Capillary GC of Triglycerides in Fats and Oils Using a High Temperature Phenylmethylsilicone Stationary Phase. Part II. The Analysis of Chocolate Fats

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The analyses of the fats and oils used in the chocolate industry are reported. Triglyceride mixtures are directly injected on an FSOT column coated with a phenylmethylsilicone gum stationary phase. On phenylmethylsilicone phase, besides a carbon number separation, the triglycerides are separated according to the different combinations of saturated and unsaturated fatty acids in the triglycerides. Cocoa butters of different origin were analyzed and their POP:POS:SOS ternary diagram is discussed. The profiles of the non-cocoa butter fats such as butter oil, oils originating from nuts (e.g., hazelnut, almond, Brazil nut, walnut) and cocoa butter equivalents are presented. The elucidation of a complex chocolate fat mixture is shown and discussed in view of the recognition of the different constituents (pattern recognition) and in view of the detection and quantification of adulterations.

Gas chromatographic analysis on apolar phases (OV-1, SE-52, SE-54) characterizes oils and fats through a carbon number (CN) separation of their triglycerides. The triglyceride separation is based on molecular weight differences. By using capillary columns, some additional peaks in a packed column CN peak can be observed on apolar phases (1-9). The nature of this fine structure has been elucidated (6,9). Resolution is achieved according to the number of unsaturated fatty acids in the triglyceride molecule (NUFA separations). Triglycerides such as SSS, SOS, SOO and OOO are separated but SOS, SLS and SLnS elute in one peak. On apolar phases, unsaturated triglycerides elute before saturated triglycerides. Packed and capillary columns with polar stationary phases were shown to give higher selectivities for triglyceride separations (10,11). The limited temperature stability of the polar "liquid" phases, however, did not allow full exploitation of the possibilities of polar phases in triglyceride analysis.

We recently reported on resolution for differences in unsaturation within the unsaturated fatty acids (12). To obtain this separation, the high efficiency of a capillary column had to be combined with the selectivity of a polarizable stationary phase. The phenylmethylsilicone gum used proved stable up to 370 C, allowing use of the columns for routine analysis of fats and oils. The potential of the technique for the analysis of vegetable oils and animal fats was presented at the Sixth International Symposium on

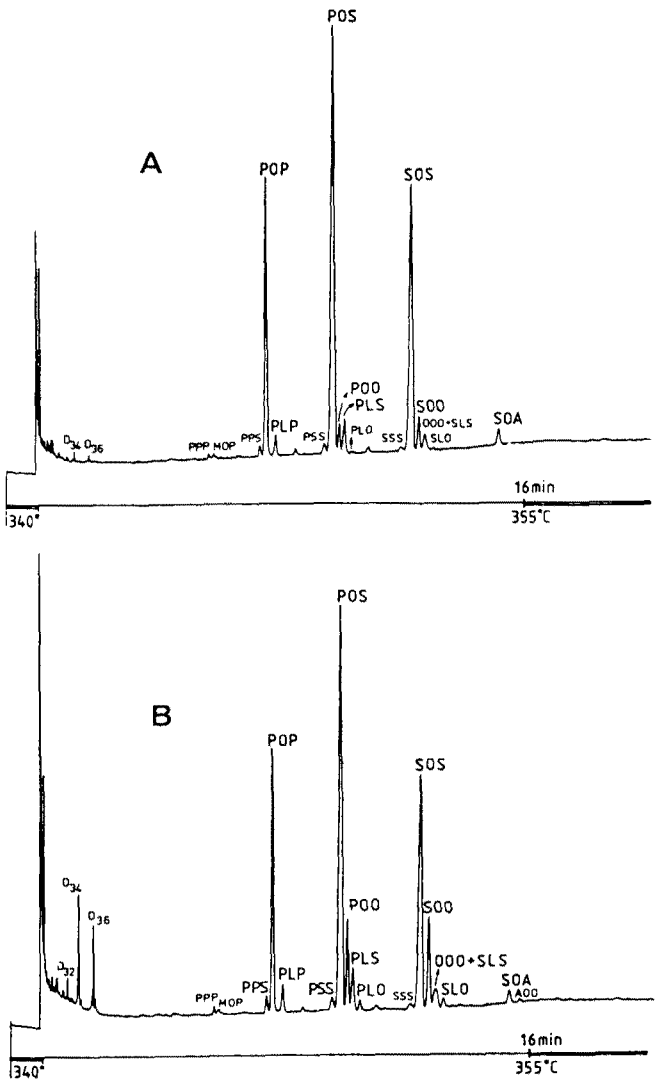

FIG. 1. Capillary GC profiles of cocoa butter with a low (A) or high (B) content of polyunsaturated triglycerides.

