The Interpretation of GLC Triglyceride Data for the Determination of Cocoa Butter Equivalents in Chocolate: A New Approach

C.C. YOUNG, Rowntree Mackintosh Plc, York YO11XY, England

ABSTRACT

A new approach to interpret triglyceride data obtained by gas liquid chromatography (GLC) in order to determine cocoa butter equivalents (CBE) in chocolate is described. The approach is based on the known straight line relationship which exists between the C_{50} and C_{54} triglycerides of cocoa butter of different origins and the realization that, for currently available CBE conforming to CAOBISCO's criteria, a similar band relationship exists. The technique described enables the quantity of unspecified CBE in a chocolate containing an unknown cocoa butter to be determined to an accuracy of ± 1.5% when present in chocolate at the 5% level. Nut oils (almond, walnut or hazelnut) are sometimes present in mainland European chocolates and, should CBE also be present, it is possible to calculate the combined percentages of nut oil and CBE in the chocolate. The method of interpretation described is not dependent on a particular GLC technique for determining triglycerides. Interpretation of other laboratories' results obtained using different GLC instruments and procedures has shown that the method enables any CBE present in the fat under examination to be determined accurately. The method compensates for variations in the composition of CBE and for the differences between cocoa butters of different origin. A detailed knowledge of CBE compositions is not required and only a few cocoa butter/CBE standards are necessary. The method described is graphical, enabling small laboratories not equipped with microcomputers to utilize the method. The calculation can, however, be programmed for a computer.

INTRODUCTION

The triglyceride content of fats can be routinely determined by gas liquid chromatography (GLC). Fincke (1–4,) and Padley and Timms (5) have published methods based on such analyses for determining cocoa butter equivalents (CBE) in chocolate. Both methods rely on the existence of a straight line relationship between C_{50} and C_{54} for all cocoa butters. Any deviation from this relationship indicates the presence of some other fat.

The quantitative determination of CBE is dependent on the identification of the CBE or CBE-type fat present, followed by use of the GLC triglyceride data for the identified fat to calculate its percentage in the extracted fat phase and hence in the chocolate. Unfortunately, the triglyceride compositions of CBE vary from month to month and year to year, depending on the raw materials from which they are manufactured. This may lead to an incorrect identification of a CBE and hence an error in the calculation of the CBE content.

Outside the chocolate industry, analysts have little access to data on the numerous CBE available, making identification more difficult and possibly resulting in errors when calculating the CBE content.

This paper describes a method by which the CBE content of a chocolate may be determined using only a limited number of CBE and cocoa butter standards.

EXPERIMENTAL

Triglyceride Analysis

In all the triglyceride analyses referred to here, the following conditions were used: GLC instrument – Perkin Elmer Sigma 3 system with autosampler; detector – flame ionization detector (FID); carrier gas – nitrogen at a flow rate of 70 mL/min; column – 18″ × 3/16″ ID glass column fitted with glass to metals seals packed with 3% SP 2100 on 100/120 mesh Supelcoport stationary phase; column temperature – 300–240 C programmed at 2 C/min; detector oven temperature – 350 C; injection oven temperature – 350 C; and sample size – 1 μL of a 1% pentane solution of the extracted fat.

The triglyceride results were normalized so that:

$$C_{50} + C_{52} + C_{54} = 100\%$$

Table I gives the determined triglyceride compositions of: (a) pressed nib cocoa butters from Ghana, Nigeria, Ivory Coast, Bahia, Cameroon and Ecuador, Trinidad, Grenada, New Guinea/Jamaica blend beans; (b) CBE – Calvetta, Illexao 30–92, Illexao 30–61, Coberine, Veberine; and (c) hazelnut oil.

INTERPRETATION PROCEDURE

Construction of Graph

In Figure 1, a straight line was constructed through the C_{50} vs C_{54} plot for the different cocoa butters. Utilizing the mean C_{50} and C_{54} values ± twice the standard deviation of the individual triglyceride results, the extreme C_{50} and C_{54} values for 95% of all cocoa butters were calculated and plotted.

