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Detection and quantification of cocoa butter equivalents in chocolate model systems: analysis of triglyceride profiles by high resolution GC

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Abstract

The detection of vegetable fats added to cocoa butters in chocolate formulations was investigated in model mixtures. Cocoa butters varying in origins, crop and treatment were analysed alone and combined at levels of 5, 10, 15 or 20% to a variety of cocoa butter equivalents. Triglyceride profiles were obtained by high resolution GC. The areas of the major triglycerides present were plotted one vs the other. The plots of percentages of specific triglycerides allowed detection of vegetable fats added to cocoa butters in these model systems, and quantification of the addition could be done down to a 5% level on a cocoa butter basis. The detection was most difficult in the case of the addition of illipe fat and the quantification could not be done below 10%. The results suggest that, in chocolate, which contains 20–25% cocoa butter, this detection system works down to 1–2%, and thus well below the proposed 5% regulatory level. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

Chocolate is usually made from the different components of the cocoa beans after processing. In chocolate the fat is therefore cocoa butter (CB) for dark chocolate, and CB with cow's milk fat in milk chocolate. However, it is possible for other vegetable fats to be used. The new draft proposal of the European Commission, replacing the Directive 73/241/EEC (EU Council Directive, 1973) relating to cocoa and chocolate products, may allow the addition of vegetable fats other than CB at levels up to 5% based on the finished product. Foreign fats normally added to chocolate are in practice similar in chemical and physical properties to CB or present specific functional properties. Cocoa butter equivalents (CBE), substitutes (CBS) or replacers (CBR) can be obtained from natural plants or produced specifically by chemical or enzymatic fractionation from plant fats (Reddy & Prabhakar, 1989; Bloomer et al., 1990; Chang et al., 1990; Reddy & Prabhakar, 1990; Sridhar et al., 1991; Chong et al., 1992; Nesaretnam & Md. Ali, 1992; Mojovic et al., 1993; Lipp & Anklam, 1998a). CBR is the general term applying to confectionery fats used in partial or whole replacement of CB, whereas the term CBE implies the total compatibility of the fat with CB (McGinley, 1991). CBEs are thus very similar in composition to CB. The major triglycerides are the same as in CB, making the detection and quantification of such an addition difficult. The regulation at a European level, and therefore the enforcement of the regulation, in turn calls for methods that are capable of detecting as well as quantifying the addition of vegetable fats other than CB to chocolate.

Much research has been dedicated to the determination of CB equivalents in chocolate. Various components of CB have been targeted as indicators for the detection of added vegetable fats other than CB to chocolate (Lipp & Anklam, 1998b). As triglycerides account for more than 95% of CB, their analysis has been studied intensively. Early work on the analysis of triglycerides from CB used gas chromatography on packed columns which separated triglyceride species by carbon number (Fincke, 1980, 1982; Padley & Timms, 1980). The use of temperature-resistant capillary columns for the analysis of intact triglycerides by gas chromatography (high resolution gas chromatography (HR-GC) improved the resolution and allowed

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determination of individual triglyceride species (Geeraert & Sandra, 1987; Termonia et al., 1987; Hammond, 1989; Frega et al. 1993; Young, 1987). Another popular analytical technique for the analysis of triglycerides is reverse phase high performance liquid chromatography (RP-HPLC) (Rezanka & Mares, 1991). Efficient separations, similar to that of HR-GC, can be achieved with two C18 columns in series, and also by using a light-scattering detection (Palmer & Palmer, 1989; Eiberger & Matissek, 1994; Anklam et al., 1996). The analysis of triglycerides can also be done by silver-ion chromatography, where separation is carried out on the basis of the degree of unsaturation of the triglycerides (Takano & Kondoh, 1987; Christie, 1990; Jeffrey, 1991). Other indicators such as sterols, 4-methylsterols, triterpene alcohols and sterol degradation products have been used for the detection of foreign fats in chocolate (Homberg & Bielfefeld, 1990; Gordon & Griffith, 1992; Artho et al., 1993; Biedermann et al., 1993; Crews et al., 1997), but their difficult identification makes the method more arduous to utilise.