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The following abbreviations are used: La, lauric acid (dodecanoic acid, C12:0); M, myristic acid (tetradecanoic acid, C14:0); P, palmitic acid (hexadecanoic acid, C16:0); S, stearic acid (octadecanoic acid, C18:0); A, arachidic acid (eicosanic acid, C20:0); O, oleic acid (*cis*-9-octadecenoic acid, C18:1); L, linoleic acid (*cis,cis*-9,12-octadecadienoic acid, C18:2); Ln, linolenic acid (*cis,cis,cis*-9,12,15-octadecatrienoic acid, C18:3); PLS, palmitolinoleostearin; D_{34}, diglyceride containing 34 fatty acid carbon numbers (for example, P+S); T_{56}, triglyceride containing 56 fatty acid carbon numbers (for example, S + L + A); CN, carbon number (number of carbon atoms in the fatty acid moieties of a triglyceride; CN for SSS, SOO, SLO = 54); NUFA, number of unsaturated fatty acids in the triglyceride (NUFA for PPS is 0, for PPO, PPL = 1).

Capillary Chromatography in 1985 (13) and at the AOCS meeting in Philadelphia in 1985. The use of the technique to characterize chocolate oils and fats is described here.

Fats and oils generally allowed in chocolate are cocoa butter, butter oil (milk chocolate) and oils originating from addition of nuts. In some countries (U.K., Denmark, Ireland, Japan), a limited addition of other fats is allowed. Foreign fats can also be found in the chocolate of coated products due to the migration of center fats. For economic reasons, there always has been an interest in using foreign fats as partial replacers for cocoa butter. To minimize the deterioration of the particular physical behavior of cocoa butter, fats with a chemical composition as close as possible to cocoa butter were developed. These are called cocoa butter equivalents (CBE). Based on the composition of existing commercial CBE's, CAOBISCO suggested an array of criteria that CBE's should meet. Based on these criteria, outlines for legal regulations are being drawn in view of the possible extension of the U.K.'s 5% vegetable fat allowance to the whole European Economic Community.

For many years, there have been discussions on analytical methods for the detection of CBE's in chocolate products. The methods proposed are based on carbon number separations of fat triglycerides by gas chromatography (GC) (14-20). These methods fail because of the inability to handle chocolate products containing fats of the UUU-type (such as nut oils) and the uncertainty about the identity of the added CBE. A method based on separation of brominated triglycerides with reversed phase high performance liquid chromatography (RP-HPLC) was developed (21). Using the specific ratios of monounsaturated triglycerides, CBE can be quantified, even in the presence of large amounts of butter oil and nut oils. The method is considered to be fast, simple and reliable (22). The present contribution also deals with this subject, and capillary GC on polarizable phases seems to be the solution for even more complex mixtures. By pattern recognition, the different fat and oil constituents of chocolate products can indeed be elucidated.

TABLE 1

Reproducibility for Cocoa Butter Triglycerides

Carbon number	Triglyceride	Mean %	Standard deviation	Coefficient of variation
50	PPS	0.60	0.05	8.9
	POP	17.79	0.17	1.0
	PLP	1.76	0.06	3.7
52	PSS	0.74	0.05	6.6
	POS	40.46	0.15	0.4
	POO	2.60	0.14	5.2
	PLS	3.14	0.08	2.5
	PLO	0.27	0.04	14.7
54	SSS	0.39	0.06	14.4
	SOS	25.77	0.23	0.9
	SOO	3.21	0.07	2.1
	OOO			
	+SLS	1.88	0.06	3.3
	SLO	0.31	0.06	19.2
56	SOA	1.06	0.08	7.4

Mean value, standard deviation and coefficient of variation for five injections.

