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Review of cocoa butter and alternative fats for use in chocolate—Part B. Analytical approaches for identification and determination

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This work reviews the literature on analytical methods suitable for the detection, identification and determination of foreign fats in cocoa butter and chocolate. Most methods are based on chromatography, analysing either the fatty acids, triglyceride or the fractions of the unsaponifiables (sterols, triterpenes, etc.). None of the methods reviewed here allows the unequivocal quantification, or just the detection, of foreign fats in chocolate without any exemption for the kind of foreign fat added. The type of foreign fats which are not detectable depends strongly on the analytical techniques applied. It is proposed to combine a number of techniques with a chemometric approach to develop unique patterns allowing the unequivocal detection and quantification of foreign fats, without having prior knowledge regarding the kind of fat added. This approach is based on the use of a complex pattern of the constituents of cocoa butter and any deviation from this pattern is attributed to the existence of foreign fat.

INTRODUCTION

A detailed introduction about the nature of fats and the nomenclature used for its description has been given in Part A (Lipp and Anklam, 1998). In Table 1 the common abbreviations for the analytical methods are explained.

The present work is divided into chapters describing the use and advantages of different analytical methods. This review puts emphasis on methods allowing an identification and quantification of fats and oils, especially in mixtures with cocoa butter. However, promising approaches for the identification of fats and oils not comprising cocoa butter are also cited here, if their application could possibly lead to some success for the detection of foreign fats in chocolate.

The majority of the techniques described here are based on chromatographic methods used for the analysis of triglycerides and/or the fraction of the unsaponifiables of the fats and oils.

Nomenclature

For the nomenclature of triglycerides the reader is referred to Part A (Lipp and Anklam, 1998) of this review. Fatty acids are abbreviated according to Table 2.

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SPECTROSCOPIC METHODS

Early data (Ahlers and O'Neill, 1954) have already revealed that neither UV- nor IR-spectroscopy provides signals based on the absorption of single wavelengths that allow an identification of oils and fats or the identification of individual fatty acids. However, more recently it was shown that NIR and FTIR are very useful techniques for certain tasks. The chocolate quality could be clearly correlated with features derived from the NIR spectra of raw cocoa beans (Davies, 1992). NIR proved to be a reliable method for the determination of moisture, fat and sucrose in powdered cocoa products. The mean error of prediction was found to range between 1 and 3% (Permanyer and Perez, 1989). FTIR was shown to be a fast and reliable method to detect the amount of *trans*-fatty acids in vegetable fats and oils. Its reproducibility was stated to be 0.7% and the accuracy 2.5% with respect to samples analysed by chemical methods (van de Voort *et al.*, 1995).

Quantification of mixtures of cocoa powders and milk powder was performed by measuring the intensity of the reflected light. Within a concentration range of 2–10%, a maximum deviation of $\pm 0.4\%$ from the actual cocoa powder content was observed. This compares well with the established HPLC method based on detection of cocoa alkaloids (Kneifel *et al.*, 1990).

Table 1. Common abbreviations used for the characterisation of analytical methods

AAS	Atomic absorption spectroscopy
c-GC	Capillary gas chromatography
CBE	Cocoa butter equivalent
DSC	Differential scanning calorimetry
ELSD	Evaporative light scattering detector
FID	Flame ionisation detector
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography
HPLC	High performance liquid chromatography
IR	Infrared
LC	Liquid chromatography (used as synonym for HPLC by some authors)
MS	Mass spectrometry
NIR	Near-infrared
RI	Refractive index detector
TLC	Thin layer chromatography
UV	Ultraviolet

The determination of metallic cations in cocoa masses, using AAS, revealed that the cocoa masses could be differentiated according to their country of origin and the processing technique (Hernández *et al.*, 1994).

CHROMATOGRAPHIC METHODS

Reviews

In the review of Hammond (1989) the application of HPLC and GC for the analysis of lipids was compared. High-temperature capillary GC using a phenylmethyl silicone phase was found to give the best resolution. However, the absolute accuracy was found to be rather poor and worsened with the degree of unsaturation present in the lipids. To achieve the maximum resolution, HPLC in the gradient mode was the method of choice. Suitable detectors were FID and ELSD. It was concluded that there is still no truly universal detector and that this hampers the achievements of good quantification results in all lipid systems.

An extensive review on the use of chromatographic and other methods for the detection of adulteration in animal and vegetable fats and oils was published by Mani and Lakshminarayana (1976). The methods described herein for the detection of adulteration of cocoa butter are all based on chromatography determining the major fatty acids and major triglycerides. However, this is restricted mainly to the addition of illipé fat to cocoa butter.

Capillary GC, HPLC with a UV detector and desorption chemical ionisation mass spectrometry (DCI–MS) were applied for the analysis of the triglycerides in cocoa butter and other vegetable fats. With the combined data of all three techniques more than 80 molecular species of triglyceride had been identified. This approach allows a very detailed analysis of fats and mixtures (Rezanka and Mares, 1991).

HPLC and GC are complementary techniques for the detection of sterol esters. Sixty different sterol esters were identified in 14 vegetable oils, cocoa butter being amongst them. The vegetable fats investigated in this study revealed a characteristic pattern of composition of sterol esters (Gordon and Griffith, 1992). The authors indicated that this technique could be very useful for the identification of foreign fat in mixtures with cocoa butter.

Gas chromatography

Results obtained by gas chromatography (GC) have to be converted by response factors. Only the results obtained by HPLC-RI do not necessarily have to be converted by response factors (Carelli and Cert, 1993).

Geographical origin

The volatile flavour components of unroasted and roasted cocoa butters were investigated. A total of 12 acidic, 218 neutral and 82 basic compounds was identified. Among those, the pyrazines were found to play a very important role in flavour. Of the 62 pyrazines identified, 57 were present in roasted cocoa butter while only 27 were present in unroasted cocoa butter. The total concentration of pyrazines in roasted cocoa butter was about twice as high as in unroasted cocoa butter (Carlin *et al.*, 1982; Hashim and Chaveron, 1994). Cocoa powders of Ghanaian and Cuban varieties could be distinguished on the basis of the GC profiles of their volatile compounds (Pino *et al.*, 1992, 1993). The characterisation of cocoa masses according to geographical origin and roasting conditions was performed by the determination of 37 volatile compounds and their subsequent evaluation by multivariate methods (Hernández and Rutledge, 1994*a*). The estimation of the linalool content by simultaneous steam distillation extraction

Table 2. Nomenclature of fatty acids

Symbol	Name	CN:DB	Symbol	Name	CN:DB
	Caprylic acid	8:0		Capric acid	10:0
La	Lauric acid	12:0	M	Myristic acid	14:0
P	Palmitic acid	16:0		Palmitoleic acid	16:1
S	Stearic acid	18:0	O	Oleic acid	18:1
L	Linoleic acid	18:2	Ln	Linolenic	18:3
A	Arachidic acid	20:0		Arachidenic acid	20:1
	Behenic acid	22:0			

CN, carbon number; DB, double bonds.

proved to be suitable for quality classification of roasted cocoa butters (Pino and Roncal, 1992).

