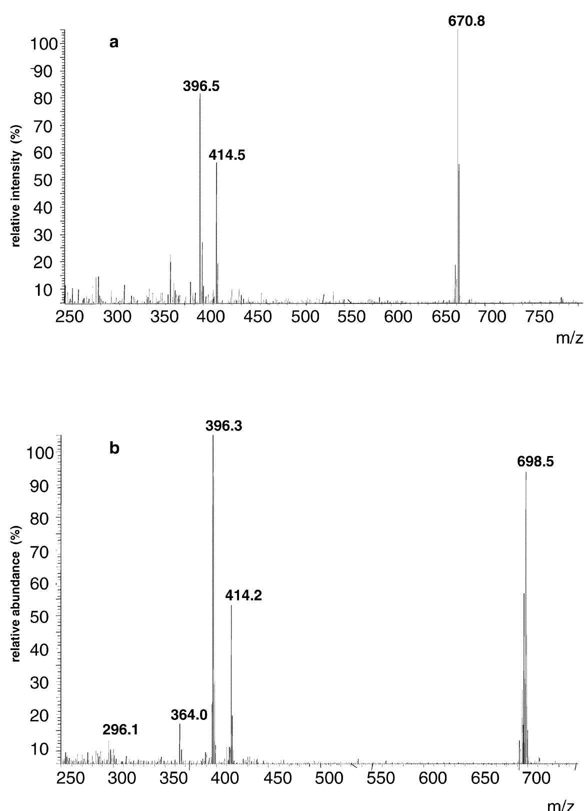

Fig. 1. Positive chemical ionization (ammonia) mass spectra of sitosteryl palmitate (a) and sitosteryl stearate (b); (for conditions see text).

$NH_4 - RCO]^+$ and $[M + NH_4 - RCO - H_2O]^+$ and thus enables determination of the molecular mass of the sterol.

The capillary gas chromatographic separation of the synthesized steryl ester standards after on-line LC–GC transfer is shown in Fig. 2. On the employed apolar GC column (DB 5ht) the steryl palmitates (**1**–**3**) are baseline resolved, whereas steryl esters of the unsaturated C_{18} fatty acids cannot be resolved according to their degree of unsaturation. The peaks for steryl stearates (18:0), oleates (18:1), linoleates (18:2) form merged peaks only (**4**–**6**). Therefore, no individual quantification for these components was possible and the sum is reported. This presence of merged peaks on an apolar stationary phase is in agreement with results obtained for cholesteryl esters [12,28].

The complete analysis of a cocoa butter sample by

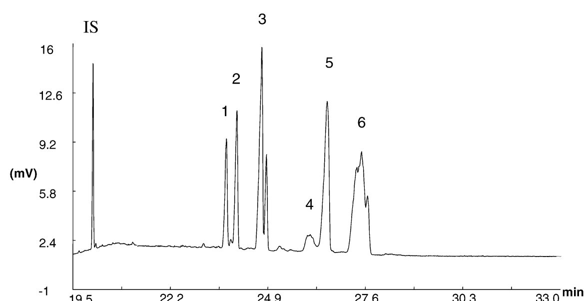

Fig. 2. Gas chromatogram of synthesized steryl ester standards after on-line LC–GC transfer (for conditions see text); campesteryl palmitate (**1**), stigmasteryl palmitate (**2**), sitosteryl palmitate (**3**), campesteryl stearate, -oleate, -linoleate (**4**), stigmasteryl stearate, -oleate, -linoleate (**5**), sitosteryl stearate, -oleate, -linoleate (**6**).

on-line LC–GC is shown in Fig. 3. The separation of the silylated cocoa butter sample achieved by normal-phase HPLC is presented in Fig. 3a. Steryl esters