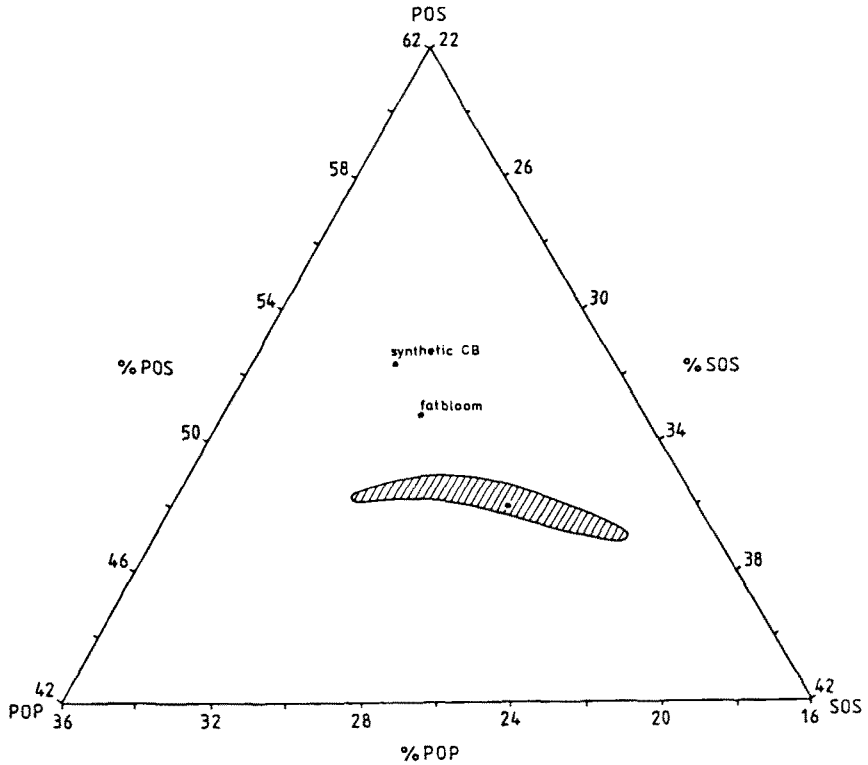

FIG. 2. Ternary diagram (enlarged) of the normalized POP, POS and SOS ratio of 100 cocoa butters from different origins.

EXPERIMENTAL

The preparation of the phenylmethylsilicone gum with a thermal stability of 370 C has been described (23). Analyses of fats and oils were carried out on 25 m × 0.25 mm FSOT columns coated with 0.12 μm phenylmethylsilicone. The columns were installed in a Hewlett Packard 5880A gas chromatograph equipped with a FID detector and a homemade movable cold on-column injector (9). Ca. 0.2 μl of 0.05% hexane solutions of the fats or oils were injected. The carrier gas was hydrogen with inlet pressure around 1 bar. Peak integration and calculation were performed on a Hewlett Packard 3354 Lab Automation System. Raw data (area slices) of all analyses were stored for subsequent custom integration.

RESULTS AND DISCUSSION

Cocoa butter. Cocoa butter, the fat of the cocoa bean, is one of the basic ingredients in chocolate products. Cocoa butter consists mainly of the triglycerides POP, POS and SOS. Some saturated triglycerides are also present together with polyunsaturated triglycerides whose amounts vary with the cocoa butter origin and growing conditions. The different triglycerides of cocoa butter cannot be separated by capillary GC on apolar capillary columns. An elegant way to realize advanced separations is RP-HPLC (21,24). The patterns obtained, however, are sometimes confusing. Moreover, the analysis times in RP-HPLC are long and the sensitivity of the method is low due to the use of RI detection.

The possibilities of capillary GC on the phenylmethylsilicone phase are illustrated in Figures 1A and 1B, showing the analyses of cocoa butters with low and high contents of polyunsaturated triglycerides in only 16 min.

