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The Determination of Cocoa Butter Equivalents in Chocolate

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ABSTRACT

A method of determining cocoa butter equivalents in chocolate and cocoa butter is described. The method relies on a new approach for interpreting data obtained by triglyceride gas liquid chromatography (GLC). This technique provides information on the composition of a fat according to the carbon number of the triglycerides (C_n). Examination of the data for a wide range of cocoa butters shows that a straight line relationship between the C_{50} and C_{54} contents exists. This relationship has been used as the basis for a quantitative method determining the amount and type of cocoa butter equivalent added to chocolate. The application of the method to both plain and milk chocolate is described. The method is also used to determine the amount of milk fat in chocolate.

INTRODUCTION

Throughout the world there is an increasing interest in the use of fats to partially replace cocoa butter in chocolate. The reasons, partly economic, are that certain fats can also provide significant product improvement (1). In the United Kingdom, Denmark and Ireland the addition of 5% non-cocoa butter fats, apart from milk fat, has been permitted for many years.

The type of fats that have been used in chocolate are very similar in chemical composition to cocoa butter. These fats mainly contain symmetrical triglycerides and for this reason it has been impossible to determine the amount of symmetrical-based fats in mixtures with cocoa butter.

Although there has been no method of monitoring the level to which added fats are used, the manufacturers have mutually agreed to abide by this 5% restriction. However it is desirable that some analytical method be made available which would quantitatively determine the amount of added fat in chocolate. The objective of the work described in this paper was to develop a method which would detect and quantify symmetrical triglyceride cocoa butter equivalents (CBE) when used at the 5% level in chocolate. Ideally, the method should also be quantitative at even lower levels of addition. The method should be suitable for both milk and plain chocolate, as well as rapid and easy enough for routine use.

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The method is required to detect the high quality symmetrical-type CBE which have physical and chemical properties similar to those of cocoa butter. These CBE include Coberine, Illexao, illipe, shea fractions and palm fractions. Ideally, the method should also detect hardened fats but other methods are presently available for their characterization.

The determination of added fats has been the subject of much research and the position was reviewed in 1959 (2). At that time, modern chromatographic methods were being applied to oils and fats for the first time and this earlier work is of little interest today. Chromatographic methods for the general characterization of mixtures of oils and fats have been reviewed by Mani (3) and more recently for confectionery fats by Fincke (4).

Early attempts to detect added fats depended on the determination of the fatty acid composition of cocoa butter and, in particular, the content of lauric acid (5). Bonar (6) was able to detect 5% Coberine in cocoa butter using a preliminary low temperature crystallization as developed by Purr and Hettich (7,8). Methods dependent on lauric acid are unsuitable when milk fat is present. Iverson and Harrill (9,10) isolated the minor acids in cocoa butter, shea and illipe by urea fractionation. Although there was a suggestion that the method could be used to detect oils in cocoa butter, most operators would find the method difficult. The use of fatty acid analysis as a means of detecting added fats is therefore severely limited because some of them have a fatty acid composition similar to cocoa butter. Large additions of, e.g., palm fraction must be added before this method will detect it with certainty.

Sterol analysis is often used for identifying specific oils and fats. Fincke (11) has reported sterol analyses for several samples of illipe, Calvetta, Coberine and cocoa butter. Bracco et al. (12) have used sterol analyses to help characterize illipe/cocoa butter mixtures. Sterol analyses do have value in specific instances, e.g., detection of shea nut oil and its fractions or rapeseed oil. They are limited for detecting replacer fats because of their inability to detect palm fractions, major components of many products.

Sterol analysis is further limited because a preliminary step to isolate sterols is necessary and this is tedious. Quantitation is also virtually impossible because of the wide

variation in sterol content caused either by natural factors or processing.

The analysis of triglycerides using silver nitrate thin layer chromatography ($AgNO_3$-TLC) should allow cocoa butter replacers to be detected. In most cases a complete triglyceride analysis would be necessary and this requires too much skilled effort and time for routine use. The method could be of great value in isolating minor glyceride components unique to certain oils, e.g., the SSU (S = saturated; U = unsaturated) fraction of lard which could then be further characterized by triglyceride gas liquid chromatography (GLC).

The addition of marker compounds also has been suggested. These include rapeseed oil, BHT, sesamol, stigmasterol and, more recently, trinonanoin. A related triglyceride triheptanoin is already used to mark Intervention Butter in the EEC (13). Marker compounds have a number of disadvantages, plus, some manufacturers could leave them out of their products.

Confectionery fats also have been characterized by high temperature GLC. The general technique has been widely documented but the reader is directed particularly to the publications by Kuksis and Breckenbridge (14) and Litchfield (15). In addition, a number of workers have used the technique to characterized confectionery fats. Luddy et al. (15) characterized fractions of tallow and this helped optimize their process. The method has also been used to characterize mixtures of fats. For example, Warmbier (17) determined the amount of lard in goose drippings using a combination of $AgNO_3$-TLC and GLC. Rapeseed, soybean and rice bran oils were detectable in cottonseed oil at the 5-10% level (18). von Eckert (19,20) has also described various applications of triglyceride GLC.

Bracco et al. (12) used triglyceride GLC to detect illipe in cocoa butter. However, in all of these applications the quantitative estimation of added fats was inaccurate because of the natural variation in the composition of the fats.