Previous research has shown the potential of triglycerides as indicators in the detection of added vegetable fats to CB. The interpretation of the results has been the subject of investigations, due to the importance of identifying the nature of added foreign fats. Research by Fincke (1980, 1982) and Padley & Timms (1980) using packed gas chromatography introduced the use of plots based on the percentage by carbon numbers (%P) and normalised to the three major triglycerides. Graphing %P50 vs %P54 gave map differences in compositions between CB and other fats. The possible identification of individual triglycerides rather than triglycerides of the same carbon number yields specific information. In particular, plots of POP (1,3 dipalmitoyl-2-oleoylglycerol) vs SOS (1,3 distearoyl-2-oleoyl-glycerol) have given encouraging results for the detection of gross additions of foreign fats to CB (Eiberger & Matissek, 1994). Problems were, however, highlighted as to the identification of the fat(s) added in the case of mixtures and quantification of the addition. Due to the fact that the composition of fats varies with their geographical origins (Chaiseri & Dimick, 1989), detection of added foreign fat may be achieved, but quantification with current techniques is at present extremely difficult, especially when the origin of CB and of other fats is unknown. The general purpose of this work was to investigate the detection and quantification of vegetable fats added to CB based on model mixtures and chocolate products using different methods and various statistical tools. This part of the research used high temperature–high pressure GC as the analytical method for the analysis of triglyceride profiles, and was based on model mixtures of CBs and vegetable fats in order to determine the effect of addition as a function of natural variations in CBs, type of foreign fat added and level of foreign fat addition.

2. Materials and methods

2.1. Materials

53 CBs and 40 vegetable fats were donated by commercial suppliers. Samples consisted of 13 pure CBs of different geographical origins such as Africa, Asia, and South America. 18 commercial CB samples consisting of 10 deodorised and eight undeodorised were also analysed. Vegetable fats consisted of 7 commercial cocoa butter equivalent (CBE) mixes as well as samples of individual CBEs such as coberine, choclin, shea fat, palm oil mid fraction, illipe fat, 1,3-dipalmitoyl-2-oleoylglycerol (POP), and a CB replacer of unknown composition.

2.2. Sample preparation

Mixtures of CBs and vegetable fats, were prepared in known proportions. All mixtures were prepared on a weight basis to calculate the exact actual percentage of the added fat. The mixtures prepared did not encompass all combinations possible of the fats received, but were selected to represent the broadest range of additions possible between types/origins of CBs and that of foreign fats. As preliminary work, this part focused on mono-additions, where only one type of foreign fat was added to CBs. Mixtures of various CBs were prepared to mimic potential mixing of various CBs in the factories. Mixtures of CBs containing added CBEs were then prepared; the range of percentage of the other fats was chosen to be 5–20% to reflect the actual situation: a 5% addition on a finished product basis (chocolate) means a 10–25% addition on a CB basis, depending on the percentage of CB in the chocolate. Mixtures were prepared from the melt at levels of 5, 10, 15 and 20% vegetable fat (w/w). Stock solutions in chloroform were then made at concentrations of 100 mg ml^{-1} followed by a further dilution to 2 mg ml^{-1} in chloroform.

2.3. Sample analysis

The analyses were performed on a Hewlett Packard gas chromatograph HP 6890 equipped with an on-column injector and flame ionisation detector. The column used was a 5% polymethylphenylsiloxane DB17HT of 30 m length, 0.25 mm internal diameter and 0.15 μm film thickness (J & W, Rancho Cordova, CA, USA). The injector was programmed in temperature to rise from 100°C to 365°C at 70°C mins. The column was programmed to rise in temperature from 100°C (held 0.2 min) to 115°C at 95°C min, then from 115°C to 175°C at 65°C min, then from 175°C to 300°C at 45°C min, and finally from 300°C to 365°C at 35°C min. The temperature was held at 365°C for 20 min. The carrier gas was Helium at 33 cm sec^{-1}.