Unsaponifiables

Ten different vegetable oils and fats were analysed for their sterols, 4-methylsterols, triterpene alcohols, tocopherols, squalene and fatty acid content. In total, 26 different compounds were quantified. The data were subsequently used to identify and quantify these oils and fats in 19 different two-, three- and six-component mixtures by a matrix inversion method. The system was fully successful for the identification of the components of the mixture. The accuracy of the quantitative determination averaged at about 80% (Abu-Hadeed and Kotb, 1988). Improved polar columns were shown to enhance the separation of the main classes of the unsaponifiable matter. The application of these columns offers an even more powerful tool for characterising the lipid sources (Frega *et al.*, 1992, 1993). For the detection of the CBEs 'Coberine', 'Choclin' and 'Clavetta' the 4,4′-dimethylsterols and triterpene alcohols are the most promising indicators (Gegiou and Staphylakis, 1981, 1985). These results have been confirmed by extensive investigation of the content of sterols, 4-methyl-sterols and triterpenes of a large number of fats and oils actually or potentially used as CBEs. However, the authors stated that no single component can be used for the quantification of CBEs in mixtures with cocoa butter, but they may well indicate their presence qualitatively (Homberg and Bielefeld, 1982*a,b*, 1990*a,b*). A thorough analysis of the unsaponifiable matter of cocoa butter with a separation by argentation TLC and subsequent analysis by GC and GC–MS resulted in the identification of 39 sterols or triterpene alcohols (Staphylakis and Gegiou, 1985).

Sterol esters are usually not greatly affected by bleaching and refining of the fats. For the determination of their sterol content, they are normally saponified. However, it could be shown that these compounds also offer great potential for the differentiation of oils and fats by isolating a sterol ester-rich fraction of different oils and fats by TLC prior to their determination by GC (Gordon and Griffith, 1992).

The addition of illipé fat to cocoa butter could be detected by the determination of the fatty acids of mixtures containing 10% and more illipé fat in cocoa butter. The analysis of the sterols, especially the quantification of α-spinasterol, allows the detection of 5% and more illipé fat to cocoa butter, while the analysis of triterpenes leads to a detection of 1% illipé fat in cocoa butter (Derbesy and Richert, 1979).

The identification of illipé and sal fat in mixtures was often tried based on the results of the sterol and desmethylsterol content. However, these components are quite similar in vegetable fats. The analysis of the triterpenes is claimed to be more important. In particular the presence of Ψ-taraxasterol is suggested as an indicator for illipé and sal fat. This compound is hardly found in vegetable fats but easy to detect (Soulier *et al.*, 1990).

Triglycerides

The three major triglycerides in cocoa butter and chocolate, POP, POS and SOS, were extensively investigated by GC (Fincke, 1980*a–c*, 1982). Evaluation of the data by linear regression allows a good quantification of cocoa butter and foreign fats in mixtures. However, the presence of illipé fat and fats obtained by interesterification of hydrogenated 1,3-palm oil-diglycerides with oleic acid could not be quantitatively detected. The papers also discuss other fat mixtures (e.g. containing hydrogenated cotton seed oil), which are virtually indistinguishable from cocoa butter. It is, therefore, necessary to complement the detection of all triglycerides (not only POP, POS and SOS) by the analysis of the fatty acids, *trans*-fatty acids and a detailed analysis of the several fractions of the unsaponifiables (sterols, methylsterols, triterpenes, etc.).

Early on, the use of packed-column GC for the determination of the CBEs in chocolate was investigated. The first attempts were made by using the peaks from C_{50} and C_{54} (Padley and Timms, 1978*a,b*, 1980; Padley, 1980 Young, 1984). This method was then extended to include the C_{52} peak. The results of a round robin study indicated that the method is precise (Chaveron *et al.*, 1981), with the relative standard deviation given to be below 0.1%. However, it does not succeed in the determination of some added fats, such as illipé fat or an equivalent produced by Procter & Gamble (Chaveron and Verdoia, 1984; Chaveron *et al.*, 1984). This work has been extended towards a more detailed analysis of the triglycerides. The difference between C_{50}, C_{52} and C_{54} originating from the fraction of disaturated monooleic triglycerides and originating from the all triglycerides has been detailed. In focusing on the fraction of disaturated monoenoic triglycerides, the mixture of cocoa butter with nut and almond oils could be clearly identified using the normalised peak area of C_{50} vs C_{54} (Chaveron *et al.*, 1984).

This approach has been modified by using capillary GC with a high-temperature phenylmethylsilicone stationary phase. This technique allows the separation of the individual triglycerides contributing to C_{50}, C_{52} and C_{54}. The authors claim that with this technique even the identification of fat content such as illipé is possible (Geeraert and Sandra, 1987).

The application of capillary GC to detect groups of triglycerides separated according to their carbon numbers was also investigated in a round robin study. The coefficient of repeatability was found to depend strongly on the concentration determined for the triglycerides. If the mean of the triglyceride concentration was found to be about 50%, the variation coefficient was well below 5%. However, for concentrations lower than 10% the variation coefficient could easily reach to above 20% (Motta *et al.*, 1987).

Analysis of fatty acids and simultaneous analysis of different classes of compound

Refined and bleached vegetable fats and oils including cocoa butter have been simultaneously analysed for their content of free fatty acid, monoglyceride, diglyceride and triglyceride using BSTFA [(*N,O*)-bis(trimethylsilyl)trifluoroacetamid] as derivatising reagent. The analysis was performed on a non-polar column resulting in a separation of the triglycerides according to their carbon numbers. The total analysis was relatively short (approx. 10 min) (D'Alonzo *et al.*, 1982).

A fast and versatile method for the simultaneous determination of free fatty acids and sterols by applying capillary GC was described by Cocito and Delfini (1994). However, it has not yet been applied for vegetable fats. The free fatty acids and sterols are extracted with chloroform and both are analysed as trimethylsilyl derivatives. The reproducibility is given as an average coefficient of variation of below 6%. The total time of analysis needed is 70 min.

Cocoa butter and illipé butter as well as mixtures thereof were analysed for their content of sterols, fatty acids and triglycerides. Two factors were established for the quantification of illipé fat in mixtures with cocoa butter: the ratio of stigmasterol and campesterol and the ratio of POS and POP (Bracco *et al.*, 1970).

The results of the determination of content of fatty acids were used to establish a set of linear equations for the quantitative determination of mixtures of cocoa butter, cocoa fat, milk fat and hazel nut oil (Saccà *et al.*, 1991).