In recent work (21), we found that triglyceride GLC could be usefully applied to confectionery fats. We described the reproducibility of the technique and typical correction factors for different triglycerides when operating under conditions similar to those described by Kuksis and Breckenbridge. In a separate brief communication (22), we have described a method of determining CBE in chocolate using triglyceride GLC data. We are aware that Fincke has independently developed a similar method which will be published shortly (personal communication).

In this paper we detail the method we have developed which overcomes inaccuracies from the natural variability of cocoa butter and allows the operator to determine quantitatively the amount of CBE added to chocolate or cocoa butter.

EXPERIMENTAL

In our early work we used a dual column technique, but if the column is properly conditioned a single column is satisfactory and gives a good baseline.

Instruments

A Pye 104 Model 64 Chromatograph equipped with a linear temperature programmer was used. Electronic integration was by a Chromalog 2 integrator (Kent Instruments).

Column

A glass, 2 foot x one-fourth inch od column was used which was fitted with graded glass-to-metal seals (Jencons, England).

Packing

Packing was 3% OV1 on 100-120 Gas-Chrom Q (Phase Separations).

Conditioning

Conditioning was basically as described by Kuksis and Breckenbridge (13). Our columns require at least 6 hr at 350 C.

Operating Conditions

A 7-cm injection needle was used so that its tip just reached into the column packing at the hottest part of the injection heater. Flow rate: 80 ml/min nitrogen; injection heater: 310-360 C, depending on the oven temperature (250-300 C), 360 C preferred injector temperature; detector heater: 380 C; programming rate: normally 4 C/min; sample: 1-2 μl of a 10% solution in chloroform.

Under these conditions, a confectionery fat containing triglyceride carbon numbers 46-56 can be analyzed in 20 min (300-355 C at 4 C/min plus 5 min hold at 355 C). Allowing ca. 10 min for the oven to cool back to 300 C, we found that 2 samples can be analyzed per hr.

The confectionery fats were not pretreated (i.e., no partial glycerides or free fatty acids, e.g., were removed) before analysis.

To obtain the highest precision and reproducibility we standardized our results using a standard cocoa butter which had been standardized by reference to a mixture of pure triglycerides. If analyses within a laboratory are self-consistent by reference to a standard cocoa butter, it is not necessary that the cocoa butter be absolutely standardized by reference to pure triglycerides. For milk chocolate analyses, a standard milk fat/cocoa butter mixture is used to insure high reproducibility. Products were sampled as described in the following sections.

Sample As Chocolate

When the sample is in the form of a chocolate bar or couverture the sample for analysis is prepared as follows: weigh out ca. 150 mg of the chocolate into a small phial and add 1 ml of analytical grade chloroform. Heat to 60 C, shake and keep at 60 C for ca. 5 min. Cool and centrifuge the solids to leave a clear or almost clear solution. Inject 2 μl of the supernatant directly into the GLC column.

We normally do not sample from the outer edges of a chocolate bar which might be contaminated on the surface or nonuniform, although our experience is that the fat phase of a chocolate bar is uniform. Duplicate samples are usually taken from different parts of the bar.

Some chocolate products such as vermicelli contain less fat than couvertures or bars. The vol of solution injected into the GLC column should be increased accordingly so that ca. 100 μg of fat is analyzed.

Sample As Fat

When the sample is available as fat, ca. 50 mg should be dissolved in 1 ml of analytical grade chloroform and 2 μl of the solution injected into the GLC column.

If a sample of cocoa butter is being analyzed, it may be in the form of a large block. Kleinert has reported nonuniformity in commercial cocoa butter blocks using the Pichard cooling curve test on samples from various parts of the block. To check this important point, we took samples from a 12-kg commercial block of cocoa butter and analyzed them in the usual way. The results in Table I show that there was no significant variation over the block.

TABLE I

Analyses of Samples from Various Parts of a Commercial Block of Cocoa Butter

Sample location	Carbon number (wt %)				
	48	50	52	54	56
Middle	0.2	16.9	47.1	34.7	1.1
Corner 1	0.2	16.8	47.3	34.6	1.1
Corner 2	0.3	16.3	47.2	35.1	1.1
Corner 3	0.2	16.7	47.2	34.8	1.1
Corner 4	0.1	16.9	47.2	34.7	1.1

NATURAL VARIABILITY OF COCOA BUTTER

Although much work has been reported on the variability of the chemical composition of cocoa butter, the majority of reported analyses refer to fatty acid methyl ester (FAME) analysis. There is little information available on the variability of the triglyceride composition.

The most comprehensive study of the variability of cocoa butter (by FAME analysis) has been reported by Woidich et al. (23,24). They analyzed 90 samples of cocoa butter which they extracted from beans of well defined origin. Large variations in the palmitic, stearic and oleic acid contents were observed for cocoa butters from different regions and from different harvests. These results were used as a starting point for our investigation of the variability of cocoa butter because we could not easily and quickly obtain such a wide variety of authentic cocoa butters.

To calculate triglyceride compositions from the FAME data we used the 1,3-random/2-random hypothesis of Van der Wal and Colemen and Fulton, which has been amply confirmed for cocoa butter. The FAME analyses at the 2-position were estimated by a method similar to that described by Litchfield (15). The method was applied to cocoa butters of known triglyceride analysis and shown to give accurate results.