3. Results and discussion

The basis for characterisation was the general composition of CB vs other vegetable fats. CB contains triglycerides for the most part (95%) and has partial glycerides (di- and mono-glycerides), fatty acids, phospholipids, antioxidants, hydrocarbons, flavours, sterols, methylsterols and triterpene alcohols as minor constituents. In this work, the analysis of triglyceride profiles was chosen as a method of potential for the determination of vegetable fats in chocolate, because triglycerides are the most abundant components of CB. CB is characterised by 3 major fatty acids: Palmitic, 16:0 (P); Stearic, 18:0 (S), and Oleic, 18:1 (O). In addition, Linoleic acid, 18:2 (L), is also present. The major triglycerides are POP, POS, and SOS. The average percentage values for commercial CBs analysed were 21.8 ± 0.1, 44.2 ± 0.3, and 24.2 ± 0.5 for POP, POS and SOS, respectively. This study particularly focused on the quantification of CBEs because they are typically the most similar in composition to CB. The need to account for natural variations was considered, since the proportions of the different TG species vary with the origin of the CB, (Chaiseri & Dimick, 1989). The variations are usually not large but they can still reach 4% for the abundant species such as POS. In addition, the variations within one single country can also be significant as function of crop, or region. These variations have to be taken into account because chocolate manufacturers can choose a type of CB (or mixtures) for their hard or soft texture, and that the texture is a direct result of the molecular composition. Therefore, a specific aim of this study was to find differences large enough to allow the detection and possible quantification of vegetable fats and CBEs in particular. Chromatographic, as well as available spectrometric techniques, were tested within the overall scope of the research and this report details the results obtained using high temperature–high pressure GC. In order to specifically map the encountered differences, model systems were selected. The model systems consisted of the simple fat component of chocolate, in order to eliminate differences potentially due to the methods of extraction which is part of an ongoing study. Nine detectable peaks on the chromatograms (Fig. 1) were used to calculate the percentage of each triglyceride species identified and subsequently to determine how variations did tend to occur.

The geographical variations between pure CBs were best revealed when plotting POO + PLS vs SOO + SLS (Fig. 2). Pure CBs were distributed on the plot according to their geographical origin. In particular, Brasilian CBs exhibited significantly higher percentages of POO/PLS and SOO/SLS, and consequently lesser amounts of SOS, POS, POP. Since POO/PLS and SOO/SLS are lower melting triglycerides, such butters tend to be softer. By the same token, south-eastern Asian CBs in general (such as Malaysian) were characterised by lower percentages of POO and SOO, which agree with their