The triglycerides are separated according to the chain length (CN separation) and each CN number is split up in function of the degree of unsaturation in the triglyceride molecules. As the polarity increases with the degree of unsaturation in the fatty acid (Ln ⋙ L ⋗ O > S) and thus also with the total number of double bonds in the triglycerides (OOO > SOO > SOS > SSS), the retention is highest for the triglycerides containing most of the highest unsaturated fatty acids. Positional isomers, e.g., POS and PSO, cannot be resolved. In Figure 1 only OOO and SLS are not separated. The structure elucidation of the different triglycerides has been discussed elsewhere (13).

Concerning the quantitative aspect of the triglyceride determinations, a cocoa butter was analyzed five times and the mean percentages, the standard deviations and the coefficients of variation were calculated (Table 1). The coefficients of variation for the main peaks are below 1%, illustrating the value of the technique.

Fifty-five cocoa butters from beans of different origins and 85 cocoa butters from 14 different suppliers were analyzed. The amounts of polyunsaturated triglycerides varied from 6.6% to 24%. Values for saturated triglycerides were normally between 1 and 2%; exceptionally, a content as high as 5% was found. The triglyceride composition clearly is a function of cocoa origin, hybrid species and growing conditions. The amount of polyunsaturated triglycerides of cocoa butters determined in this way correlates very well with solid fat content values obtained by pulse-NMR (E. Geeraert and D. De Schepper, unpublished results).

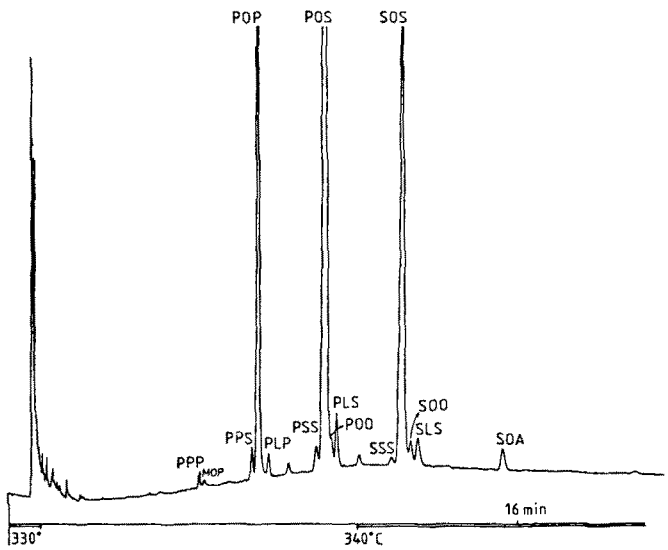

FIG. 3. Capillary GC profile of fat bloom from a dark chocolate.