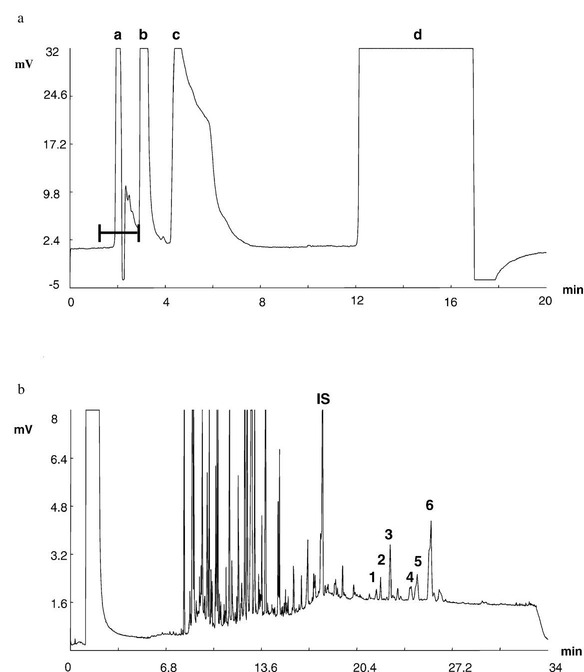

Fig. 3. Analysis of steryl esters in a cocoa butter sample. Separation on normal-phase LC (Fig. 3a) with indication of apolar fraction (a) containing silylated sterols and steryl esters and range of transfer window. Other fractions represent elution range of diglycerides (b) and triglycerides (c); (d) backflush with MTBE. GC chromatogram (Fig. 3b) after on-line LC–GC of fraction (a); (for conditions see text).

and components of similar polarity (e.g. silylated sterols) elute prior to di- and triglycerides and were transferred in one fraction. The GC chromatogram of the transferred fraction is shown in Fig. 3b. The peaks in the first part of the chromatogram are apolar components (e.g. silylated sterols and tocopherols, hydrocarbons) [29]. As the focus of this study was on the determination of the steryl esters, optimum resolution and identification of the compounds in this region of the chromatogram were not pursued.

The identities of the steryl esters were confirmed by off-line LC–GC–MS. The apolar fraction (indicated as 'a' in Fig. 3a) was collected manually and analyzed by GC–MS with positive chemical ionization. The chromatogram was recorded in the full scan mode and ion chromatograms for the steryl esters (**1–6**) are given in Fig. 4.

3.2. Linearity

Linearity of response in the concentration range of interest was checked using synthesized stigmasteryl palmitate as reference substance and cholesteryl laurate as internal standard. The calibration function ($y = 0.940x + 0.099$) showed a good linearity, illustrated by a high correlation coefficient (0.997).

3.3. Method evaluation

The repeatability was evaluated by sixfold analysis of a cocoa butter sample. As shown in Table 1, low standard deviations were determined proving the good repeatability of the on-line LC–GC method.

The recovery of the compounds of interest was investigated by the addition of a known amount of