The C_{50} vs C_{54} plots of the CBE examined fell within an empirical CBE band constructed so that the lower line of the band ran from 70% on the C_{50} axis to 80% on the C_{54} axis and the upper line from 79% on the C_{50} axis to 89% on the C_{54} axis.

The explanation for the CBE triglyceride data lying within a band almost parallel to the cocoa butter line is that CBE are generally prepared from blends of mid-palm and mid-shea fractions with, in some instances, small additions

TABLE I

Triglyceride Compositions (%)

	C_{50}	C_{52}	C_{54}
Cocoa Butters			
Ghana	19.0	47.6	33.4
Ecuador	19.6	47.0	33.4
Trinidad	21.0	48.0	31.0
Nigeria	19.9	47.5	32.6
Bahia	19.1	47.1	33.8
New Guinea/Jamaica	21.2	47.5	31.3
Grenada	21.0	48.0	31.0
Ivory Coast	20.0	47.8	32.2
Cameroon	20.2	47.1	32.7
Mean value	20.1	47.5	32.4
Range (± 2X SD)	18.5–21.7	47.0–48.0	30.2–34.5
CBE			
Clavetta	74.5	21.8	3.7
Illexao 30/61	44.9	17.8	37.3
Illexao 30/92	2.6	12.5	84.9
Coberine	35.9	25.0	39.1
Veberine	44.6	26.7	28.7
Nut oil			
Hazelnut oil	1.5	17.5	81.0