Two approaches of linear regression, multiple weighted linear regression and mixed integer programming, have been applied to the quantification of mixtures of vegetable and animal fat (de Jong and de Jong, 1991). The fatty acid profile of several mixtures has been investigated. A maximum number of 10 different ingredients was set and the regression tools assigned for each mixture the approximate compositional data for all ingredients. However, the authors stated that the result was not always unequivocal and major improvements could be expected if the sterol content is also taken into consideration.

Milk fat can be detected and quantified in chocolate, even in the presence of lauric fats, by a combined evaluation of medium chain triglycerides (C_{40}, C_{42} and C_{44}) and the methyl esters of lauric acid and the minor fatty acids situated between myristic and palmitic acid (Pontillon, 1995).

The fatty acid, sterol and tocopherol content of edible oils, as determined by GC, was used to investigate the composition of oil mixtures by means of a weighted least square estimator. The model was tested on 93 samples containing sunflower seed, groundnut, soybean, cotton seed maize, olive and palm oil. Satisfactory results were obtained with 79 out of 93 samples. The model is described to be superior to the matching of fatty acid composition, but still needs development (van Niekerk and Burger, 1985).

High performance liquid chromatography

HPLC–RI was found to give accurate results, even without the use of conversion factors. HPLC–ELSD yields inaccurate results for the lower concentration range, but yields satisfactory results at higher concentrations (Carelli and Cert, 1993).

Triglycerides

HPLC with an RI-detector and proprionitrile as mobile phase was used to quantify the triglycerides by from soxhlet extraction of the cocoa beans. The main triglycerides—POP, SOS, LOO and PSS—were used in combination with discriminant analysis to separate the samples according to their geographical origin (Hernández *et al.*, 1991). It was demonstrated that use of signals of the triglycerides POP, POS and SOS is superior to determination of the corresponding signals of C_{50}, C_{52} and C_{54} packed column GC. The presence of hazel nut oil and milk fat, in particular, does not interfere, as they do not contain significant amounts of monounsaturated triglycerides, but contain triglycerides with the CN of 50, 52 or 54, respectively (Podlaha *et al.*, 1984).

HPLC with an RI-detector was found to give an optimal resolution for different vegetable and animal fats for a C_{18}-column and the solvent mixture acetone–acetontrile. However, the total time of analysis was about 90 min (Defense, 1984). Detailed investigations on different columns revealed that separation is improved by increasing coverage of the silica particles (coated with longer hydrocarbons), smaller particle size of the silica particles and increase in mobile phase polarity (El-Hamdy and Perkins, 1981). The addition of chloroform to the eluent shortens the time of analysis to about 75 min (Balesdent and Kapseu, 1989). It has been shown that a UV-detector can also be applied under these conditions for the separation of triglycerides in edible oils (Marini and Balestrieri, 1989).

An earlier investigation evaluated the use of a UV-detector equipped to an HPLC system. The UV absorption was measured at 220 nm and separation was performed using acetonitrile and tetrahydrofuran under isocratic conditions as eluent and an RP18-column (Shukla *et al.*, 1983). However, the time needed for a complete, separation was about 90–100 min.

Some of the disadvantages of using a UV-detector can be avoided by brominating the triglycerides prior to their separation. In particular, brominating leads to a better separation of the monounsaturated triglycerides from the di- and polyunsaturated triglycerides. The brominated monounsaturated triglycerides give a four times stronger absorption at 215 nm and the analysis time is considerably shorter (Geeraert and De Shepper, 1983).

Palm oils and palm mid-fraction were characterised by HPLC equipped with a RI-detector. A clear differentiation between palm oils and palm oleins, as well as various other palm oil fractions and their mixtures was obtained using signals of selected triglycerides (OOO, PPP, PLO, PLP, POP, POO) (Aitzetmüller *et al.*, 1988).

Multivariate optimisation was used to find optimal conditions for the separation of triglycerides from vegetable fats using reversed phase HPLC connected to a ELSD. A negative exponential gradient from acetonitrile/isooctane to acetonitrile/ethanol/isooctane at a temperature of 50°C, with a flow rate of 1.5 ml min^{-1} was found to be the optimal condition. For identification of the compounds, a version of the equivalent carbon number concept utilising the Snyder polarity index was used (Bergqvist and Kaufmann, 1993).

A rapid analysis (< 30 min) of the triglycerides in cocoa butter, using reversed phase HPLC with an ELSD, was achieved by applying a gradient of mobile phase consisting of acetonitrile and dichloromethane. Due to the relatively small particle size of the column used, the resolution obtained was very high and the authors claim that this approach is useful for detection of the adulteration of cocoa butter with other vegetable fats (Palmer and Palmer, 1989).

The application of isocratic mobile phase conditions in combination with an ELSD offers the possibility of a fast separation of triglycerides in vegetable fats and oils. The time needed for analysis is only about 20 min, providing the possibility of separating triglycerides containing α- and γ-linolenic acid (Caboni *et al.*, 1991).

Another method developed for HPLC with an ELSD was using a gradient for the composition of the mobile phase consisting of acetonitrile and dichloromethane. A complete separation of the triglycerides OOO, LnLnO and LnLnLn from animal and vegetable fats was achieved within 20 min (Letter, 1993).

The accuracy of determining the addition of foreign fats (except fats as illipé) to chocolate is estimated to be at least ±2%. The method is based on the determination of the three main triglycerides POP, POS and SOS. However, in the presence of bigger amounts of fats such as illipé the error will increase to much higher values (Eiberger and Matissek, 1994*a*,*b*).

HPLC equipped with an ELSD was used for the quantitative analysis of the content of foreign fat in cocoa butter. An attempt was made to quantify the amount of foreign fat added while not differentiating which kind fat was actually added. Partial least square regression on the signal of 17 peaks revealed a mean error of prediction of 2.5%. The data were equally well analysed using neural nets and only two out of 14 were classified incorrectly (Anklam *et al.*, 1996).

A very detailed analysis of triglycerides from cocoa butter and other vegetable fats can be obtained by preseparation on a mini-column impregnated with silver ions followed by HPLC analysis. The first step separates the triglycerides according to their degree of unsaturation in four fractions. The application of HPLC with an RI-detector in the isocratic mode (proprionitrile as solvent) allows the identification of 56 different triglycerides. Each HPLC separation was optimised to be concluded within 10–20 min (Kemper *et al.*, 1988).

The separation of natural diglycerides obtained from cocoa butter as the 3,5-dinitrophenylurethanes by chiral phase HPLC–MS allows the unequivocal identification of the exact paring of the fatty acids in cocoa butter (Itabashi *et al.*, 1991).