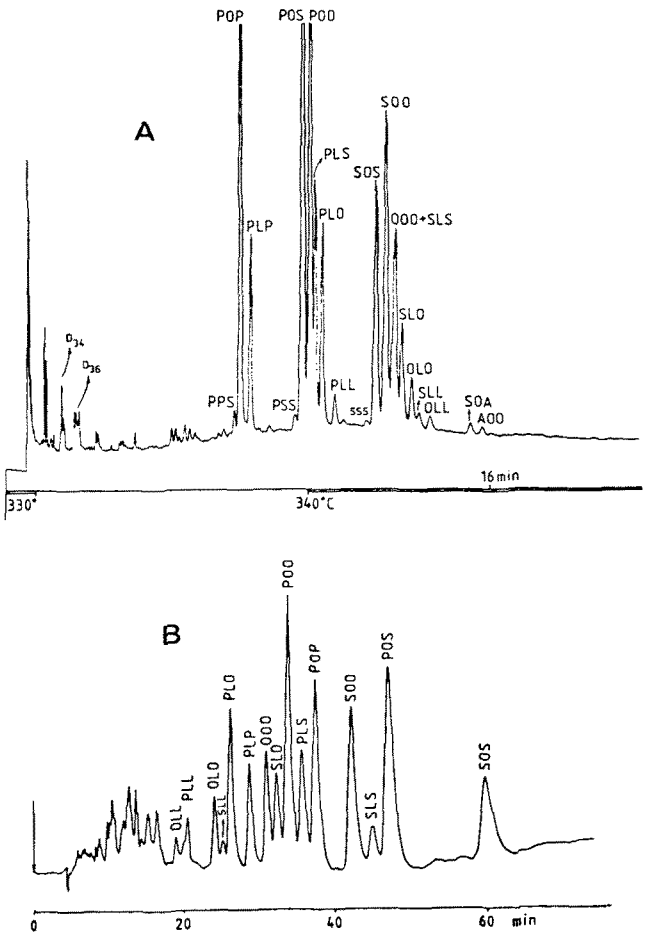

FIG. 4. Analyses of mowrah (*Madhuca latifolia*) oil. A, capillary GC profile; B, RP-HPLC profile.

Figure 1 also reveals differences in diglyceride content (D_{34}, D_{36}).

Although the total amount of the monounsaturated triglyceride POP, POS and SOS may differ in different cocoa butters, the relative proportions of POP, POS and SOS prove quite constant for all cocoa butters. The amount of POP, POS and SOS of 100 cocoa butters from different origins was normalized to 100%, and the calculated values were put into a ternary diagram. The relevant part of the ternary diagram is shown in Figure 2.

All cocoa butters fall within a small area (shaded area). An experimental synthetic cocoa butter equivalent shows a POP:POS:SOS ratio very close to that of natural cocoa butters. Differentiation from natural sources with the help of the ternary diagram is easy. The two other dots in the ternary diagram correspond with the bulk fat (dot in the shaded area) and the fat bloom of a dark chocolate. The triglyceride profile of the fat bloom is represented in Figure 3.

Compared to the bulk fat (not shown), the amount of polyunsaturated triglycerides (POO, PLO, SOO, OOO, SLO) is strongly reduced. Fat bloom occurs as a very thin layer ($\sim$ 5μm) on the chocolate surface. Sampling was performed by gently rubbing a few square centimeters with a paper tissue. The paper tissue was put in hexane and the hexane solution was directly injected into the capillary column.

*CBEs.*CBEs are obtained by fractionation of palm oil or exotic wild crop fats like shea (*Butyrospermum parkii*), sal (*Shorea robusta*), mango (*Mangifera indica*), illipé (*Madhuca longifolia*) or mowrah (*Madhuca latifolia*), or by using exotic fats such as tenkawang (*Shorea stenoptera*). A typical example is mowrah oil (Fig. 4). Besides POP, POS and SOS, the main compounds of natural cocoa butter, large amounts of polyunsaturated triglycerides (PLP, POO, SOO, etc.) are present.

The superior separation obtained by capillary GC on a polarizable phase compared to RP-HPLC is illustrated by Figures 4A and 4B showing, respectively, the capillary GC and RP-HPLC analyses of the same mowrah oil on an RSil C18 HL 5 μ column (250 $\times$ 4.6 mm I.D.) from Alltech Europe. The best resolution is obtained at 14 C with propionitrile as mobile phase (21). The disadvantages of RP-HPLC are mainly the long analysis times, the low sensitivity of RI detection and the disappearance of the trisaturated triglycerides which are nearly insoluble in propionitrile, the high selectivity mobile phase we need.

To be useful as a CBE, mowrah oil has to be fractionated. Figure 5A shows the chromatogram of a commercial CBE probably obtained by fractionation of mowrah oil. Tenkawang, on the other hand, does not need fractionation to be useful as a CBE, as can be deduced from Figure 5B. POP, POS and SOS are the main triglycerides. Commercial CBEs are also prepared by blending fractions originating from different oils or fats. A typical example is shown in Figure 5C. A palm midfraction, rich in POP, was blended with a shea stearin, rich in SOS.