Using this method, triglyceride compositions by carbon number and hence P_{50}, P_{52} and P_{54} values could be determined for a wide range of cocoa butters:

$$P_{50} = \frac{\%\text{ of carbon no. } 50}{\%\text{ of carbon nos. } 50 + 52 + 54} \times 100\%$$

i.e., the data for the 3 major peaks, 50, 52 and 54, is normalized so that $P_{50} + P_{52} + P_{54} = 100$. Examination of the data showed that P_{52} was practically constant for wide variations of P_{50} and P_{54}, and there was a linear relationship between P_{50} and P_{54}.

Following this encouraging result, we determined directly the triglyceride analyses by carbon number of a range of commercial cocoa butters. The Woidich et al. data is in many ways too comprehensive for our purpose as commercial samples of cocoa butter are not observed with such a wide variation in FAME analysis. This presumably is because the beans are blended together to give more uniform and consistent properties. We obtained 39 samples of cocoa butter or beans from chocolate manufacturers and cocoa butter producers in Great Britain and the Netherlands. The samples were chosen to be as near as possible to a random sample of the cocoa butters or beans which were available commercially in the years 1971-1974. The number of samples from each producing country was in approximate proportion to that country's production.

The 39 analyses were fitted to a straight line by the conventional least squares method giving:

$$P_{50} = 43.798 - 0.737^{1}\ P_{54}, \qquad [I]$$

with a residual standard deviation of 0.1275.

The fit of the data to the line is shown in Figure 1. The equation of this line is similar to the line previously determined from our calculations using the Woidich et al. results. Workers in other laboratories could determine a slightly different line depending on how close their results are to absolute triglyceride percentages. Providing all the data in a given laboratory is referred to the same standard cocoa butter as already explained, this is unimportant since the results will be internally self-consistent. The important point is that the variability of the triglyceride composition of cocoa butter can be expressed by an equation of the form:

$$P_{50} = a - b\ P_{54},$$

where a and b are constants. We conclude that all analyses of pure cocoa butter from whatever source must lie on this line. We can also describe the distribution of the cocoa butter analyses by the means and standard deviations of P_{50} and P_{54}:

$$\text{mean } P_{54} = 34.752 \qquad \sigma_{54} = 1.022$$
$$\text{mean } P_{50} = 18.183 \qquad \sigma_{50} = 0.764$$

These parameters can then be used to describe the distribution of the analyses along the line if we assume the natural variability of cocoa butter analyses to be normally distributed. Figure 2 illustrates the distribution of the P_{54} values with a histogram. The normal distribution

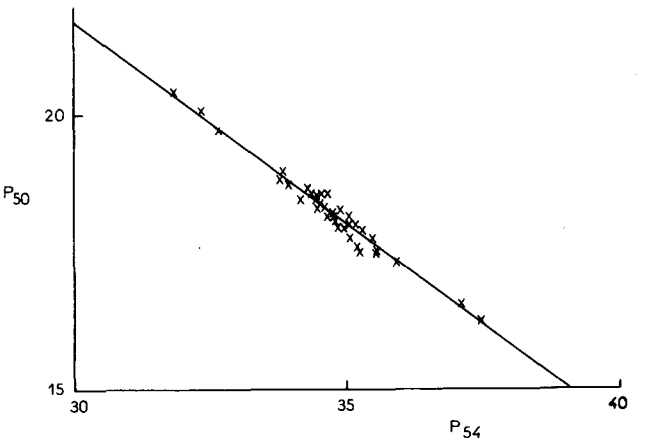

FIG. 1. The natural variability of cocoa butter: linear relationship between P_{50} and P_{54}.

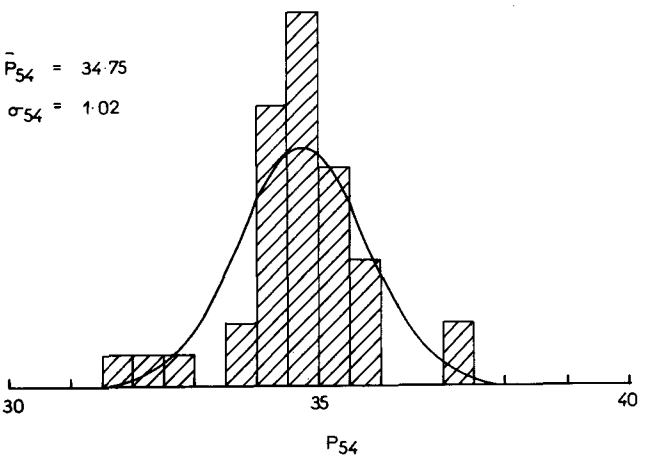

FIG. 2. The natural variability of cocoa butter: comparison of histogram distribution with normal distribution.

curve deduced from the mean and standard deviation just given is superimposed for comparison. In view of the limited amount of data, there is a reasonable approximation to a normal distribution. All the data lies within the limits which would contain 99.8% of the normal distribution. Eighty-eight percent of the data lies within the limits which would contain 95% of the normal distribution. The extreme analyses do seem to be associated with the smaller producing regions. For example, the highest P_{54} value comes from Sabah (Malaysia) and the lowest from Grenada (West Indies). Subsequent analyses of more than 40 cocoa butters extracted from commercial chocolates have all been within the 99.8% limits with mean and standard deviation similar to those already given.