Unsaponifiables

HPLC was used to analyse steradienes (dehydration products of sterols formed during the fat refining process). The excess of other lipids present is removed by chromatography on silica gel columns with petrol ether. The steradienes are subsequently eluted on a reversed phase column with acetonitrile and tert-butylmethyl ether using UV-detection at 235 nm. These conditions are so specific that virtually only the peaks of the steradienes appear in the last part of the chromatogram. The time needed for analysis is about 25 min (Schulte, 1994).

LC–GC coupling and combined data evaluation of LC and GC methods

Unsaponifiables

For the direct analysis of the minor components of edible oils and fats, silylation was shown to be superior to acylation (Artho *et al.*, 1993). It eliminates the problem of the esterification of the free alcohols with free fatty acids in oils and fats of high acidity. Moreover, the LC fraction is widened to include squalene and tocopherols.

Another approach using transesterification and online LC–GC for the determination of the sum of free and esterified sterols in edible oils and fats, avoids many of the problems obtained by direct analysis. Transesterification with sodium methanolate circumvents the extraction of the neutral matters from a soap solution, which is often tedious and not quantitative. It is carried out at room temperature, i.e. under far milder conditions. This automated method provides a very low standard deviation (typically about 1–3%) and the manual sample preparation is reduced to less than 5 min, allowing the analysis of up to 25 samples each day, instead of about two with the direct method (Biedermann *et al.*, 1993). However, bleaching of the samples degrades the content of sterols and its esters, but the composition of the dehydroxylation products of the sterols still reflects their composition in the oils and fats (Grob *et al.*, 1994*b*). In particular, the determination of the olefinic degradation products stigmastadiene, stigmastatriene, campestadiene and campestatriene can be used for the identification of a bleaching process (Grob *et al.*, 1994*a*).

A method for the rapid and direct identification of wax esters, sterols and sterolesters circumventing saponification and any off-line pre-separation is described (Grob *et al.*, 1989). The sterols are esterified with pivalic acid in the oil. The diluted oil is then injected in a LC and the fraction containing the above classes of compounds is cut and transferred in a GC. A complete analysis was performed within 45 min.

With an off-line LC–GC method for the analysis of steradienes, using silica gel columns for pre-separation and capillary GC for a sensitive off-line detection, it is possible to obtain the same results as with on-line coupling at a lower price (Haase-Aschoff *et al.*, 1995).

Separation of the enantiomers

Enantiomeric diglycerides were identified by preparative separation of the corresponding 3,5-dinitro-phenylurethane derivatives on a chiral LC phase. This was followed by a GC separation of the trimethylsilyl derivatives of these diglycerides (Itabashi *et al.*, 1990). However, this type of analysis is rather complex and tedious. It was shown that a separation of the diglycerides with HPLC–MS is much easier and faster (Itabashi *et al.*, 1991).

The results of the determination of fatty acids obtained by GC analysis and results of the determination of triglycerides obtained by HPLC were combined using multivariate statistical data analysis. Three vegetable oils under investigation—corn, rapeseed and soybean oils—could be clearly differentiated (Kaufmann and Herslöf, 1991).

The combined use of the fatty acid concentration and the concentration of triglycerides is necessary for an identification of cocoa butters and cocoa butter replacer. It was emphasised that none of the methods individually reveals sufficient information (Nikolova-Damyanova and Amidzhin, 1992).

The fatty acid composition from vegetable fats obtained by GC analysis correlates closely to the one calculated from the triglyceride analysis by HPLC. For this calculation the triglycerides of some vegetable oils were determined and their concentration multiplied by the percentage of each fatty acid present in the triglyceride (Konishi *et al.*, 1993).

Silver ion chromatography

The impregnation of the solid phase with silver ions, followed by a stepwise elution, gave pure trisaturated and disaturated monoene species, together with a mixed fraction of saturated dimonoene and disaturated diene and a fraction of highly unsaturated species (Christie, 1990).

This principle, adapted to HPLC, is applied together with chiral chromatography for the structural analysis of oils and fats. Enantiomers of the fat component can be achieved by a multi-step reaction involving several specific enzymes or by the reaction of the non-chiral 1,2- or 2,3-*sn*-diglycerides with enantiomerically pure amines such as naphtyl-ethyl urethane. Nevertheless, this is a rather tedious process and only limited but promising information is available (Christie *et al.*, 1991; Semporé and Bézard, 1991; Christie, 1994; Takagi and Ando, 1991, 1995).

Silver ion HPLC is used to determine the amount of *cis*- and *trans*-triglycerides in vegetable fats. Triglycerides containing *trans*-fatty acids are formed during hardening of fats and oils, which is based on hydrogenation of double bonds. This technique could be applied for the detection of the addition of hardened vegetable fats (Smith *et al.*, 1994).

Thin layer chromatography

A thin layer chromatography (TLC) method for the detection of at least 5% kokum butter in cocoa butter was developed using a mobile phase of benzene, ethylactetate and acetic acid. A bluish green spot indicated the presence of kokum butter. This phenomenon could not be observed with other fats, such as fats from mango kernel or mahua (Deotale *et al.*, 1990).

DIFFERENTIAL SCANNING CALORIMETRY

The melting thermograms of palm mid-fraction, anhydrous milk fat and cocoa butter show distinct differences among the three fats. Further evaluation revealed that a characterisation of a two-component mixture is possible by calculating the partial melting enthalpy. The data do not allow a quantification of all three components in a ternary mixture. This method requires a tempering of the samples for a considerable time span (several hours to days), making it less convenient (Ali and Dimick, 1994*a*,*b*).

The pure triglycerides, POP, POS and SOS, were investigated for their crystallisation and melting behaviour. The speed of cooling and subsequent heating has a great influence on the shape of the thermograms. The method was described as a useful tool for quality control, but special care should be taken for the interpretation of the results (Cebula *et al.*, 1992*b*). As an extension, the effect of blends and minor components was investigated. Differential scanning calorimetry was described to be a very convenient tool to classify the different types of confectionery fats. Differential scanning calorimetry (DSC) can be used to identify and quantify the progressive changes in the formulation of a typical cocoa butter equivalent and the effects caused by the presence of trisaturated triglycerides and diglycerides (Cebula and Smith, 1992*a*).

A direct correlation of the mechanical hardness of cocoa butters and peak area of the polymorph II endotherm (a specific signal with a maximum at 21°C) could be established. Soft cocoa butters were characterised by a high content of POO and SOO, a high iodine value and a low percentage area of the polymorph II endo-

therm. Hard cocoa butters show the opposite phenomenon, i.e. low content of POO and SOO, low iodine value and a high percentage area of the polymorph II endotherm. In general, the cocoa butter of South America could be classified as soft, the one of Asia and Oceania as hard using this method. The specimens originating from Africa, and North and Central America were ranging in between (Chaiseri and Dimick, 1989).