By calculating the POP:POS:SOS ratios, we can deduce the content of each ingredient. From the ratio of mono- to polyunsaturated triglycerides, an estimation

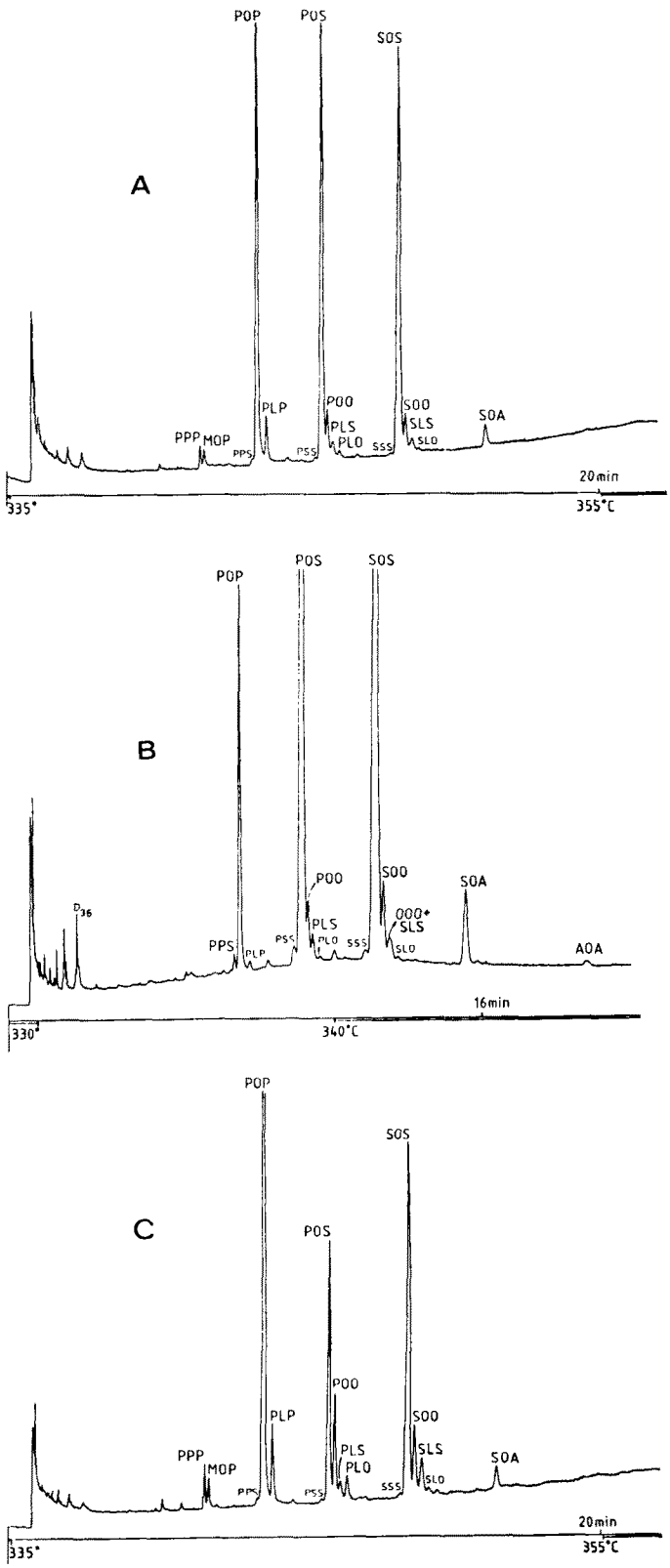

FIG. 5. Analyses of cocoa butter equivalents. A, commercial cocoa butter equivalent derived from mowrah oil; B, tenkawang (Borneo tallow, *Shorea stenoptera*); C, commercial cocoa butter equivalent obtained by blending a palm midfraction and shea stearin.