INTERPRETATION OF GLC DATA

All CBE added to cocoa butter will cause the triglyceride analysis to deviate from the line described by Equation I to the extent that the P_{52} value of the CBE is different from the P_{52} value of cocoa butter. All possible commercial CBE made solely from vegetable fats contain fewer carbon-number-52 triglycerides than are found in cocoa butter. Hence all CBE will cause deviations from Equation I and will be detected (Fig. 3). Unlike FAME analyses, where the deviation resulting from a fat rich in palmitic acid can be cancelled out by adding a fat rich in stearic acid, deviations from Equation I cannot be cancelled out by adding a second CBE of different triglyceride analysis. Only lard and tallow and their fractions have high contents of carbon-number-52 triglycerides such that in mixtures with vegetable-based CBE they might escape detection by our method. Animal fats usually can be detected by sterol analyses or by $AgNO_3$-TLC. Typical analyses for several CBE are given in Table II.

It is possible to quantify the deviation from the line and thus determine the percentage and type of CBE added. Consider a CBE, A, with analysis $P_{50}{}^{A}$, $P_{52}{}^{A}$, $P_{54}{}^{A}$. A mixture of A and cocoa butter is made to contain a fraction x (by weight) of A. By mass balance (neglecting triglycerides with carbon numbers other than 50, 52 and 54 as negligible, which is discussed in more detail in Discussion, we have:

$$P_{50}{}^{CB+A} = P_{50}{}^{CB}(1-x) + P_{50}{}^{A} \cdot x$$
$$P_{54}{}^{CB+A} = P_{54}{}^{CB}(1-x) + P_{54}{}^{A} \cdot x \qquad [II]$$

where $P_{50}{}^{CB+A}$, $P_{54}{}^{CB+A}$ and $P_{50}{}^{CB}$, $P_{54}{}^{CB}$ are the analyses of the mixture and the cocoa butter, respectively.

Solving Equations II and III with Equation I we have:

$$P_{50}{}^{CB+A} = 43.798(1-x) + x(P_{50}{}^{A} + 0.737^{1}\ P_{54}{}^{A}) - 0.737^{1}\ P_{54}{}^{CB+A} \qquad [IV]$$

Equation IV is the equation of a family of straight lines parallel to the line described by Equation I.

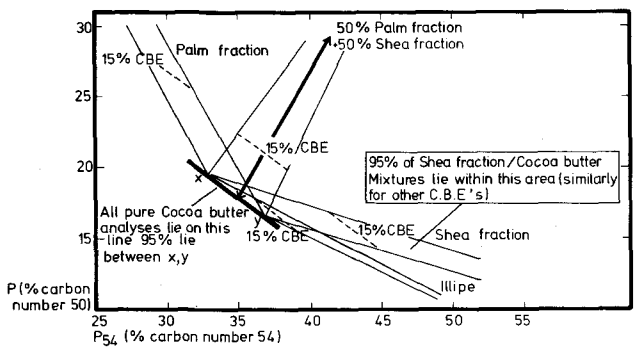

FIG. 3. Effect of adding CBE to cocoa butter.

TABLE II

The Composition of Different CBE

CBE	No. of samples	Average P_{50}	Average P_{54}
Calvetta	17	75.52	3.04
Shea fraction	14	0.76	90.51
Coberine	21	38.22	34.37
Choclin	21	49.65	28.72
Illipe	22	7.24	54.84
Shea oil	8	0.81	89.27
Sal oil	5	1.45	81.80
Mowrah oil	1	19.10	34.95
Kokum oil	1	0.57	93.75
Illexao 30-61	1	38.0	38.0
Illexao 30-96	2	21.30	64.77

Compositions must be determined periodically because of variation in composition.

To determine the locus of a given point $P_{50}{}^{CB+A}$, $P_{54}{}^{CB+A}$ as A is added, i.e., the line connecting $P_{50}{}^{CB}$, $P_{54}{}^{CB}$ and $P_{50}{}^{A}$, $P_{54}{}^{A}$ we eliminate x between Equations II and III to give:

$$P_{50}{}^{CB+A} = \frac{P_{54}{}^{CB+A} \cdot (P_{50}{}^{A} - P_{50}{}^{CB})}{(P_{54}{}^{A} - P_{54}{}^{CB})} + \frac{P_{50}{}^{CB} \cdot P_{54}{}^{A} - P_{54}{}^{CB} \cdot P_{50}{}^{A}}{P_{54}{}^{A} - P_{54}{}^{CB}} \qquad [V]$$

Using Equations IV and V and given any CBE whose analysis is known, a family of lines can be drawn rapidly showing the effect of adding various percentages of CBE to any sample of pure cocoa butter. In practice, CBE are variable in analysis because of blending, processing or natural variability. It is possible to express this variability statistically, leading to a modification to Equation V.

Quantitative Estimation with the Aid of Diagrams

To illustrate the use of Equations IV and V, Figure 4 shows the data calculated for the 3 important CBE or CBE components; palm fraction, shea fraction, illipe (Borneo Tal-