OTHER TECHNIQUES

Mass spectrometry

Direct chemical ionisation in combination with mass spectrometry (MS) was used for the identification of triglyceride patterns of fats. This technique prevents fragmentation of the triglycerides and the identification of the triglycerides is achieved according to their carbon numbers and the number of double bonds (Schulte *et al.*, 1981).

Atom bombardment mass spectrometry combined with principal component analysis was applied for the differentiation of triglycerides in edible oils (Lamberto and Saitta, 1995). Five different vegetable oils were analysed and the method was claimed to be fast (typically < 3 min for each analysis) and suitable for the identification of edible oils as well as the discovery of counterfeits in those oils.

Pyrolysis mass spectrometry was used for the classification of different fats and oils comprising cocoa butter. This fast method (typical time for analysis below 3 min) is based on the thermal degradation of the compound followed by a mass spectrometric analysis of the fragments. Neural networks and principal component analysis were applied for the data evaluation. With both methods a good discrimination of cocoa butter from all other vegetable fats could be achieved (Anklam *et al.*, 1997).

X-ray microanalysis

Milk powder, cocoa solids and chocolate were investigated for their metal content using X-ray microanalysis. Particles from milk powder were found to contain similar amounts of potassium and calcium, whereas cocoa solid particles are rich in potassium but contain very small amounts of calcium. Neither the particles originating from milk powder nor the one originating from cocoa solids are homogeneous in their composition. Thus a large number or particles have to be investigated for being representative (Brooker, 1990). The time needed for this procedure and the remaining inaccuracy, however, seems to make the application of X-ray microanalysis less suitable for routine control of the milk fat content in chocolate.

Transmission electron microscopy, scanning differential calorimetry and X-ray diffraction have been used for a characterisation of a mixture of 50% cocoa butter and 50% hydrogenated vegetable fat. The addition of vegetable fats clearly affects both the molecular structure and the morphology of the fat crystals (Hicklin *et al.*, 1985).

Nuclear magnetic resonance

Nuclear magnetic resonance (NMR) can be used to investigate the polymorphic forms of solid fats. Polymorphic forms of the fats include the unstable α and the more stable β and β'-modification. ^{1}H-spin lattice relaxation times could be clearly related to the amount (determined by X-ray diffraction) of the β-modification present in a fat sample (Colquhoun and Grant, 1989). NMR analysis of chocolate also offers the spatial detection of polymorphic states of cocoa butter in chocolate and clearly resolves the various components present (Duce *et al.*, 1990).

Low resolution pulse NMR was used for the analysis of the fluidification of cocoa butter at 27.5°C. The parameters of the resulting bimodal curve were used for a separation of cocoas according to the process and the type of roasters used (Hernández and Rutledge, 1994*a*).

Pulsed NMR is one of the standard methods to determine the solid fat content in fats. Analysis has shown that the cocoa butter equivalents Illexao A and B can be distinguished from cocoa butter with respect to their solid fat content at different temperatures (Shukla, 1991).

CONCLUSIONS

Various analytical methods are described for their potential use of detecting and quantifying foreign fats and oils in cocoa butter or chocolate. The technique investigated in most detail is the analysis of triglycerides by either GC or HPLC. The most common approach is to detect the major three triglycerides of cocoa butter—POP, POS and SOS—and to develop characteristic ratios for the identification of the cocoa butter as well as the quantification of the content of foreign fat in cocoa butter. This method, however, does not allow the detection of the addition of illipé fat which has a triglyceride structure very similar to cocoa butter. The analysis of the fractions of the unsaponifiables in cocoa butters and mixtures of foreign fats was shown to be very feasible for most of the added fats. However, most difficult to detect again were products made of illipé fat. One author identified one compound, Ψ-taraxasterol, of the fraction of the unsaponifiables, which could be used for the identification of illipé fat. It is claimed that this compound is typical for illipé and sal fat and is hardly found in other fats.

There is no unique approach for the identification and quantification of foreign fats in cocoa butter or

chocolate. No single method could provide means which allow the unequivocal identification of those mixtures. However, it seems most probable that the combined application of several methods and subsequent chemometric data evaluation could provide the means for a reliable quantification of mixtures of cocoa butter and other vegetable fats. Of particular interest would be the combination of a thorough analysis of the triglycerides *and* the fraction of the unsaponifiables. In addition, this approach would reveal a pattern typical for the cocoa butter being based on a rather big number of components and making adulteration of cocoa butter much more difficult.

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REFERENCES

Abu-Hadeed, A. M. and Kotb, A. R. (1988) A method for the quantitative determination of individual oils in a blend. *Journal of the American Oil Chemists' Society* **65**, 1922–1926.

Ahlers, N. H. E. and O'Neill, L. A. (1954) The structure of oil and resins spectroscopy. *Journal of the Oil and Colour Chemists' Association* **37**, 533–599.

Aitzetmüller, K., Wessels, H. and Werner, G. (1988) Unterscheidung von Palmölen und Palmölfraktionen durch HPLC der Triglyceride. *Fat Science and Technology* **90**, 442–447.

Ali, A. R. Md. and Dimick, P. S. (1994*a*) Thermal analysis of palm mid-fraction, cocoa butter and milk fat blends by differential scanning calorimetry. *Journal of the American Oil Chemists' Society* **71**, 299–302.

Ali, A. R. Md. and Dimick, P. S. (1994*b*) Melting and solidification characteristics of confectionery fats: anhydrous milk fat, cocoa butter and palm kernel stearin blends. *Journal of the American Oil Chemists' Society* **71**, 803–805.

Anklam, E., Lipp, M. and Wagner, B. (1996) HPLC with light scatter detector and chemometric data evaluation for the analysis of cocoa butter and vegetable fats. *Fett/Lipid* **98**, 55–59.

Anklam, E., Bassani, M. R., Eiberger, T., Kriebel, S., Lipp, M. and Matissek, M. (1997) Characterisation of cocoa butters and other vegetable fats by pyrolysis mass spectrometry. *Fresenius Journal of Analytical Chemistry* **357**, 981–984.

Artho, A., Grob, K. and Mariani, C. (1993) On-line LC–GC for the analysis of the minor components in edible oils and fats—the direct method involving silylation. *Fat Science and Technology* **95**, 176–180.

Balesdent, D. and Kapseu, C. (1989) Charactérisation des triglycérides de l'huile de coton par chromatographie liquide haute performance (CLHP) en une seule étape. *Revue Français Corps Gras* **36**, 27–33.

Bergqvist, M. H. J. and Kaufmann, P. (1993) A multivariate optimisation of triacylglycerol analysis by high-performance liquid chromatography. *Lipids* **28**, 667–675.

Biedermann, M., Grob, K. and Mariani, C. (1993) Transesterification and on-line LC–GC for determining the sum of free and esterified sterols in edible oils and fats. *Fat Science and Technology* **95**, 127–133.