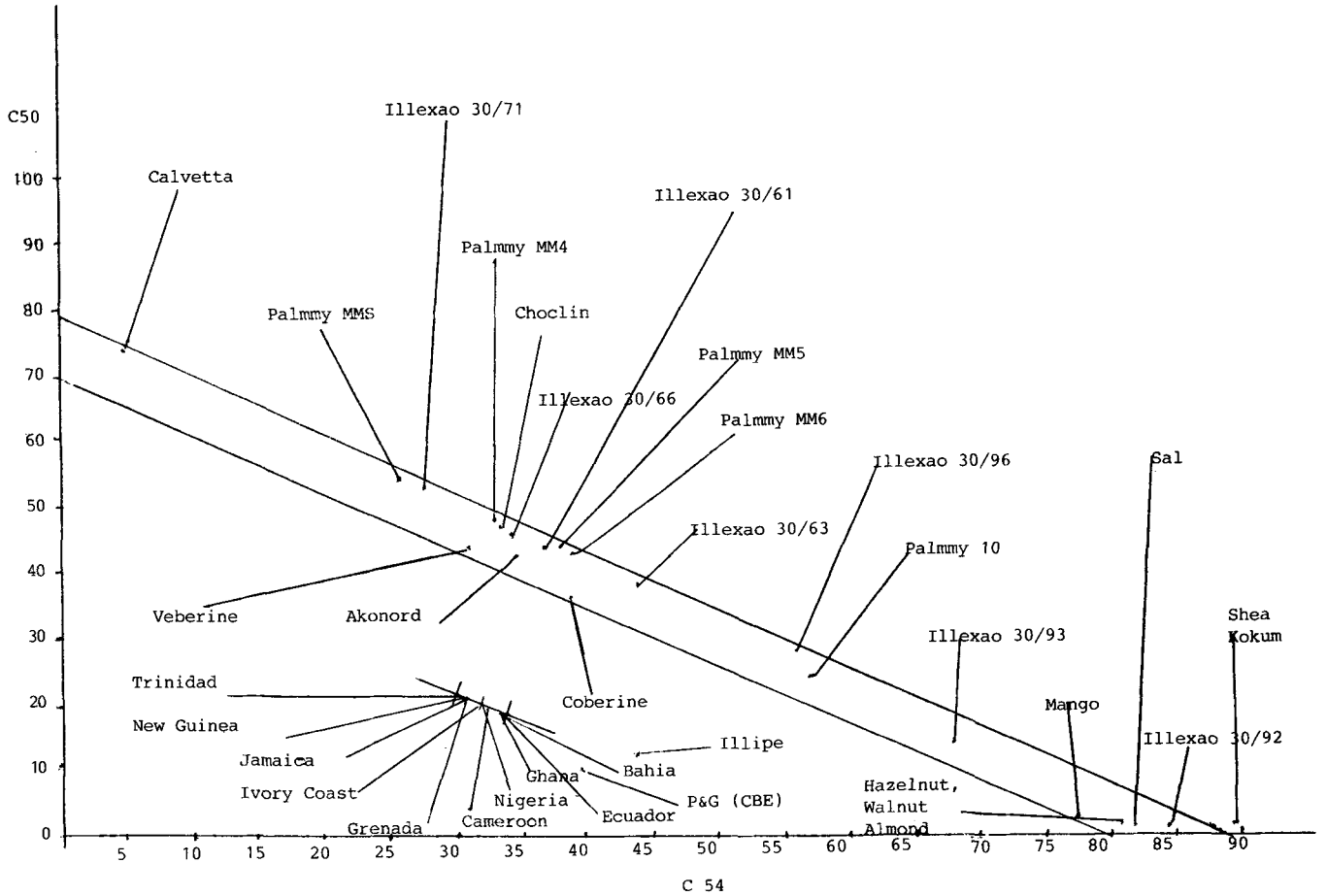

FIG. 1. C_{50} vs C_{54} plot of the GLC triglyceride data of CBE and cocoa butters.

of illipe or other fats containing 2 oleo disaturated triglycerides. Although different feedstocks and different manufacturing techniques result in scatter, the C_{50} vs C_{54} plots fall within a clearly defined band.

The validity of the concept of this band was demonstrated when data obtained from 10 other laboratories, which had examined different CBE on different columns operated under different conditions, were found to fall within it. With the exception of the experimental Procter & Gamble fat, available data indicate that the parameters used to construct the band in Figure 1 will embrace all currently commercially available CBE.

Use of Graph when Analyzing Chocolate of Unknown Composition

The normalized C_{50}, C_{52} and C_{54} triglyceride values of the fat extracted from the chocolate are corrected to compensate for the quantity of milk fat present in the fat phase. The corrected, renormalized C_{50} and C_{54} values are plotted on the graph. If the plot does not lie on the cocoa butter line, the fat is deemed to contain another fat.

A line is constructed from the extreme cocoa butter plot A on the left-hand side (LHS) of the cocoa butter line through the sample plot and extended through the CBE band. The C_{52} values of the two points at which the line intercepts the CBE band are calculated from $100-(C_{50} + C_{54})$. Two similar lines are drawn from the extreme cocoa butter plot B on the right-hand side (RHS) of the cocoa butter line and from the point representing the mean value for all the cocoa butters and the corresponding C_{52} values calculated. In some instances, the extended line may only intercept the band at one point and there may even be occasions when the line does not intercept the band at all. This only occurs when the CBE is essentially a mid-palm or mid-shea type and suggests that the specific cocoa butter having the C_{50} and C_{54} values from which the line originates is probably not present in the fat phase.

The C_{52} values so obtained are used in the following calculation to determine the quantities of CBE present in the chocolate fat phase.

$$\%\ \text{CBE in the fat blend} = \frac{(C_{52}\ \text{for mean CB} - C_{52}\ \text{sample}) \times (100 - \%\ \text{milk fat in fat phase})}{C_{52}\ \text{for mean CB} - C_{52}\ \text{for each CBE band intercept}}$$

The calculation is repeated for each extreme cocoa butter C_{52} and the corresponding sample C_{52} values.

EXAMPLE

The butyric acid content of an extracted fat having the following normalized triglyceride data:

C_{50} =	22.4
C_{52} =	41.8
C_{54} =	35.8
	100.0

was determined by the method of Phillips and Sanders (6), so as to determine the percentage of milk fat in the fat phase. The fat was found to contain 20% milk fat.

Milk fat contains an average of: C_{50} = 12%; C_{52} = 13%; C_{54} = 8%. Correction of the determined C_{50}, C_{52}, C_{54} values to allow for the milk fat was as follows:

	Normalized	Renormalized
C_{50} =	22.4 − (12 X 20/100) = 20.0	21.4
C_{52} =	41.8 − (13 X 20/100) = 39.2	42.0
C_{54} =	35.8 − (8 X 20/100) = 34.2	36.6
	100.0 93.4	100.0

Figure 2 demonstrates the approach using the renormalized triglyceride data of the extracted fat.