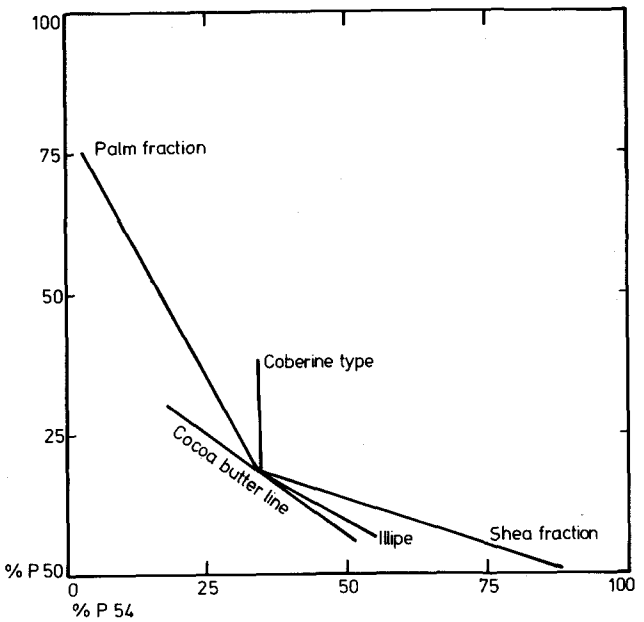

FIG. 4. Detection of CBE by triglyceride GLC method.

low), together with the data for a typical CBE consisting of a mixture of palm fraction and shea fraction. The lines show the limits within which 95% of all CB/CBE mixtures lie assuming the variability of the CBE is as we have determined by sampling from Unilever factories. Similar diagrams can be drawn for any other CBE of interest. To use the diagrams, the analysis is plotted on transparent graph paper and placed over each diagram in turn.

If the analysis lies on the cocoa butter line (Equation I), it is deemed to be pure cocoa butter. If it deviates from the line (for vegetable fats it can do so in an upward direction only) then each CB/CBE diagram is checked to see within which CB/CBE field it lies. Finally, the CBE percentage is read; for clarity only the 15% lines are shown in Figure 4. Sometimes, especially for low additions of CBE, it will not be possible to distinguish unequivocally between similar CBE. In such cases, alternative results must be quoted. Additional evidence will then be required if a firmer conclusion is necessary.

To provide more information than is easily obtained using the graphical method of interpretation just described, we have developed a rigorous mathematical interpretation of the data using standard statistical methods. Using a computer, the data may be rapidly processed and results given including confidence limits. All the results given in the following section have been obtained this way. The method of calculating 95% confidence limits and of deciding whether an analysis lies on the cocoa butter line is given in the following section.

Deciding if a Sample Is Pure Cocoa Butter

Pure cocoa butter is assumed to be defined by Equation I. If the data is normally distributed, then 99% of the analyses are beneath the average value plus 2.326 x residual standard deviation. Therefore, pure cocoa butter should comply with:

$$P_{50} < 43.798 - 0.737^1\, P_{54} + 2.326 \times 0.1275,$$
$$\text{i.e., } P_{50} < 44.095 - 0.737^1\, P_{54},$$

for 99% of all analyses. Greater values of P_{50} are taken to mean that the sample is not pure cocoa butter.

Calculation of % CBE in a CB/CBE Mixture

If we solve Equation VI for x we get:

$$x = \frac{c}{d} = \frac{P_{50}{}^{CB+A} - 43.798 + 0.737^1\, P_{54}{}^{CB+A}}{P_{50}{}^{A} - 43.798 + 0.737^1\, P_{54}{}^{A}}, \qquad [VI]$$

where x is the fraction of CB/CBE mixture.

In the derivation of Equation IV it was assumed that components other than triglycerides with carbon numbers 50, 52 and 54 were negligible and could be neglected. This is a reasonable assumption for cocoa butter and many CBE but for certain oils, other glycerides or unsaponifiable matter must be considered. One such oil is sal oil (*Shorea robusta* seed oil), which contains 15-20% of triglycerides with carbon number 56.

To calculate x more rigorously we define f^{CB}, f^{A} or f^{CB+A} as the fraction of the CB, CBE or mixture which consists of triglycerides with carbon numbers 50, 52 and 54. Then, instead of our earlier Equations II and III we have:

$$P_{50}{}^{CB+A}.f^{CB+A} = P_{50}{}^{CB}.f^{CB}(1-x) + P_{50}{}^{A}.f^{A}.x \qquad [VII]$$
$$P_{54}{}^{CB+A}.f^{CB+A} = P_{54}{}^{CB}.f^{CB}(1-x) + P_{54}{}^{A}.f^{A}.x \qquad [VIII]$$

Summing Equations VII and VIII and the equivalent equation for P_{52} we deduce:

$$f^{CB+A} = (1-x)f^{CB} + xf^{A}, \qquad [IX]$$

since $P_{50} + P_{52} + P_{54} = 100$ by definition.

Combining Equations VII, IX and I, we deduce that:

$$x = \frac{x'}{f^{A}/f^{CB} + (1-f^{A})x'}, \qquad [X]$$

where x′ is the value of x deduced from the approximate Equation VI and x is the exact value. To estimate f, one has to take into account triglycerides other than 50, 52 and 54, partial glycerides and unsaponifiable matter. Our experience is that the correction implied by Equation X is negligible for most CBE except for sal oil (mentioned previously) or unfractionated shea oil which is rich in unsaponifiables and partial glycerides.

Accuracy of Estimated % CBE

If we assume that all variations are normally distributed and that the unknown sample of chocolate has been mixed with a randomly chosen batch of CBE, then we can calculate confidence limits for % CBE using standard statistical methods.

Although to be exact one should apply Equation X after Equation VI, x and x′ are so similar that for the estimation of the confidence limits we can just use Equation VI.

From Equation VI, since we do not know the values of P_{50} and P_{54} for the particular CBE which has been used, the best estimate of x which we can make is: $x = \frac{c}{\mu_d}$, where $\mu_d = \bar{P}_{50}^{A} - 43.798 + 0.737^1\, \bar{P}_{54}^{A}$ and $\bar{P}_{50}^{A}$ and $\bar{P}_{54}^{A}$ are the mean values obtained from the analysis of a random selection of samples of the CBE.