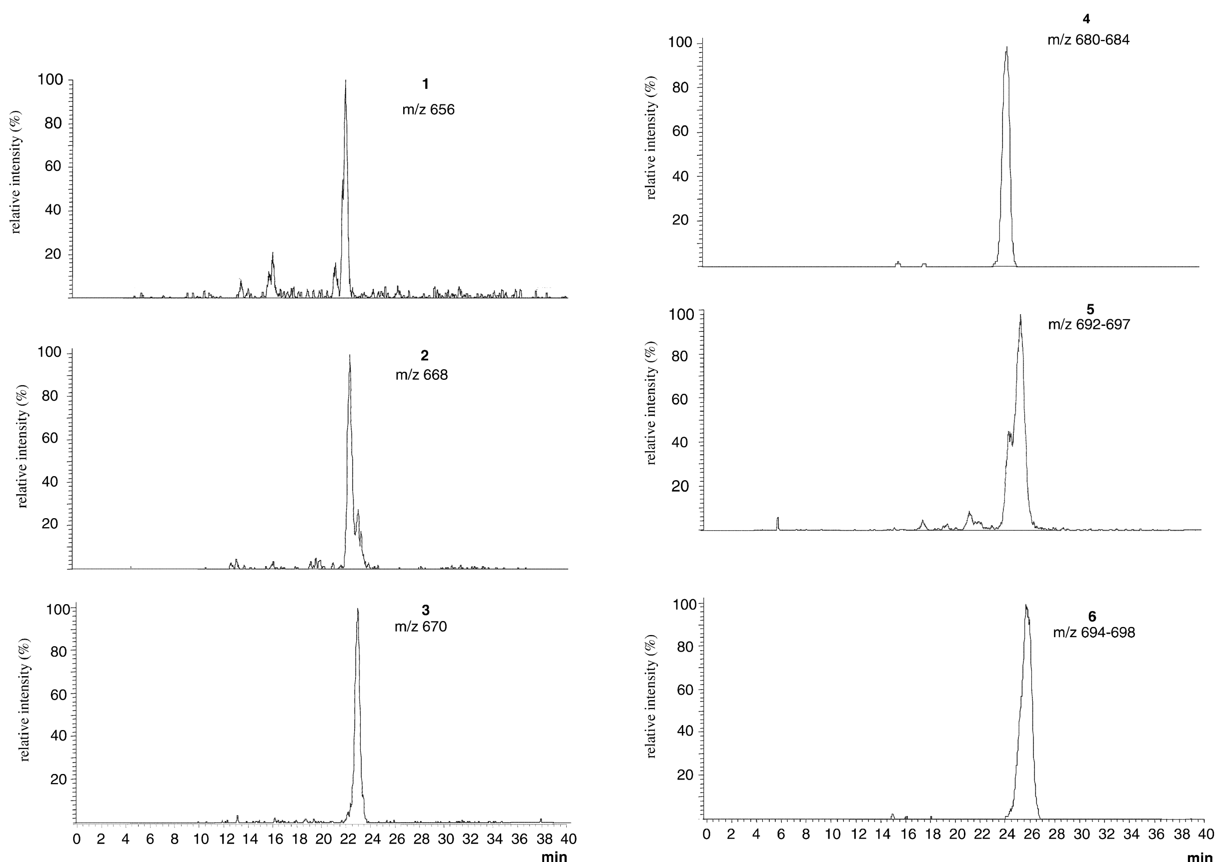

Fig. 4. Positive chemical ionization (ammonia) mass spectra of steryl esters in a cocoa butter sample after off-line LC–GC–MS. Confirmation of the individual steryl esters (**1**–**6**, identification see Fig. 2) in full scan mode (conditions for ion chromatograms, see text).

Table 1
Results obtained by repeated LC–GC analysis ($n=6$) of a cocoa butter sample

	Steryl esters (mg/kg)[a]
Campesteryl palmitate	14 (2)
Stigmasteryl palmitate	28 (1)
Sitosteryl palmitate	90 (5)
Campesteryl stearate, -oleate, -linoleate	40 (1)
Stigmasteryl stearate, -oleate, -linoleate	66 (2)
Sitosteryl stearate, -oleate, -linoleate	253 (3)
Sum	491 (8)

[a] Mean values (standard deviations).

stigmasteryl palmitate to cocoa butter and was found to be 98%.

The limit of detection (defined as the peak area being three times the standard deviation of the noise of the blank) was 3 mg/kg and the limit of quantification (defined as the peak area being 10 times the standard deviation of the noise of the blank) was 10 mg/kg.

3.4. Steryl esters in cocoa butter

The contents of steryl esters determined in commercial cocoa butter samples are listed in Table 2. Only slight differences in the absolute amounts of steryl esters depending on the geographical origin of the material were observed. According to the suggested classification of vegetable oils on the basis of their steryl ester content [11], cocoa butter belongs to the group with a low content (<1200 mg/kg). The average amount of 548 mg/kg determined in the material available for analysis is in the range reported for soybean oil [11].