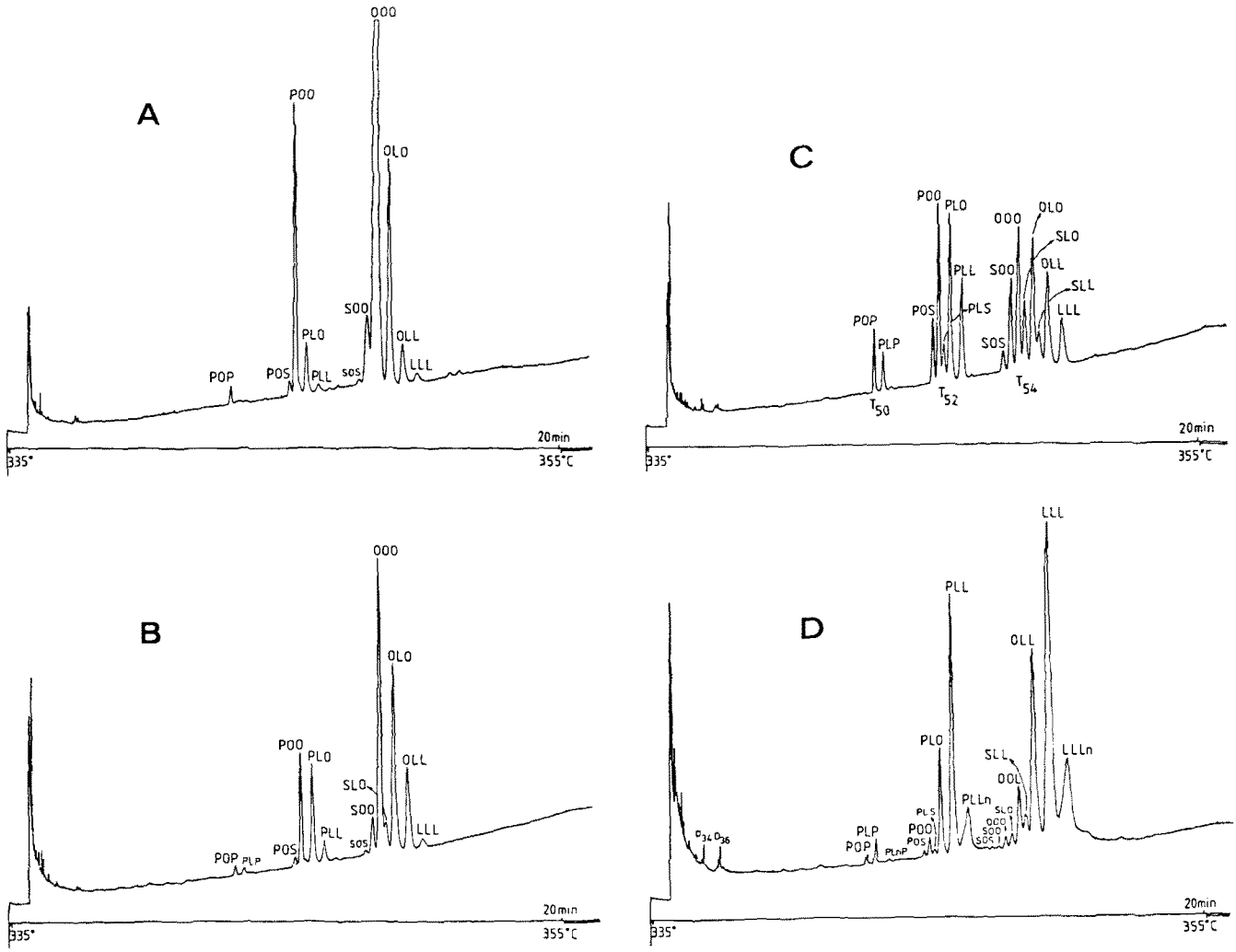

FIG. 6. Triglyceride profiles of nut oils. A, hazelnut oil; B, almond oil; C, Brazil nut oil; D, walnut oil.