To obtain confidence limits for x we need a function of x whose mean and standard deviation can be calculated. A suitable function is: $\theta = d.x - c$. If x is the true proportion of CBE, it follows from Equation VI that $\mu_\theta = 0$. For any determination, θ is estimated by $(\mu_d x - c)$ and will differ from 0 because d for the particular CBE will not equal μ_d and because c varies between determinations with a standard deviation which we can assume to be the variation about Equation I for cocoa butter. Hence:

$$\sigma_\theta{}^2 = \sigma_d{}^2.x^2 + \sigma_c{}^2,$$

where $\sigma_c = 0.1275$, $\sigma_d{}^2 = (\sigma_{50}{}^A)^2 + (\sigma.7371\ \sigma_{54}{}^a)^2 + 1.4742\ \sigma^A_{5054}$ and $\sigma_c{}^2$, $(\sigma_{50}{}^A)^2$, e.g., are the variances of c, percentage of carbon number 50 in A, e.g., respectively and σ^A_{5054} is the covariance of the percentages of carbon numbers 50 and 54 in A. $|\mu_d.x - c| < 1.96\ \sigma_\theta$ in 95% of determinations. It follows that confidence limits (probability = 0.95) are given by solutions of:

$$(\mu_d.x - c)^2 = 3.8416\ (\sigma_d{}^2.x^2 + \sigma_c{}^2)$$

∴ 95% confidence limits =

$$\frac{\mu_d.c \pm \sqrt{\mu_d{}^2.c^2 - (\mu_d{}^2 - 3.8416\ \sigma_d{}^2)(c^2 - 3.8416\ \sigma_c{}^2)}}{\mu_d{}^2 - 3.8416\ \sigma_d{}^2}$$

Milk Chocolate

When milk chocolate is analyzed it is necessary to correct the observed triglyceride analysis for the presence of milk fat triglyceride in the carbon number 50, 52 and 54 region. This correction is small and we use the sum of the analyses of the carbon-number-40-, 42- and 44-triglycerides both to estimate the amount of milk fat and to determine the necessary correction to the carbon-number-50, 52- and 54-triglycerides. The sum of triglycerides 40, 42 and 44 is used because it shows smaller variability with different milk fats than the single triglyceride 40 or a wider range of glycerides because it minimizes interference from the diglyceride or triglyceride peaks of the cocoa butter or CBE.

We have found that the total butter fat peaks between C_{26} and $C_{60} = (C_{40} + C_{42} + C_{44}) \times 4.270$ and this relation-

ship can be used to calculate the percentage butterfat, e.g.,

$$\% \text{ Butterfat} = \frac{(C_{40} + C_{42} + C_{44}) \times 4.270}{\sum_{20}^{60} C_i}$$

Each operator should, however, determine this factor using appropriate standard mixtures.

In order to correct the peaks at 50-54 resulting from the presence of butterfat we have used the following relationship: 10% (C_{40} + C_{42} + C_{44}) is equivalent to: C_{50} = 4.7; C_{52} = 4.7; and C_{54} = 2.6. Using this relationship, it is possible to correct the peaks in the 50-54 region before determining the level of CBE.

RESULTS

To confirm the accuracy of the method, several fat and chocolate samples of known composition were prepared using the CBE Calvetta. Various types of chocolate products were prepared including vermicelli and flake chocolate (Table III).

The agreement between observed and calculated analyses is good. Only one analysis lies outside the estimated 95% confidence limits. Vermicelli-type products are prepared by dry mixing or kneading without melting. The ingredients are thus not as well mixed as in normal chocolate and this may have caused the observed discrepancy.

Besides Calvetta, other CBE were possible as alternative interpretations of the data especially at low levels of adulteration. This is not usually a problem as at the 15% level of CBE in fat (∿5% on chocolate) which is of greatest interest, the CBE analyses overlap less or not at all. Other analyses such as sterol analysis can then be used if required to help to confirm a particular CBE.

In Table IV we give the results of the analysis of chocolates purchased in Great Britain and Canada. Results are given in terms of the main CBE sold for use at the 5% level in chocolate in Britain, e.g., coberine, illipe (Borneo Tallow), Illexao 30-96, Illexao 30-61. Calvetta is also included. Where "zero" is given, there is less than a 0.2% (1 in 500) chance of the product consisting of a mixture of cocoa butter and that particular CBE; where "low" is given, a less than 5% (1 in 20) chance.

The results in Table IV allow the percentage of CBE in the chocolate to be calculated in terms of commercial products when the fat content of the chocolate is known. The fat content can be determined by standard procedures, but for routine screening of products it is sufficient to assume that one-third fat is present.

In the United Kingdom the present legislation permits the addition of 5% vegetable fat CBE to chocolate. From the results it is clear that most manufacturers have been adding a CBE and that many are adding the full 5% permitted by law.

The limited results in Table IV on 3 products from 2 manufacturers do indicate that CBE are not present in Canadian chocolate, in marked contrast to the results for British chocolate.

DISCUSSION

The method of analysis and the interpretation of the results outlined in this paper have 3 main advantages over existing methods for the detection and determination of CBE in chocolate.