The intercepts obtained from Figure 2 when used in the above equation give the following.

LHS cocoa butter (plot A).

1st intercept:

$$\frac{(48.0 - 42.0) \times (100 - 20)}{48.0 - 24.0} = 20.0\% \text{ CBE}$$

2nd intercept:

$$\frac{(48.0 - 42.0) \times (100 - 20)}{48.0 - 13.1} = 18.6\% \text{ CBE}$$

Mean cocoa butter.

1st intercept:

$$\frac{(47.5 - 42.0) \times (100 - 20)}{47.5 - 23.8} = 18.6\% \text{ CBE}$$

2nd intercept:

$$\frac{(47.5 - 42.0) \times (100 - 20)}{47.5 - 14.2} = 13.2\% \text{ CBE}$$

RHS cocoa butter (plot B).

1st intercept:

$$\frac{(47.0 - 42.0) \times (100 - 20)}{47.0 - 25.6} = 18.7\% \text{ CBE}$$

2nd intercept:

$$\frac{(47.0 - 42.0) \times (100 - 20)}{47.0 - 15.3} = 12.6\% \text{ CBE}$$

The range obtained is 12.6–20.0% with a mean of 16.3%.

The CBE content of the fat phase is therefore 16.3 ± 3.7%. Assuming the milk chocolate to contain 30.0% fat, the percentage CBE in the chocolate is:

$$(16.3 \pm 3.7) \times \frac{30}{100} = 4.9 \pm 1.2\%$$

VERIFICATION

To test the reliability of the method, the following fat blends and chocolate were prepared (Tables II and III). The fats used as standards were different to those used as ingredients for the chocolates. The cocoa butters used in the chocolates were blends of different origin resulting in each chocolate containing a different type of cocoa butter. In so doing, the chocolates were representative of products purchased by consumers.

The fat blends and the fats extracted from the chocolates were analyzed to determine the: (a) total fat content of the chocolate, (b) milk fat in the fat phases (Phillips and