Bracco, U., Rostagno, W. and Egli, R. H. (1970) A study of cocoa butter—Illipé butter mixtures. *International Chocolate Review* **25**, 41–48.

Brooker, B. E. (1990) Identification and characterisation of cocoa solids and milk proteins in chocolate using X-ray microanalysis. *Food Structure* **9**, 9–21.

Caboni M. F., Conte L. S. and Lercker G. (1991) HPLC analysis of lipids in food by light scattering detection in quality control. In *Strategies for Food Quality Control and Analytical Methods in Europe,* Proceedings of the 6th European Conference on Food Chemistry, Hamburg, Germany, Sept. 22–26, 468–473.

Carelli, A. and Cert, A. (1993) Comparative study of the determination of triacylglycerol in vegetable oils using chromatographic techniques. *Journal of Chromatography* **630**, 213–222.

Carlin, J. T., Lee, K. N., Hsieh, O. A.-L., Wang, L. S., Ho, C.-T. and Chang, S. S. (1982) Cocoa butter flavors. *The Manufacturing Confectioner* **6**, 64–73.

Cebula, D. J. and Smith, K. W. (1992) Differntial scanning calorimetry of confectionery fats: Part II—effects of blend and minor components. *Journal of the American Oil Chemists' Society* **69**, 992–998.

Cebula, D. J., Smith, K. W. and Talbot, G. (1992) DSC of confectionery fats, pure triglycerides. *The Manufacturing Confectioner* **9**, 135–139.

Chaiseri, S. and Dimick, P. S. (1989) Lipid and hardness characterisitics of cocoa butters from different goegraphic origins. *Journal of the American Oil Chemists' Society* **66**, 1771–1776.

Chaveron, H., Adenier, M., Padley, F. B., Dallas, M. S. J., Meijboom, P. W. and Jongenotter, G. A. (1981) Métthode de détermination des triglycérides monooléodisatures symétriques et disymétriques. *Ann. Fals. Exp. Chim.* **74**, 455–464.

Chaveron, H. and Verdoia, C. (1984) Détermination des matières grasses végétales (M.G.V.) dans les chocolats. *Ann. Fals. Exp. Chim.* **77**, 401–428.

Chaveron, H., Verdoia, C. and Meili, M. (1984) Influence des matières grasses polyinsaturées sur la detection et la dosage des matières grasses végetales dans le chocolat. *Ann. Fals. Exp. Chim.* **77**, 571–585.

Christie, W. W. (1990) Silver ion chromatography of triacylglycerols on solid phase extraction columns packed with a bonded sulphonic acid phase. *Journal of the Science of Food and Agriculture* **52**, 573–577.

Christie, W. W. (1994) Silver ion and chiral chromatography in the analysis of triacylglycerols. *Progress in Lipid Research* **33**, 9–18.

Christie, W. W., Nikolova-Damyanova, B., Laakso, P. and Herslof, B. (1991) Stereospecific analysis of triacyl-sn-glycerols via resolution of diastereomeric diacylglycerol derivatives by high-performance liquid chromatography on silica. *Journal of the American Oil Chemists' Society* **68**, 695–701.

Cocito, C. and Delfini, C. (1994) Simultaneous determination by GC of free and combined fatty acids and sterols in grape musts and yeasts as silanised compounds. *Food Chemistry* **50**, 297–305.

Colquhoun I. J. and Grant A. (1989) Solid state N.M.R. spectroscopy of fats. *Agric. Food Chem. Consum. Proc. Eur. Conf.* 27–29 Sept., 1989 Versailles, France, INRA, Paris, Vol. 2, 668–672.

D'Alonzo, R. P., Kozarek, W. J. and Wade, R. L. (1982) Glyceride composition of processed fats and oils as determined by glass capillary gas chromatography. *Journal of the American Oil Chemists' Society* **59**, 292–295.

Davies, A. M. C. (1992) Prediction of chocolate quality from near-infrared spectroscopic measurements of raw cocoa beans. *Analytical Proceedings* **29**, 198–200.

de Jong, S. and de Jong, Th. J. R. (1991) Computer assisted fat blend recognition using regression analysis and mathematical programming. *Fat Science and Technology* **93**, 532–536.

Defense, E. (1984) Application de la chromatographie liquide haute performance à l'analyse des triglycérides des graisses végétales et animales et de leur fraction obtenues par cristallisation fractionné. *Revue Français Corps Gras* **31**, 123–129.

Deotale, M. Y., Patil, M. N. and Adinarayaniah, C. L. (1990) Thin layer chromatographic detection of kokum butter in cocoa butter. *Journal of Food Science and Technology* **27**, 230–236.

Derbesy, M. and Richert, T. (1979) Détection du beurre de karité dans la beurre du cacao. *Oléagineux* **34**, 405–409.

Duce, S. L., Carpenter, T. A. and Hall, L. D. (1990) Nuclear magnetic resonance imaging of chocolate confectionery and the spatial detection of polymorphic states of cocoa butter in chocolate. *Lebensmittelwissenschaften und- Technologie* **23**, 545–549.

Eiberger, T. and Matissek, R. (1994*a*) Zum Nachweis von Kakaobutteräquivalenten mittels HPLC. *Lebensmittelchemie* **48**, 49.

Eiberger, T. and Matissek, R. (1994*b*) Ansätze und Daten zum Nachweis von Kakaobutteräquivalenten (CBE). *Lebensmittelchemie* **48**, 126.

El-Hamdy, A. H. and Perkins, E. G. (1981) High performance reversed phase chromatography of natural triglycerides. *Journal of the American Oil Chemists' Society* **58**, 49–53.

Fincke, A. (1980*a*) Möglichkeiten und Grenzen einfacher gaschroma-tografischer Triglyceridanalysen zum Nachweis fremder Fette in Kakaobutter und Schokoladenfette. 1. Mitteilung: Verteilung der nach C-Klassen klassifizierte Triglyceride in Kakaobutter. *Deutsche Lebensmittel Rundschau* **76**, 162–167.

Fincke, A. (1980*b*) Möglichkeiten und Grenzen einfacher gaschromatografischer Triglyceridanalysen zum Nachweis fremder Fette in Kakaobutter und Schokoladenfette. 2. Mitteilung: Verteilung der nach C-Klassen klassifizierte Triglyceride in Kakaobutter-Austauschfetten und anderen Fetten. *Deutsche Lebensmittel Rundschau* **76**, 187–192.

Fincke, A. (1980*c*) Möglichkeiten und Grenzen einfacher gaschromatografischer Triglyceridanalysen zum Nachweis fremder Fette in Kakaobutter und Schokoladenfette. 3. Auswertung gaschromatografischer Triglyceridanalysen von Kakaobutter. *Deutsche Lebensmittel Rundschau* **76**, 384–389.