First, the method is almost foolproof because it depends on the analysis of triglycerides, the essential and major component of fats. Methods depending on the detection of minor components such as sterols can often give misleading or wrong results because such components may be removed without noticeably affecting the physical characteristics of the fat. Thus, solvent crystallization, bleaching and deodorization, processes which are all used in the production of CBE, can significantly reduce the level of minor components such as sterols. Methods depending only on the addition of marker compounds are inherently vulnerable to unscrupulous manufacturers who may not add the marker compound.

Second, the method is quantitative, and reliable confidence limits can be assigned to the results. The interpretation of the data provided by the method will depend in part on the additional information that can be provided—e.g., by the chocolate or CBE manufacturer. If no information is available about the sample then a list (normally limited to 2 or 3 possibilities) of possible CBE and the amounts present can be calculated (see Table IV). If the type of CBE is known, it will be estimated quantitatively and the other alternatives can be ignored.

Third, the method is rapid and capable of automation. Total time for sampling, analysis and interpretation of the results using a computer is no more than 45 min for milk or plain chocolate. The GLC analysis can easily be automated so that ca. 50 analyses can be made in 24 hr. Auto-

TABLE III

Comparison of Observed (from GLC Analysis) and Calculated (from Recipe) Results

Product type	% Milk fat in total fat	Pure cocoa butter present	% Calvetta in CB/CBE (i.e., excluding milk fat)		
			Found	Range given by 95% confidence limits	Calculated
Bar	-	No	1.8	1.0 - 2.6	2.1
Vermicelli	19.7 ± 2.6	No	10.8	9.8 - 11.8	13.3
Vermicelli	22.6 ± 3.1	No	11.8	10.8 - 12.8	12.6
Vermicelli	-	No	14.0	13.0 - 15.0	14.7
Vermicelli	-	Yes	-	-	0
Flake	13.2 ± 1.5	Yes	-	-	0
Flake	-	Yes	-	-	0
Bar	9.5 ± 1.7	Yes	-	-	0
Bar	-	Yes	-	-	0
Fat	-	No	6.4	5.4 - 7.4	6.0
Fat	-	No	7.5	6.5 - 8.5	7.0
Fat	-	No	5.8	4.8 - 6.8	5.0
Fat	-	Yes	-	-	0
Fat	-	Yes	-	-	0

mation should lead to even higher precision than we have achieved using manual injection with relatively unsophisticated GLC equipment available in most analytical laboratories.

Using the equations and method of calculation outlined in Interpretation of GLC Data, it is possible to give minimum detection limits and 95% confidence limits at the 15% CBE level for any CBE. Samples of the particular CBE must be analyzed to establish the variability of the CBE. The average results, calculated for 5 CBE, assuming each CBE is added separately to cocoa butter, are shown in Table V. The presence of milk fat has no significant effect on the minimum amount which can be detected or the confidence limits, when they are expressed as the % CBE in the CB/CBE mixture, excluding milk fat.

These figures clearly show the difficulty of detecting and quantifying illipe alone. This is because illipe has a triglyceride analysis similar to the cocoa butter analysis. The problem is clearly shown in Figure 3. In practice, as indicated in Table IV, it is often possible to show that illipe cannot be present on its own, so that the problem may not be very important, especially considering the relatively small quantity of illipe available.

Should it be possible to obtain a sample of the cocoa butter used to formulate the chocolate, the precision of detection and quantification of illipe and other fats can be much improved (see Table V). For instance, the minimum detection limit and the confidence limits can then be made comparable with the figures given for Coberine. *By analyzing several chocolates from a given chocolate manufacturer containing different levels of the same CBE it is possible to deduce the analysis of the cocoa butter used*, assuming it is the same for all the chocolates analyzed. This follows from equation V. The information is then available to improve the precision of the illipe analysis. The precision of detection of palm oil may be similarly improved. Alternatively, palm oil can be detected at a level of 1% or less if the triglyceride analysis is done on a trisaturated triglyceride fraction isolated by crystallization or $AgNO_3$-TLC. A similar procedure has been used to determine lard in goose drippings (17).

In some countries, poor quality solvent-extracted cocoa butter is available and may be added to chocolate. This poor quality cocoa butter contains cocoa shell fat. We have

TABLE IV

The Composition of the Fat Phase of Some British and Canadian Chocolate Products

Country	Type of product	Pure cocoa butter present	Milk fat[a] (%)	Alternative CBE possible[b]				
				Calvetta (%)	Coberine (%)	Illipe (%)	Illexao 30-92 (%)	Illexao 30-96 (%)
UK	Milk chocolate bar	No	21.3 ± 2.3	zero	16.4 ± 2.8	zero	zero	12.9 ± 1.1
UK	Milk chocolate bar	No	20.8 ± 2.0	9.5 ± 0.9 low	17.0 ± 2.9	zero	zero	13.4 ± 1.1 low
UK	Milk chocolate bar	No	27.6 ± 3.5	11.0 ± 1.0	19.4 ± 3.1	zero	zero	zero
UK	Milk chocolate bar	No	29.9 ± 3.3	zero	24.6 ± 3.9	zero	zero	zero
UK	Chocolate-coated toffee bar	No	19.5 ± 2.0	zero	22.5 ± 3.5	zero	zero	17.6 ± 1.2
Canada	Molded chocolate shapes	Yes	3.3 ± 0.7	-	-	-	-	-
UK	Chocolate-coated peppermint cream	Yes	5.9 ± 0.9	-	-	-	-	-
UK	Chocolate-coated peppermint cream	No	-	5.3 ± 0.8 low	9.3 ± 1.9	zero	zero	7.3 ± 1.1 low
UK	Plain chocolate bar	No	14.3 ± 1.3	1.2 ± 0.8 low	2.1 ± 1.3	11.0 ± 8.0	1.7 ± 1.1	1.6 ± 1.0
UK	Chocolate-coated wafer	No	24.7 ± 2.4	zero	20.1 ± 3.3	zero	zero	15.7 ± 1.1 low
Canada	Chocolate-coated wafer	Yes	28.1 ± 3.2	-	-	-	-	-
Canada	Plain chocolate bar	Yes	9.8 ± 0.8	-	-	-	-	

[a]Percentage in total fat.