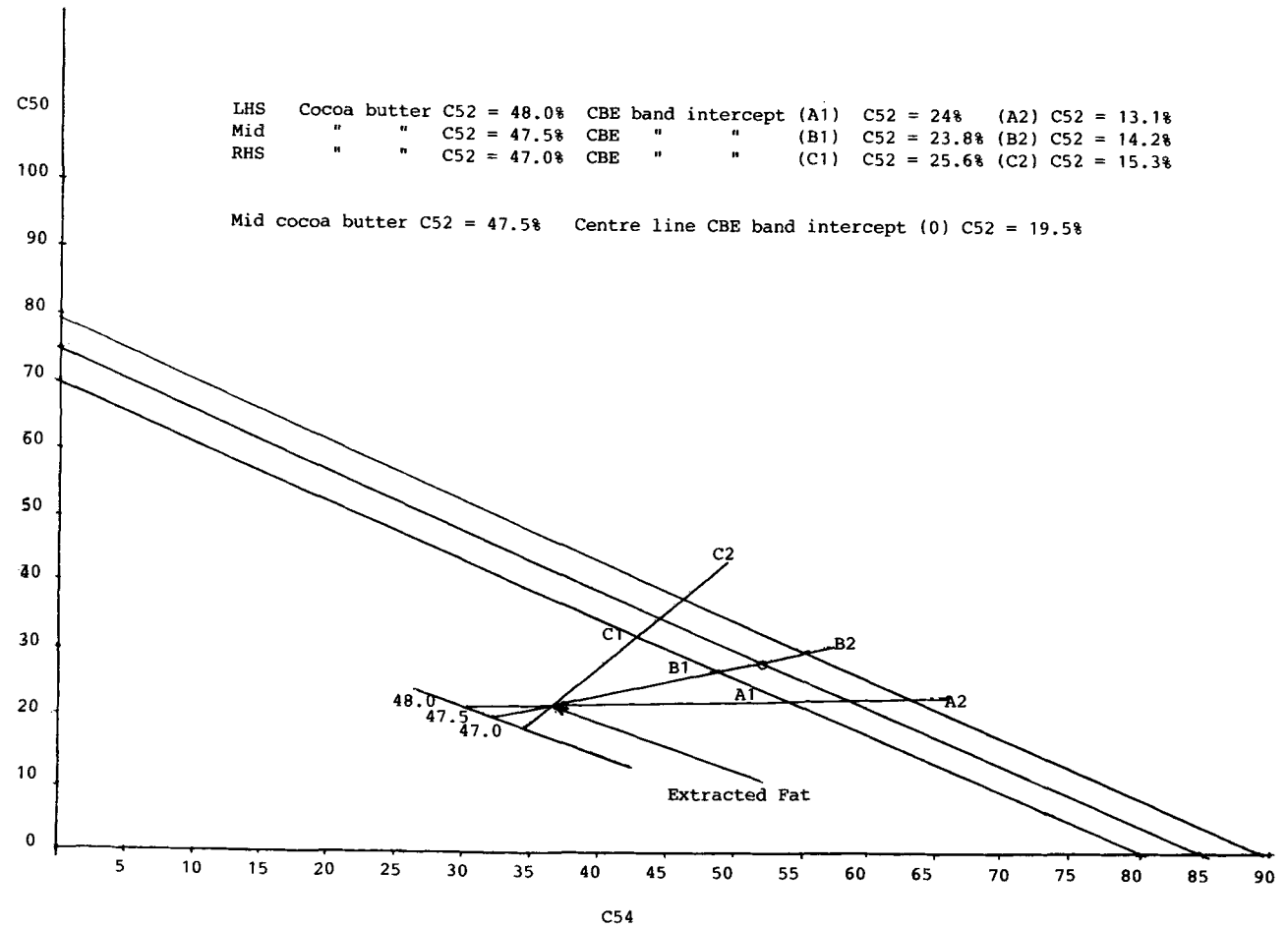

FIG. 2. Example of the CBE band approach.

TABLE II

Fat Blends Preposed

Blend	Cocoa butter (%)	CBE % type		Milk fat (%)
		CBE (1)	CBE (2)	
1	82.5	Calvetta 17.5%	–	–
2	87.9	Illexao 30/96 12.1%	–	–
3	87.9	Illexao 30/92 6.05%	Calvetta 6.05%	–
4	73.1	Illexao 30/92 11.1%	–	15.8
5	59.2	Illexao 30/61 15.8%	–	25.0
6	60.8	Illexao 30/93 6.85%	Illexao 30/63 6.85%	25.3
7	72.2	Calvetta 5.5%	Hazelnut oil 6.5%	15.8

TABLE III

Chocolates Preposed

Chocolate	Cocoa butter (%)	CBE % type		Milk fat (%)	Total fat in chocolate (%)
		CBE (1)	(CBE (2)		
1	82.5	Calvetta 17.5%	–	–	31.6
2	87.9	Illexao 30/96 12.1%	–	–	31.6
3	87.9	Calvetta 6.05%	Illexao 30/92 6.05%	–	31.6
4	73.1	Illexao 30/92 11.1%	–	15.8	31.6
5	59.1	Illexao 30/61 15.8%	–	25.0	31.6
6	60.8	Calvetta 6.95%	Illexao 30/93 6.95%	25.3	31.6
7	65.3	Akonord 10.9%	Hazelnut oil 8.0%	15.8	31.6

TABLE IV

Triglyceride Results

	C_{50}	C_{52}	C_{54}
Fat blend			
1	28.3	43.0	28.7
2	18.8	43.1	38.1
3	20.9	43.2	35.9
4	17.5	42.7	39.8
5	24.5	40.9	34.6
6	20.1	41.6	38.3
7	23.1	43.3	33.6
Chocolate			
1	27.5	42.7	29.8
2	20.6	43.3	36.1
3	21.2	43.3	35.5
4	17.4	42.6	40.0
5	23.7	41.3	35.0
6	24.1	41.9	34.0
7	20.0	40.7	39.3

Sanders method), (c) triglycerides of the fats (Table IV), and (d) percentage of CBE using the method described in this paper.