Fincke, A. (1982) Möglichkeiten und Grenzen einfacher gaschromatografischer Triglyceridanalysen zum Nachweis fremder Fette in Kakaobutter und Schokoladenfette. 4. Auswertung gaschromatografischer Triglyceridanalysen von Milchschokoladenfetten. *Deutsche Lebensmittel Rundschau* **78**, 389–396.

Frega, N., Bocci, F. and Lercker, G. (1992) Direct gas chromatographic analysis of the unsaponifiable fraction of different oils with a polar capillary column. *Journal of the American Oil Chemists' Society* **69**, 447–450.

Frega, N., Bocci, F., Giovannoni, G. and Lercker, G. (1993) High resolution GC of unsaponifiable matter and sterol fraction in vegetable oil. *Chromatographia* **36**, 215–217.

Geeraert, E. and De Shepper, D. (1983) Structure elucidation of triglycerides by chromatographic techniques. *Journal of High Resolution Chromatography and Chromatography Communications* **6**, 123–132.

Geeraert, E. and Sandra, P. (1987) Capillary GC of triglycerides in fats and oils using a high temperature phenylmethylsilicone stationary phase. Part II. The analysis of chocolate fats. *Journal of the American Oil Chemists' Society* **64**, 100–105.

Gegiou, D. and Staphylakis, K. (1981) Determination of cocoa butter substitutes in chocolate. *The Quality of Foods and Beverages* **2**, 301–309.

Gegiou, D. and Staphylakis, K. (1985) Detection of cocoa butter equivalents in chocolate. *Journal of the American Oil Chemists' Society* **62**, 1047–1051.

Gordon, M. H. and Griffith, R. E. (1992) Steryl ester analysis as an aid to the identification of oils in blends. *Food Chemistry* **43**, 71–78.

Grob, K., Giuffré, A. M., Biedermann, M. and Bronz, M. (1994*a*) The detection of adulteration with desterolised oils. *Fat Science and Technology* **96**, 341–345.

Grob, K., Giuffré, A. M., Leuzzi, U. and Mincione, B.(1994*b*) Recognition of adulterated oils by direct analysis of the minor components*Fat Science and Technology* **96**, 286–290

Grob, K., Lanfranchi, M. and Mariani, M. (1989) Determination of free and esterified sterols and of wax esters in oils and fats by coupled liquid chromatography—gas chromatography. *Journal of Chromatography* **471**, 397–405.

Haase-Aschoff, K., Haase-Aschoff, I. and Patz, Cl.-D. (1995) Bestimmung von Steradienen in Speiseöl und Fetten zum Nachweis der Naturbelassenheit. *Lebensmittelchemie* **49**, 89–90.

Hammond, E. W. (1989) Chromatographic techniques for lipid analysis. *Trends in Analytical Chemistry* **8**, 309–313.

Hashim, L. and Chaveron, H. (1994) Extraction and determination of methylpyrazines in cocoa beans using coupled steam distillation—microdistillator. *Food Research International* **27**, 537–544.

Hernández, B., Castellote, A. I. and Permanyer, J. J. (1991) Triglyceride analysis of cocoa beans from different geographical origin. *Food Chemistry* **41**, 269–276.

Hernández, C., Bermond, A. and Ducauze, C. J. (1994) Discrimination de masses de cacao par l'utlisation de données chimiométriques: détermination de leur origine géographique et de leur traitement technologique à partir des teneurs en cations métallique. *Analusis* **22**, 15–22.

Hernández, C. V. and Rutledge, D. N. (1994*a*) Characterisation of cocoa masses: low resolution pulse NMR study on the effect of geographical origin and roasting on fluidification. *Food Chemistry* **49**, 83–93.

Hernández, C. V. and Rutledge, D. N. (1994*b*) Multivariate statistical analysis of gas chromatograms to differentiate cocoa masses by geographical origin and roasting conditions. *Analyst* **119**, 1171–1176.

Hicklin, J. D., Jewell, G. G. and Heathcock, J. F. (1985) Combining microscopy and physical techniques in the study of cocoa butter polymorphs and vegetable fat blends. *Food Microstructure* **4**, 241–248.

Homberg, E. and Bielefeld, B. (1982*a*) Sterine und Methylsterine in Kakaobutter und Kakaobutter-Ersatzfetten*Dtsch. Lebensm. Rundsch.* **78**, 73–77.

Homberg, E. and Bielefeld, B. (1982*b*) Freie und gebundeneSterine in rohen und raffinierten Palmölen. *Teil I. Fette Seifen Anstrichmittel* **84**, 141–146.

Homberg, E. and Bielefeld, B. (1990*a*) Hauptkomponenten der 4-Methylsterin- und Triterpenfraktion von 12 Pflanzenfetten und ihr Einfluß auf die Sterinanalytik. *Fat Science and Technology* **92**, 478–480.

Homberg, E. and Bielefeld, B. (1990*b*) Sterin- und Fettsäurezusammensetzung in Keimölen. *Fat Science and Technology* **92**, 118–121.

Itabashi, Y., Kuksis, A. and Myher, J. J. (1990) Determination of molecular species of enantiomeric diacylglycerols by chiral phase high performance liquid chromatography and polar capillary gas–liquid chromatography. *Journal of Lipid Research* **31**, 2119–2126.

Itabashi, Y., Marai, L. and Kuksis, A. (1991) Identification of natural diacylglycerols os the 3,5-dinitrophenylurethanes by chiral phase liquid chromatography with mass spectrometry. *Lipids* **26**, 951–956.

Kaufmann, P. and Herslöf, B. G. (1991) A multivariate identification of natural triglyceride oils. *Fat Science and Technology* **93**, 179–183.

Kemper, K., Melchert, H.-U., Rubach, K. and Hoffmeister, H. (1988) Charakterisierung der Triglyceridmuster von pflanzlichen und tierischen Fetten sowie Human- und Tierseren mittels HPLC nach Vortrennung an $AgNO_3$-imprägnierten Kieselgel-Minisäulen. *Fresenius' Zeitschrift für Analytische Chemie* **331**, 634–641.

Kneifel, W., Ulberth, F. and Hoch, G. (1990) A rapid colorimetric method for the estimation of cocoa content in milk-powder–cocoa mixtures. *Lebensmittelwissenschaften und-Technologie* **23**, 189–191.

Konishi, H., Neff, W. E. and Mounts, T. L. (1993) Correlation of reversed-phase high-performance liquid chromatography for fatty acid composition of some vegetable oils. *Journal of Chromatography* **629**, 237–241.

Lamberto, M. and Saitta, M. (1995) Principal component analysis in fast atom bombardement—mass spectrometry of triacylglycerols in edible oils. *Journal of the American Oil Chemists' Society* **72**, 867–871.