[b]Percentage in CB/CBE phase, excluding milk fat.

TABLE V

Effect on Confidence Limits if Either CB or CBE Is Known Precisely[a]: 95% Confidence Limits at 15% CBE Addition

CBE	Neither CB nor CBE known			CB known, CBE unknown			CBE known, CB unknown			CB and CBE known		
	Graph[b]	P_{50}	P_{54}	Graph	P_{50}	P_{54}	Graph	P_{50}	P_{54}	Graph	P_{50}	P_{54}
Calvetta	1.1		6.5	0.9		0.7	0.8		6.5	0.4		0.6
Illexao 30-92	1.1		3.7	0.7		0.6	1.1		3.7	0.5		0.4
Coberine	2.5		-[c]	2.4		-[c]	1.3		-[c]	0.6		48
Illipe	8.9		10.7	6.9	1.8	2.8	6.5	7.2	10.2	3.1	1.4	1.0
Illexao 30-96	1.1		17.2	0.8		1.9	1.0		17.1	0.5		1.5

[a]e.g., 15% Calvetta would be determined with 95% confidence to give a result 15 ± 1.1% using the line method and no prior knowledge.

[b]Method described in this paper.

[c]Effectively 100%.

analyzed several cocoa shell fats and poor quality cocoa butters. Such fats do not affect our method of analysis.

Nut oils from nuts added to chocolate will be detected and in most cases it is not possible to distinguish them from oils such as shea oil by this method. In this case slightly more sophisticated methods would have to be used if a correction for nut oils was required.

The accuracy and speed of this method for the detection and the determination of CBE in chocolate make it ideal for the routine analysis of chocolate for the purpose of minotoring the addition of CBE. In just a few cases, the method will not yield unequivocal results and supplementary analyses such as sterol or *trans* acid determination would be required.

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⁂Selective Hydrogenation with Copper Catalysts: V. Kinetics and Mechanism at High Pressure[1]

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ABSTRACT

The mechanism of hydrogenation at 900-950 psi with copper-chromite catalyst was investigated with pure methyl esters as well as their mixtures. A comparison of double bond distribution in *trans*-monoenes formed during hydrogenation of linoleate and alkali-conjugated linoleate revealed that 85-95% of the double bonds in linoleate conjugated prior to hydrogenation. The mode of hydrogen addition to conjugated triene and diene at high pressure is similar to that at low pressure but positional and geometric isomerizations of unreduced conjugated esters were less at high pressure. Geometric isomerization of methyl linoleate and linolenate was considerable at high pressure whereas it was negligible at low pressure. The absence of conjugated products during hydrogenation of polyunsaturated fatty acid esters resulted from their high reactivity. Conjugated dienes are 12 times more reactive than the triene, methyl linolenate, and 31 times more reactive than the diene, methyl linoleate. The products of methyl linolenate hydrogenation were the same as those predicted by the conjugation mechanism.

INTRODUCTION

Studies on the selective hydrogenation of soybean oil with copper catalysts at pressures between 500 and 3000 psi (1) revealed a number of features that are different from low-pressure hydrogenation. Minor amounts of conjugated dienes that appear in the product during low-pressure hydrogenation were eliminated at high pressures. The amount of *trans* isomers formed for each unit of iodine value drop was significantly greater at high pressures. Despite these differences, the selectivity for the reduction of linolenate remained the same. According to theory (2), increased pressure decreases linoleate selectivity (S_L) and *trans* isomerization. In the hope of explaining the anomalous behavior of copper catalysts at high pressures, the mechanism of hydrogenation was investigated with pure unsaturated fatty acid esters varying in number, position and geometric configuration of double bonds as model compounds. The relative reaction rates were determined by hydrogenating mixtures of fatty acid esters. The results of these studies were compared with those obtained at low pressure (3-5) where conjugation was shown to be an essential step prior to hydrogenation.

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EXPERIMENTAL PROCEDURES

Preparation of Pure Methyl Esters

Methyl linolenate (6) and methyl linoleate (7) were prepared by counter double-current distribution of linseed and safflower oil esters, respectively. β-Eleostearic (*t*9,*t*11,*t*13-octadecatrienoic) acid was prepared from tung oil by low-temperature crystallization (8) and esterified with methanolic hydrochloric acid and 2,2-dimethoxy propane (9). Methyl *c*9,*t*11- and *t*10,*c*12-octadecadienoate mixture was obtained by conjugation of methy linoleate with alkali.

Hydrogenation. Reductions were carried out in a 150-ml, magnetically stirred, Magna-Dash autoclave. The fatty ester and 0.5% commercial copper chromite catalyst (Harshaw-CU 1106P) were heated electrically to 170 C under vacuum.