Corrected triglyceride values allowing for milk fat percentages determined by analysis are shown in Table V.

The percentages of CBE in the fat phases and hence the chocolates were calculated as described earlier. Table VI summarizes the results obtained.

DISCUSSION

The method as interpretation given in this paper enable competent analysts to determine CBE in chocolate to an accuracy within the 5.0 ± 2.0% limits discussed by CAOBISCO for chocolates.

The method appears to compensate for the effects of variations in triglyceride compositions of cocoa butters, CBE and milk fats, provided that the GLC triglyceride analysis itself is satisfactory. Fincke (private communication) and Chaveron (private communication) found similar normalized cocoa butter triglyceride values, specifically the C_{52} value, to those obtained for the cocoa butter standards used in the present work.

Fincke: $C_{50} = 19.5$, SD = 1.6;
$C_{52} = 47.5$, SD = 0.5;
$C_{54} = 33.0$, SD = 2.1.

Chaveron: $C_{50} = 19.3$, SD = 1.2;
$C_{52} = 47.5$, SD = 0.4;
$C_{54} = 33.2$, SD = 1.5.

It is paramount that analysts obtain similar values when determining the GLC triglyceride content of cocoa butters. Failure to achieve similar results indicates that the GLC analysis is unsatisfactory, which would lead to erroneous CBE determination.

The effectiveness of the proposed method is demonstrated by using the cocoa butter calibration lines of Fincke and Chaveron to determine the quantity of CBE present in the chocolate fat phase given in Figure 2 (see Table VII).

TABLE V

Corrected Triglyceride Values

	C_{50}	C_{52}	C_{54}
Fat blend			
4	16.4	42.9	40.7
5	23.5	41.0	35.5
6	18.6	41.9	39.5
7	22.4	43.5	34.1
Chocolate			
4	16.4	42.8	40.8
5	22.6	41.5	35.9
6	23.1	42.1	34.8
7	19.1	40.8	40.1

TABLE VI

Summary of CBE Analysis of Fat Blends and Chocolates

Sample	Theoretical Values				Analysis values			
	CBE in fat phase	CBE in chocolate	Milk fat in fat phase	Total fat in chocolate	CBE in fat phase	CBE in chocolate	Milk fat in fat phase	Total fat in chocolate
Fat blend								
1	17.5	–	–	100.0	19.7 ± 4.0	–	–	–
2	12.1	–	–	100.0	15.1 ± 3.6	–	–	–
3	12.1	–	–	100.0	15.5 ± 3.7	–	–	–
4	11.1	–	15.8	100.0	12.1 ± 2.5	–	15.4	–
5	15.8	–	25.0	100.0	18.2 ± 3.2	–	25.2	–
6	13.7	–	25.3	100.0	14.6 ± 3.2	–	25.0	–
7	12.0	–	15.8	100.0	13.0 ± 3.3	–	15.0	–
Chocolate								
1	17.5	5.5	–	31.6	20.3 ± 4.6	6.2 ± 1.4	–	30.7
2	12.1	3.8	–	31.6	15.2 ± 3.9	4.7 ± 1.2	–	30.9
3	12.1	3.8	–	31.6	15.5 ± 3.7	4.7 ± 1.1	–	30.6
4	11.1	3.5	15.8	31.6	13.0 ± 3.2	4.1 ± 1.0	15.5	31.6
5	15.8	5.0	25.0	31.6	16.8 ± 3.8	5.2 ± 1.2	24.7	30.8
6	13.9	4.4	25.3	31.6	15.8 ± 4.0	4.9 ± 1.3	24.2	31.0
7	18.9	6.0	15.8	31.1	20.1 ± 4.2	6.2 ± 1.3	14.8	31.1

TABLE VII

CBE Values

Analyst	CBE in fat phase	CBE in chocolate
Fincke	16.5 ± 4.0	5.0 ± 1.2
Chaveron	16.7 ± 3.5	5.0 ± 1.1
RM	16.3 ± 3.7	4.9 ± 1.2

In each case, using the different cocoa butter calibration lines, similar values are found for a fat phase containing an unknown cocoa butter and CBE, i.e., ca. 5.0% with a possible maximum error of ± 1.1%. When the maximum possible error for the same fat is calculated without the use of the CBE band, using Fincke's extreme C_{52} values and RM's extreme C_{52} values observed for CBE i.e., 12.5% and 26.3%, the maximum possible error is increased from ca. ± 1.1% to ± 1.9%. This indicates the magnitude of the increased error to be expected when an analyst incorrectly identifies a CBE using an alternative method to the proposed CBE band approach.