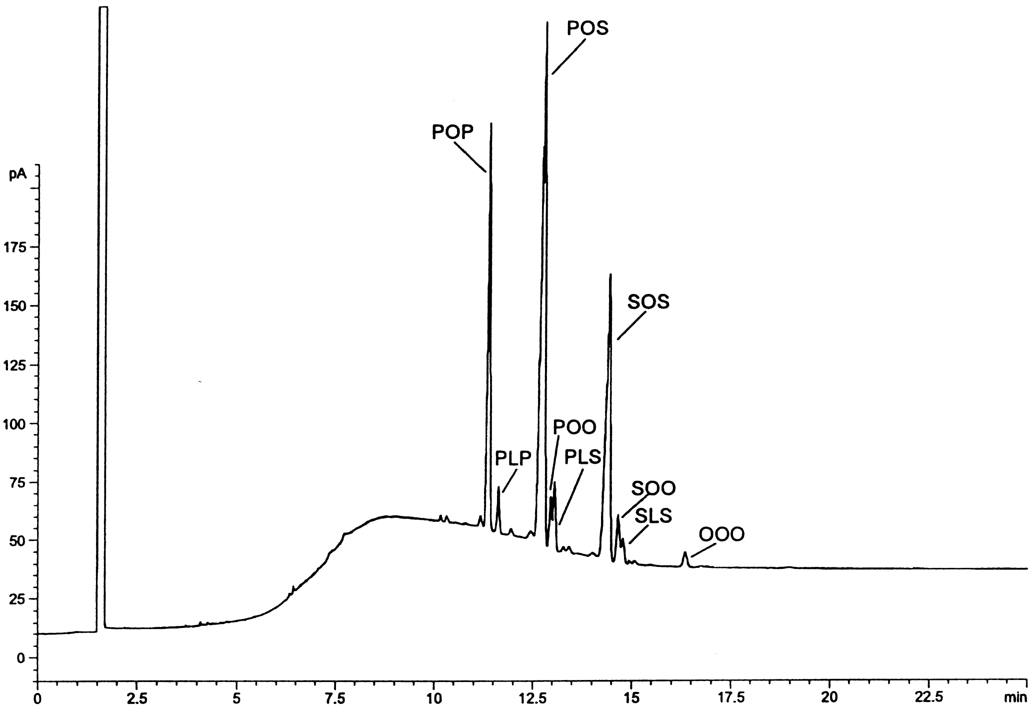

Fig. 1. Typical chromatogram obtained by HR-GC for pure cocoa butter. POP: 1,3 dipalmitoyl-2-oleoylglycerol; PLP: 1,3 dipalmitoyl-2-linoleoyl glycerol; POS: 1palmitoyl-2-oleoyl-3-stearoylglycerol; POO: 1palmitoyl-2,3-dioleoylglycerol; PLS: 1palmitoyl-2-linoleoyl-3-stearoylglycerol; SOS: 1,3 distearoyl-2-oleoylglycerol; SOO: 1 stearoyl-2,3-dioleoylglycerol; SLS: 1,3 distearoyl-2-linoleoylglycerol; OOO: trilinolein.

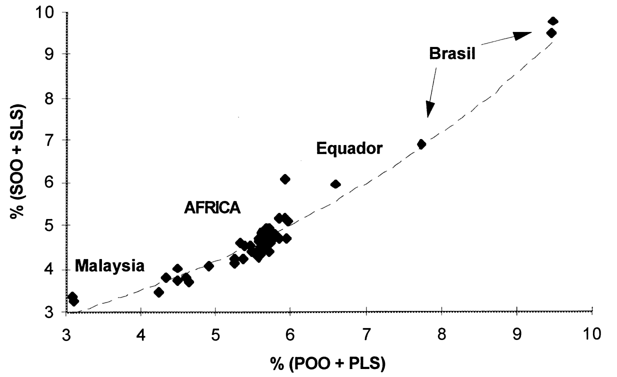

Fig. 2. Geographical origin for 53 cocoa butters seen by POO + PLS vs SOO + SLS. South American cocoa butters, typically softer, contained significantly higher percentages of di-unsaturated long chain triglycerides, whereas hard cocoa butters such as Malaysian cocoa butter had low percentages of unsaturated triglycerides.

tendency to be called hard CBs. These results are in agreement with a previous study which correlated texture and triglyceride composition (Chaiseri & Dimick, 1989). Commercial CB mixes were fairly consistent with one another in terms of composition. Both undeodorised or deodorised CB were clustered in a region without significant variations.

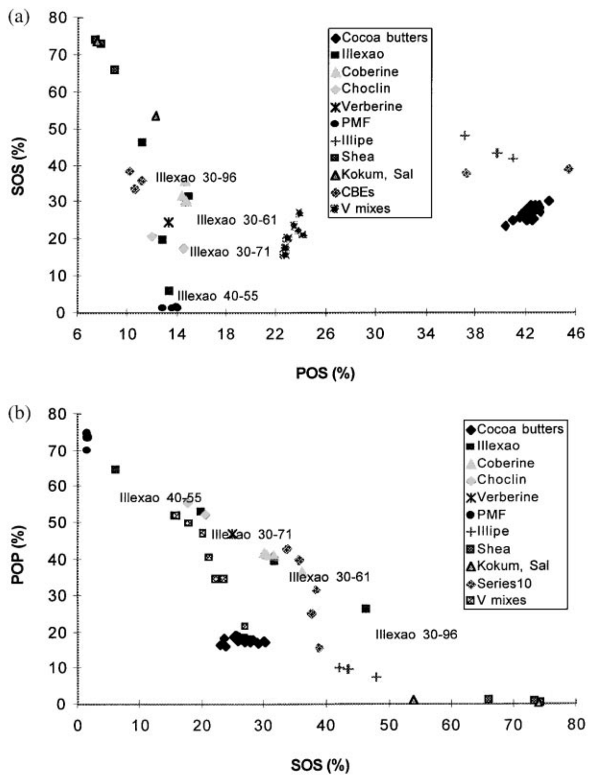

Fig. 3. Comparison of all cocoa butters vs all vegetable fats. PMF: palm oil mid fraction, Illexao: commercial mixes of different types, V mixes: mixes randomly produced for the purpose of the study. CBEs: cocoa butter equivalents from commercial source was not specified. (a) Plot representing POS vs SOS; (b) plot of SOS vs POP developed by Eiberger and Matissek (1994).