The relative proportions of the steryl esters were also consistent for all samples. The percentage distributions of campesteryl, stigmasteryl and sitosteryl esters as well as of C_{16} and C_{18} esters are in good agreement with the fatty acid and sterol compositions reported for cocoa butter [30]. For rapeseed oil differences in fatty acids in steryl esters and the whole oil as well as differences in sterol isolated after saponification from the steryl esters and the whole oil have been observed [11].

A comparison of data obtained for three pairs of deodorized and non-deodorized samples revealed this refining step to have no influence on the pattern and on the amounts of steryl esters in cocoa butter (Table 3). This is in agreement with previous publications reporting that normal refining conditions have only a minor impact (5–10% loss) on the amounts of steryl esters in fats and oils [13,31].

4. Conclusion

The described on-line LC–GC method shows distinct advantages compared to the commonly applied analysis of steryl esters involving saponification and investigation of the cleavage products. The technique yields information on the composition of intact steryl esters and allows their quantification with high sample throughput required for screening large amounts of samples. The detection limit for steryl esters is 3 mg/kg and the quantification limit

Table 2
Distribution of steryl esters in commercial cocoa butter samples

Steryl esters	South America[a]		Asia[a]		Africa[b]		African/Asian[b]	
	mg/kg	Rel.%	mg/kg	Rel.%	mg/kg	Rel.%	mg/kg	Rel.%
Campesteryl palmitate	16	2.7	16	3.1	18 (5)	3.1 (0.2)	14 (1)	2.9 (0.5)
Stigmasteryl palmitate	43	7.1	31	5.9	42 (16)	7.0 (1.2)	32 (11)	6.5 (0.7)
Sitosteryl palmitate	115	19.3	96	18.3	119 (28)	20.2 (1.2)	94 (27)	19.1 (1.1)
Campesteryl stearate, -oleate, -linoleate	41	6.8	45	8.6	44 (10)	7.5 (0.6)	37 (7)	7.7 (0.4)
Stigmasteryl stearate, -oleate, -linoleate	87	14.5	69	13.1	82 (19)	14.0 (1.0)	69 (18)	14.1 (0.7)
Sitosteryl stearate, -oleate, -linoleate	296	49.5	267	51.0	278 (38)	48.2 (3.2)	241 (45)	49.5 (1.9)
Total	598		524		583 (109)		487 (111)	

[a] Mean values of two samples.
[b] Mean values (standard deviations) of seven samples.

Table 3
Distribution of steryl esters in non-deodorized (1a–3a) and deodorized cocoa butter samples (1b–3b) of African/Asian origin

	Steryl esters (mg/kg)					
	1		2		3	
	a	b	a	b	a	b
Campesteryl palmitate	13	14	15	16	12	13
Stigmasteryl palmitate	28	29	33	34	25	24
Sitosteryl palmitate	84	90	100	103	75	76
Campesteryl stearate, -oleate, -linoleate	33	36	37	37	34	33
Stigmasteryl stearate, -oleate, -linoleate	61	65	71	71	57	62
Sitosteryl stearate, -oleate, -linoleate	211	232	244	243	219	216
Sum	430	466	500	504	422	424

10 mg/kg, respectively. Sample preparation is reduced to a minimum and due to the on-line procedure a loss of analytes is avoided. This is especially important if only low amounts of steryl esters are present as it is the case for cocoa butter. The applied procedure is based on the LC–GC approach involving silylation of the sample, thus the method can easily be extended to the simultaneous determination of steryl esters and free sterols in cocoa butter.

Due to a recent EU legislation [32] the detection of vegetable fats other than cocoa butter in chocolate products is of increasing importance. A combination of approaches rather than a single technique will probably be required [33–35]. The method described in this paper can be another valuable piece of information. The analytical basis has been created, screening of a broader spectrum of alternative fats for use in chocolate will be required to determine the usefulness of steryl ester analysis for authenticity assessment.