The results given in Table VI clearly indicate that the CBE band method holds for combinations of CBE and hazelnut oil. The method is unsuitable for the determination of the experimental Procter & Gamble type CBE, illipe or hydrogenated fats, because they lie outside the CBE band. CBE component fats such as mid-palm and mid-shea fractions, sal, mango and kokum can be determined as total CBE.

If fats other than CBE and nut oil are suspected in the chocolates, perhaps through center fat migration in enrobed products, additional analysis such as GLC fatty acid, GLC isomer fatty acid and thin layer chromatography is essential.

The CBE band can be broadened, but the maximum possible error is increased. Table VIII gives a comparison of the CBE results given in Table VI with those calculated using two alternative CBE bands, constructed so that the lower line remained the same with the upper line constructed as follows – CBE band (a): C_{50} = 84.0%, C_{54} = 94.0%; CBE band (b): C_{50} = 90.0%, C_{54} = 100.0%. The results indicate that it is possible for a wider band to

TABLE VIII

Comparison of CBE Results

Samples	CBE results given in Table VI		CBE results using CBE band (a)		CBE results using CBE band (b)	
	CBE in fat phase	CBE in chocolate	CBE in fat phase	CBE in chocolate	CBE in fat phase	CBE in chocolate
Fat blend						
1	19.7 ± 4.0	–	19.7 ± 4.0	–	19.7 ± 4.0	–
2	15.1 ± 3.6	–	14.0 ± 3.9	–	13.3 ± 4.6	–
3	15.5 ± 3.7	–	14.8 ± 4.2	–	14.0 ± 5.0	–
4	12.1 ± 2.5	–	12.1 ± 2.5	–	12.1 ± 2.5	–
5	18.2 ± 3.2	–	17.5 ± 4.7	–	16.5 ± 5.6	–
6	14.6 ± 3.2	–	13.5 ± 3.5	–	12.8 ± 4.3	–
7	13.0 ± 3.3	–	12.2 ± 3.6	–	11.5 ± 4.5	–
Chocolate						
1	20.3 ± 4.6	6.2 ± 1.4	19.4 ± 4.7	6.0 ± 1.4	18.4 ± 6.7	5.6 ± 2.1
2	15.2 ± 3.9	4.7 ± 1.2	14.7 ± 4.5	4.5 ± 1.4	13.9 ± 5.7	4.3 ± 1.8
3	15.5 ± 3.7	4.7 ± 1.1	14.8 ± 4.5	4.5 ± 1.4	14.0 ± 5.3	4.3 ± 1.6
4	13.0 ± 3.2	4.1 ± 1.0	12.6 ± 3.8	4.0 ± 1.2	12.6 ± 3.8	4.0 ± 1.2
5	16.8 ± 3.8	5.2 ± 1.2	16.2 ± 4.5	5.0 ± 1.4	15.3 ± 5.1	4.7 ± 1.6
6	15.8 ± 4.0	4.9 ± 1.3	15.4 ± 4.7	4.8 ± 1.5	14.6 ± 5.5	4.5 ± 1.7
7	20.1 ± 4.2	6.2 ± 1.3	19.0 ± 5.1	5.9 ± 1.6	18.0 ± 6.1	5.6 ± 1.9

be used, while still meeting the discussed CAOBISCO requirement of 5.0 ± 2.0% CBE in the chocolate.

Calculation of the CBE content using the extreme cocoa butter C_{52} values and the corresponding C_{52} values for the CBE band intercepts expresses the results as percentages including the maximum possible errors. For routine analysis, the calculation may be simplified by using only the mean C_{52} cocoa butter value and the corresponding intercept values on a line running through the center of the CBE band. For the example shown in Figure 2, for instance, values of 15.7% in the fat phase or 4.7% in the chocolate would result. If values greatly exceed 5.0% in the chocolate, the entire calculation procedure must be completed to determine the maximum possible error.