Variations engendered by the addition of other vegetable fats were revealed by plots of the major triglycerides. The general trends produced by the addition of vegetable fats were shifts to higher percentages of POP and lower percentages of POS. These trends were exemplified by plotting the pure cocoabutters vs the pure vegetable fats (Fig. 3). Cocoa butters for this comparison included both commercial mixes and pure cocoa butter from a reference collection. The identification of the nature of the fat added seemed best exhibited by plots of SOS vs POP (Fig. 3(a)), as was revealed by the GC method on packed columns originally developed by Padley & Timms (1980). However, the data suggested that plots of POS vs SOS (Fig. 3(b)), could more effectively quantify smaller additions, even with the detriment of exactly identifying the fat added.

Taking all CBs into account, plots of POS vs SOS of CB mixes typically used in the chocolate industry and their mixtures with the various foreign fats (Fig. 4) showed measurable differences for 5% increments while allowing an improved detection of the nature of the fat added. The addition of illipe fat seemed best described by an increase of the percentage of SOS without significant effect on either the percentage of POP or POS. Plots representing the packed column GC approach were also drawn in the range of the addition of vegetable fats. The trends on a plot SOS vs POP were as visible as with the plot of POS vs SOS, but the tendency to spread the data according to the nature of the fat rather than by the quantity added posed a problem for the quantification of an unknown addition of vegetable fat (Fig. 5).

Regressions were then calculated in order to determine if the quantification could be performed beyond a reasonable doubt. The results taking into account the three major triglycerides, in the case of the addition of palm oil mid fraction, showed excellent correlation

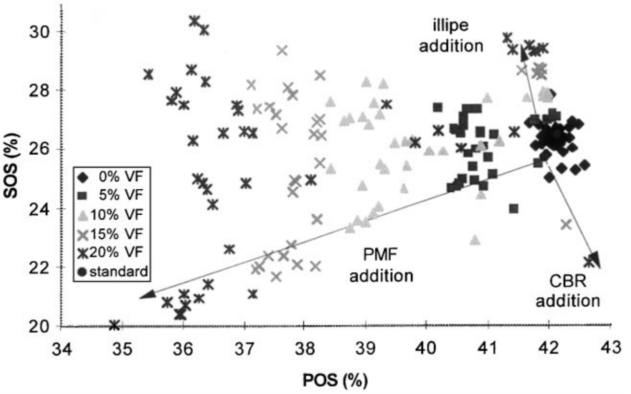

Fig. 4. Commercial CBs and their mixtures with other vegetable fats using POS and SOS as indicators. A standard of pure cocoa butter was analysed every 10 samples to assess the effect of column ageing and instrument drifts. The two for the first and last standard after 450 samples are shown on the graph and values represent the maximum variation from the technique.

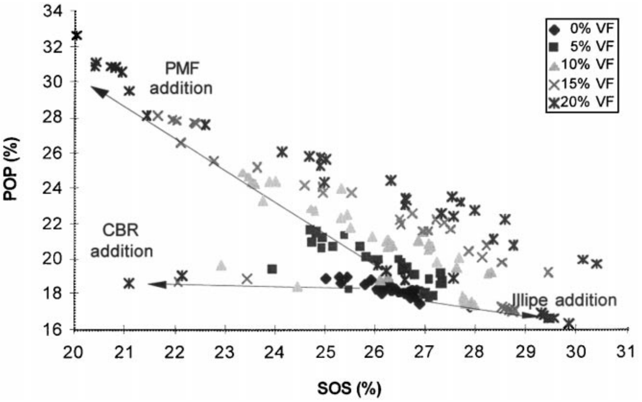

Fig. 5. Comparison of commercial CBs and their mixtures with other vegetable fats using SOS and POP as indicators developed by Eiberger and Matissek (1994).

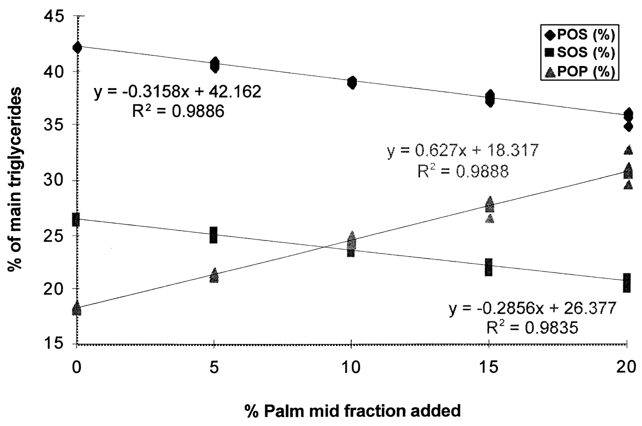

Fig. 6. Variation in the percentage of major triglyceride species as a function of the percentage of palm oil mid fraction added.