References

[1] G. Lercker, M.T. Rodriguez-Estrada, J. Chromatogr. A 881 (2000) 105.
[2] A. Cert, W. Moreda, M.C. Pérez-Camino, J. Chromatogr. A 881 (2000) 131.
[3] W. Kamm, F. Dionisi, C. Hischenhuber, K.-H. Engel, Food Rev. Int. (2001), in press.
[4] European Union Commission, J. Eur. Communities L248 (1991) 2568.
[5] R. Aparicio, R. Aparicio-Ruíz, J. Chromatogr. A 881 (2000) 93.
[6] A. Johansson, Lipids 14 (1978) 285.
[7] A. Johansson, L.-A. Appelqvist, Lipids 13 (1978) 658.
[8] D. Boskou, I. Vlachopoulou, Lebensm.-Wiss. Technol. 19 (1986) 156.
[9] M.H. Gordon, R.E. Griffith, Fat Sci. Technol. 94 (1992) 218.
[10] M.H. Gordon, R.E. Griffith, Food Chem. 43 (1992) 71.
[11] M.H. Gordon, L.A.D. Miller, J. Am. Oil Chem. Soc. 74 (1997) 505.
[12] R.P. Evershed, V.L. Male, L.J. Goad, J. Chromatogr. 400 (1987) 187.
[13] R.A. Ferrari, W. Esteves, K.D. Mukherjee, E. Schulte, J. Agric. Food Chem. 45 (1997) 4753.
[14] L.A.D. Miller, M.H. Gordon, Food Chem. 56 (1996) 55.
[15] A. Kuksis, J.J. Myher, L. Marai, J.A. Little, R.G. McArthur, D.A.K. Roncari, Lipids 21 (1986) 371.
[16] C. Mariani, E. Fedeli, K. Grob, A. Artho, Riv. Ital. Sost. Grasse 68 (1991) 179.
[17] P. Schuhmann, R. Schneller, Mitt. Gebiete Lebensm. Hyg. 87 (1996) 708.
[18] K. Grob, On-line Coupled LC–GC, Hüthig, Heidelberg, 1991.
[19] K. Grob, M. Lanfranchi, C. Mariani, J. Chromatogr. A 471 (1989) 397.
[20] A. Artho, K. Grob, C. Mariani, Fat Sci. Technol. 95 (1993) 176.
[21] C. Plank, E. Lorbeer, J. Chromatogr. A 683 (1994) 95.
[22] M. Lechner, B. Reiter, E. Lorbeer, Fett/Lipid 101 (1999) 171.
[23] K. Staphylakis, D. Gegiou, Fette Seifen Anstrichmittel 87 (1985) 150.
[24] K. Staphylakis, D. Gegiou, Lipids 20 (1985) 723.
[25] G. Höfle, W. Steglich, H. Vorbrüggen, Angew. Chem. 90 (1978) 602.
[26] W.R. Lusby, M.J. Thomson, J. Kochansky, Lipids 19 (1984) 888.
[27] R.P. Evershed, L.J. Goad, Biomed. Environ. Mass Spectrom. 14 (1987) 131.
[28] N.B. Smith, J. Chromatogr. 254 (1983) 195.
[29] K. Grob, A.M. Giuffré, U. Leuzzi, B. Mincione, Fat Sci. Technol. 96 (1994) 286.

[30] D. Firestone, in: D. Firestone (Ed.), Physical and Chemical Characteristics of Oils, Fats and Waxes, AOAC Press, Washington DC, 1999, p. 28.
[31] R.A. Ferrari, E. Schulte, W. Esteves, L. Brühl, K.D. Mukherjee, J. Am. Oil Chem. Soc. 73 (1996) 587.
[32] European Union Commission, Directive, J. Eur. Communities L197 (2000) 19.
[33] M. Lipp, E. Anklam, Food Chem. 62 (1998) 73.
[34] M. Lipp, E. Anklam, Food Chem. 62 (1998) 99.
[35] C. Simoneau, M. Lipp, F. Ulberth, Eur. Food Res. Technol. 211 (2000) 147.