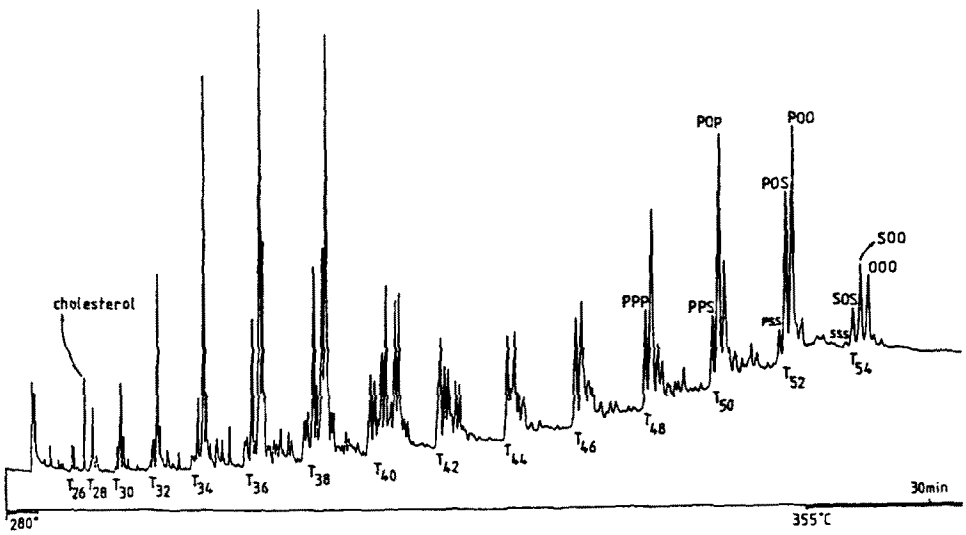

FIG. 7. Analysis of butter oil from milk collected in June.

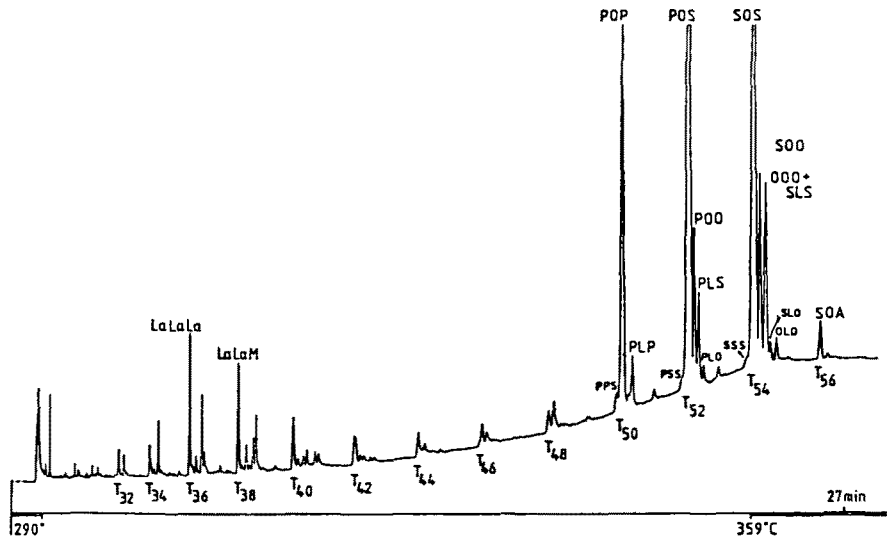

FIG. 8. Analysis of a composite fat mixture.

of the "sharpness" of the fractionation can be made. None of the CBEs developed to date possesses a POP:POS:SOS ratio equal to the natural cocoa butters. Additions of 5% CBE easily can be detected by placing the relative POP:POS:SOS percentages in the ternary diagram (Fig. 2).

Nut oils. The triglyceride profiles of hazelnut oil, almond oil, Brazil nut oil and walnut oil are represented in Figures 6A, 6B, 6C and 6D. The knowledge of these profiles and their natural variations can be helpful in identifying nut oils in a chocolate product through pattern recognition.

Butter oil. The triglyceride profile of butter oil is extremely complex because butter oil contains long as well as short chain fatty acids. Over 200 peaks are detected in the triglyceride profile (Fig. 7) and the structure elucidation is still going on. The butter oil profile is so typical that it can be recognized easily in chocolate products.

Composite mixture. The possibilities of capillary GC on FSOT columns coated with phenylmethylsilicone gum can best be illustrated through the analysis of a complex fat mixture. A mixture containing 57% cocoa butter, 18% butter oil, 15% CBE (shea stearin), 5% palm kernel stearin and 5% hazelnut oil was made and analyzed (Fig. 8).

All components can be detected from the chromatogram. The lauric fat (hydrogenated palm kernel stearin) is detected through the triglycerides LaLaLa and LaLaM. Both triglycerides are well separated from the typical T_{36} and T_{38} butter oil triglycerides. The high OOO peak reveals the presence of a nut oil.

From the corrected, normalized POP:POS:SOS ratios, the addition of a shea stearin CBE (high SOS content) is easily detected and quantified.

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