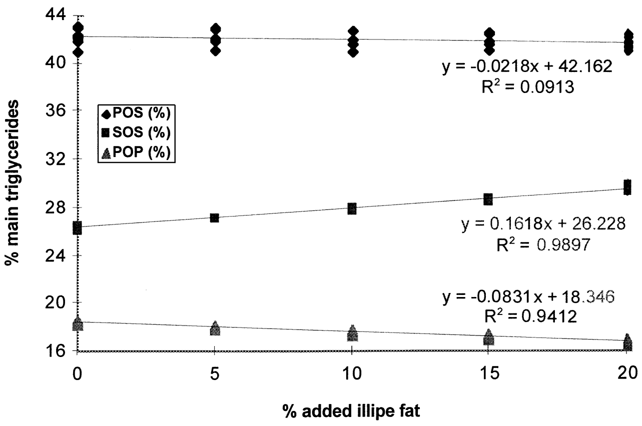

Fig. 7. Variation in the percentage of major triglyceride species as a function of the percentage of illipe added.

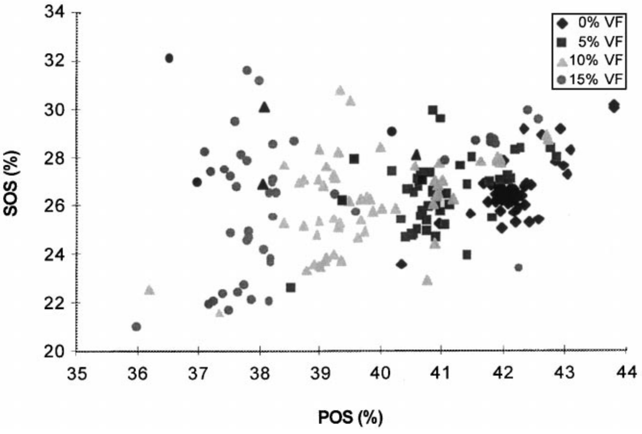

Fig. 8. Test of the quantification of mixtures of unknown nature and quantities at level 5, 10 or 15%.

between the percentage of palm mid fraction, added and the decrease in the percentage in SOS ($r^2 = 0.98$) or the increase in the percentage of POP ($r^2 = 0.98$) (Fig. 6). Trends for the other types of CB equivalents always showed a decreased percentage of POS but showed lower regression coefficients due to natural variations. A regression was calculated particularly in the case of illipe fat. However, the results suggested (Fig. 7) that the addition of illipe fat could not be quantified with a sufficient degree of certainty as was shown by low values of regression coefficients. In addition, the reduction in % POP engendered by the addition of illipe fat in cocoa butter might be overcast by natural variation in cocoa butters and the instrumental variation such as column-ageing (Simoneau et al., unpublished results).

Finally, unknown samples were analysed to test the power in identification of added foreign fat as well as quantification of such an addition. Three unknown mixtures consisted of a cocoa butter and either one of three fats of ‘unknown’ nature. In order to simplify the experiment, the samples had been prepared at either the 5, 10 or 15% of addition level. The quantification was attempted based on a POS vs SOS plot (Fig. 8). Out of those three mixtures, two were quantified in excess by about 5%, and one mixture would have resulted in an inaccurate quantification. Verification of the nature of the unknown revealed that the inaccurate quantifications resulted from the addition of illipe fat. In the other cases, where the fats added were Coberine and Choclin, respectively, the quantification showed a tendency to overestimate rather than underestimate the amount of fat.

4. Conclusion

High resolution gas chromatography (HR-GC) gave results that allowed the detection of added vegetable fats in CB based on model systems. Using the technique of HR-GC and based on model systems of cocoa butters and vegetable fats, quantification of the addition could be achieved down to a 5% level on a CB basis, which translates into very low amounts on the basis of the final chocolate product. Because various CBEs exhibit different deviating values from those of CBs, several plots might be necessary to provide an optimal detection and quantification level. The identification of the nature of the foreign fat added was possible for various vegetable fats because of the unique trends that their addition engendered, and because only one type of foreign fat was added in this experiment scheme. Current research is focusing on multi-indicator data using integrated statistical tools.

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