Letter, W. S. (1993) A qualitative method for triglyceride analysis by HPLC using an evaporative light scattering detector. *Journal of Liquid Chromatography* **16**, 225–239.

Lipp M. and Anklam E. (1998) Review on cocoa butter and alternative fats for use in chocolate—Part A. Compositional data. *Food Chemistry*.

Mani V. V. S. and Lakshminarayana G. (1976) Chromatographic and other methods for detection of adulteration of oils and fats. *Journal of the Oil Technology Association of India* **5**, 84–103.

Marini, D. and Balestrieri, F. (1989) Analisi di miscele di trigliceridi mediante HPLC con rilevatore UV. *Rivista delle Sostanze Grasse* **66**, 11–16.

Motta, L., Carelli, G., Lercker, G., Mariani, C., Cozzoli, O. and Zelinotti, T. (1987) Considerazioni sull'analisi gascromatografica dei trigliceridi con utilizzo di colonne capillari. *Rivista delle Sostanze Grasse* **16**, 545–549.

Nikolova-Damyanova, B. M. and Amidzhin, B. S. (1992) Comparative analysis of cocoa butter and cocoa butter replacers. *Bulgarian Chemical Communications* **25**, 361–365.

Padley F. B. (1980) Characterisation of cocoa butter equivalents and their determination in chocolate. Paper presented at the General Assembly of the Technical Committee, June 1980, Cambridge.

Padley, F. B. and Timms, R. E. (1978*a*) Analysis of confectionery fats—Part II. Gas liquid chromatography of triglycerides. *Lebensmittelwissenschaften und- Technologie* **11**, 319–330.

Padley, F. B. and Timms, R. E. (1978*b*) Determination of cocoa butter equivalents in chocolate. *Chem. Ind.* **12**, 918–919.

Padley, F. B. and Timms, R. E. (1980) The determination of cocoa butter equivalents in chocolate. *Journal of the American Oil Chemists' Society* **57**, 286–293.

Palmer, A. J. and Palmer, F. J. (1989) Rapid analysis of triacylglycerols using high-performance liquid chromatography with light scattering detector. *Journal of Chromatography* **465**, 369–377.

Permanyer, J. J. and Perez, M. L. (1989) Compositional analysis of powdered cocoa products by near infrared spectroscopy. *Journal of Food Science* **54**, 768–769.

Pino, J. and Roncal, E. (1992) Linalool content in roasted cocoa butter as a characteristic of several flavour grade cocoas. *Die Nahrung* **36**, 299–304.

Pino, J., Nuñez de Villavicencio, M. and Roncal, E. (1992) Pattern recognition of GC profiles for classification of cocoa powder of Ghanaian and Cuban varieties. *Die Nahrung* **36**, 302–306.

Pino, J., Nuñez de Villavicencio, M. and Roncal, E. (1993) Pattern recognition of gc profiles for classification of cocoa butter of ghanian and cuban cultivars. *Journal of Food Quality* **16**, 125–132.

Podlaha, O., Töregård, B. and Püschl, B. (1984) TG-type composition of 28 cocoa butters and correlation between some of the TG-type components. *Lebensmittelwissenschaften und- Technologie* **17**, 77–81.

Pontillon, J. (1995) Determination of milk fat in chocolates by gas-liquid chromatography of triglycerides and fatty acids. *Journal of the American Oil Chemists' Society* **72**, 861–866.

Rezanka, T. and Mares, P. (1991) Determination of plant triacylglycerols using capillary chromatography, high-performance liquid chomatography and mass spectrometry. *Journal of Chromatography* **542**, 145–159.

Saccà, B., Spata, M. and Ruffini, B. (1991) Determinazione quantitativa di oli e grassi di natura diversa in miscela. *Rassenga Chimica* **1**, 27–34.

Schulte, E. (1994) Determination of edible fat refining by HPLC of Δ3,5-steradienes. *Fat Science and Technology* **96**, 124–128.

Schulte, E., Höhn, M. and Rapp, U. (1981) Mass spectrometric determination of triglyceride patterns of fats by the direct chemical ionisation technique (DCI). *Fresenius' Zeitschrift für Analytische Chemie* **307**, 115–119.

Semporé, G. and Bézard, J. (1991) Determination of molecular species of oil triacylglycerols by reversed-phase and chiral-phase high-performance liquid chromatography. *Journal of the American Oil Chemists' Society* **68**, 701–709.

Shukla V. K. S. (1991) Application of NMR spectrometry: pulsed NMR. In *Analysis of Fats, Oils and Lipoproteins*, ed. E. G. Perkins. American Oil Chemists Society, Champaign, Illinois, U.S.A., p. 458–463.

Shukla, V. K. S., Schiotz Nielsen, W. and Batsberg, W. (1983) A simple and direct procedure for the evaluation of triglyceride composition of cocoa butters by high performance liquid chromatography—a comparison with the existing TLC–GC method. *Fette Seifen Anstrichmittel* **85**, 274–278.

Smith, K. W., Perkins, J. M., Jeffrey, B. S. J. and Phillips, D. L. (1994) Separation of molecular species of cis- and trans-triacylglycerols in trans-hardened confectionery fats by silver ion high performance liquid chromatography. *Journal of the American Oil Chemists' Society* **71**, 1219–1222.

Soulier, P., Farines, M. and Soulier, J. (1990) Triterpene alcohols, 4-methylsterols, and 4-desmethylsterols of sal and illipe butters. *Journal of the American Oil Chemists' Society* **67**, 388–393.

Staphylakis, K. and Gegiou, D. (1985) Sterols in cocoa butter. *Fette Seifen Anstrichmittel* **87**, 150–155.

Takagi, T. and Ando, Y. (1991) Stereospecific analysis of triacylglycerols by chiral-phase HPLC direct derivatisation of partially hydrolysed products. *Journal of the Japanese Oil Chemists' Society* **40**, 288–292.

Takagi, T. and Ando, Y. (1995) Stereospecific analysis of monounsaturated triacylglycerols in cocoa butter. *Journal of the American Oil Chemists' Society* **72**, 1203–1209.

van de Voort, F. R., Ismail, I. I. and Sedman, J. (1995) A rapid automated method for the determination of cis and trans content of fats and oils by Fourier transform infrared spectroscopy. *Journal of the American Oil Chemists' Society* **72**, 873–880.

van Niekerk, P. J. and Burger, A. E. C. (1985) The estimation of the composition of edible oil mixtures. *Journal of the American Oil Chemists' Society* **62**, 531–538.

Young, C. C. (1984) The interpretation of GLC triglyceride data for the determination of cocoa butter equivalents in chocolate: a new approach. *Journal of the American Oil Chemists' Society* **61**, 576–581.