ACKNOWLEDGMENTS

C. Bishop determined the triglyceride analysis and B. Stubbs prepared the chocolate samples.

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APPENDIX

Definition

The following definition of vegetable fats has been drawn up for the purposes of the EEC directive: edible vegetable oils and fats are lipids obtained from vegetables, the predominant glycerides being triglycerides.

They may contain small amounts of other components of lipids such as mono- and diglycerides, polar lipids, free fatty acids and unsaponifiable matter.

They may be fractionated, hydrogenated, inter- or intraesterified and/or refined.

Analytical Criteria

The vegetable fats for use in chocolate within the Community must comply with the following analytical criteria in order to allow qualitative and quantitative control:

(a) Level of triglycerides type SOS $\geqslant$ 65%.
(b) Fractions of the 2-position of triglycerides, occupied by unsaturated fatty acids, $\geqslant$ 85%.
(c) Total content of unsaturated fatty acids, $\leqslant$ 45%.
(d) Unsaturated fatty acids with 2 or more double bonds, $\leqslant$ 5% (this figure is included in [c]).
(e) Level of lauric acid, $\leqslant$ 1%.
(f) Level of *trans* fatty acids, $\leqslant$ 2%.

Work is continuing on certain of these analytical criteria and if this leads to any proposed changes in the figures now indicated, the EC Commission will be informed.

Level of Use and Declaration

The use of added vegetable fats should be limited to 5% of the total weight of chocolate in the product. The presence of added vegetable fats should be indicated in the list of ingredients. (CAOBISCO has already accepted that a full declaration of ingredients should be applied to chocolate products, in accordance with the provisions of the labelling directive.)

♣Gas Chromatographic Separation of Long-Chain Fatty Nitriles and Long-Chain Acid Amides

C.N. WANG and **L.D. METCALFE**, Akzo Chemie America*, Research Laboratories, 8401 W. 47th Street, McCook, IL 60525

ABSTRACT

A method is described for the chromatographic separation of long-chain fatty nitriles and long-chain acid amides on a cyanopropyl silicone column. We found that better separations were obtained for these compounds using a cyano column than with any column previously reported.

INTRODUCTION

The long-chain nitriles are neutral materials and are easily chromatographed on many types of columns. Apiezon, silicone type and Carbowax 4000 monostearate have been reported to give excellent separations (1,2). If the separation of saturates from unsaturates and polyunsaturates is desired, a polyester column can be used (3-5). The nitrile group is highly electronegative, probably the most polar of all functional groups, and this polarity becomes apparent when a separation is made on polar and nonpolar type columns. On a DEGS column, the retention time for a nitrile of a given chain length is almost double that for a methyl ester of equal chain length. From this observation, one would predict that liquid phases containing nitriles would be useful, highly polar substrates. This observation has been verified by Tenny and others (6-8).

In order to obtain rapid separations of nitriles without losing the valuable saturate-unsaturate separation, phosphoric acid treated polyesters are useful (4). However, the limited resolution of saturates and unsaturates, as well as the retention time of this column, does not offer the optimum condition for nitrile separations. The described cyanopropylsilicone column, as predicted earlier, will give superior separation of fatty nitriles by gas chromatography.

Long-chain acid amides derived from fatty acids are high-melting, waxlike substances. They are used in many commercial applications. These materials have a very low degree of volatility. One would not generally consider the amides as suitable samples for gas chromatographic analysis. Considerable research effort has been made to chromatograph these compounds. The amides have been separated on Apiezon L-KOH columns (9). Amides have also been resolved on the Versamid 900 column (10). The trifluoroacetyl derivatives of fatty amides have been made and separated (11). Short-chain amides have been resolved on the Dowfax 9N9 column (12). When unsubstituted amides are chromatographed on a DEGS (13,14) and a polyester phosphoric acid column, the peaks that emerge are reported to be nitriles resulting from on-column dehydration of the

*Formerly Armak Company.