

Determination of Milk Fat in Chocolates by Gas-Liquid Chromatography of Triglycerides and Fatty Acids

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ABSTRACT: The combination of two routine methods is proposed to determine the content of milk fat (MF) in chocolates, which is applicable even in the presence of lauric fats or others. The content of MF is obtained from the sum of C_{40} , C_{42} , and C_{44} medium-chain triglycerides, determined by capillary gas-liquid chromatography (GLC). A new method, based on methyl esters of lauric acid and on minor acids situated between myristic and palmitic, is proposed. It enables detection and estimation of potential lauric fats, as well as the determination of the actual content of MF. The influence of other vegetable and animal fats is discussed. We analyzed 45 MF samples extracted from industrial milk powders and from pure or fractionated MF for chocolate manufacturing or pastry by GLC of triglycerides. We also analyzed by capillary GLC the methyl esters from 22 of those fats. Mixtures of these 22 MF samples with a cocoa butter also were used for chromatographic analyses of methyl esters and triglyceride. Results from the various analytical methods have been presented.

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During routine analysis of a dark or a milk chocolate, the analyst must determine the content of milkfat (MF) and the purity of the cocoa butter (CB). Whenever at least one other fat is present, because of legally authorized use of vegetable fats or because of incorporation of nuts (almonds, hazelnuts, groundnuts, etc.) or other oilseeds (grated coconut, for instance), the analyst also must determine the content of the latter fat. The reasons are twofold—first, to check if the allowed legal values are respected (but this issue will not be taken into consideration in this article); second, the analyst also must make sure that these fats do not influence the determination of MF. To be as accurate as possible, this determination must be made with a two-step procedure: one, the primary determination of the gross content; and two, the determination of fats that are likely to bias this gross result. The necessary corrections then can be made. Many authors have proposed

methods for primary determination of MF, based on the content of butyric acid (1), lauric acid (2), all acids from butyric to lauric (3), or on the ratio between myristic and palmitic (4). Others considered the composition of triglycerides obtained by gas-liquid chromatography (GLC) and established a method that is regarded as official and known as the Caobisco method (see Ref. 8).

The Caobisco method is based on the sum of triglycerides C_{40} , C_{42} , and C_{44} for the determination of MF content. In addition, the total content of triglycerides C_{50} , C_{52} , and C_{54} minus the corresponding carbon numbers from MF are used for verification of the purity of the CB present (5-8). This last method is of particular interest for routine analysis in the laboratories of the chocolate industry because it is rapid and answers two out of three questions: What is the apparent content of MF? What is the purity of cocoa butter? However, this method is not totally satisfactory for the third question (Is there presence of fat other than CB and MF, interfering with this one, and how much?), determining the purity of the MF because, in the case of lauric fat addition, the content of triglycerides C_{40} , C_{42} , and C_{44} represents the sum of those coming from milk and lauric fats.

The purpose of this paper is to check some of the classical analyses and to propose a simple and efficient method for verification of the purity of MF contained in chocolates. For countries where regulations accept the addition of vegetable fats, particularly lauric fats, and for chocolate imitations, we propose a way of calculating the estimated contents of lauric fat and pure MF. The method is based on the determination of the ratio between lauric acid and the sum of minor fatty acids present between the peaks of myristic and palmitic, which are quantitated by GLC of their methyl esters.

EXPERIMENTAL PROCEDURES

Methyl ester analysis was performed on a DELSI 30 gas chromatograph (Suresnes, France), equipped with split injector and flame-ionization detector (FID). The carrier gas was He at 0.4 bar. The capillary column used was a 25 m \times 0.32 mm column coated with BP 70, 0.25 μ , REF 25QC3/BP \times 70 (SGE, Ringwood, Victoria, Australia). The 1- μ L injections were from a solution of methyl esters at approximately 200

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mg/mL hexane. The temperature parameters were: injector, 250°C, detector 250°C, column 185°C isothermal. The integration was performed by an SP 4290 (Spectra Physics, San Jose, CA).

The GLC triglyceride analysis was performed on a DELSI 3000 gas chromatograph equipped with a ROSS needle injector (PERICHRON, Longjumeau, France) and FID. The carrier gas was He at 0.65 bar. The capillary column used was 5 m × 0.33 mm, coated with 3P1, 0.25 μ (SGE). Injections of 1 μL were of a solution at 1 mg/mL hexane. Temperature parameters were: injector, 380°C; detector, 360°C; column, 255 to 355°C at 10°C/min. Integrations were performed by an SP 4290.

Thirty-eight MF samples were collected (primarily from France); each material lot was used in a French chocolate factory last year. Some samples were carefully extracted from roller or spray milk powders according to the Roese-Gottlieb Official IDF-ISO AOAC method (9). Others were pure MF. In addition, five commercial MF stearins were analyzed from dry fractionation, used mainly for "millefeuille" (puff) pastry and authorized in chocolates by European Economic Community regulation. Two MF oleins were also collected, which included a special one for "croissant." One sample of a pure cocoa butter from Asia was utilized in the study. Fifteen out of the 38 MF samples were selected so that the whole year was represented. The MF samples were mixed with the CB at a 15:85 (MF/CB) ratio. The same procedure was followed for the stearins and the oleins.

RESULTS AND DISCUSSION

Compositional analysis data for different medium-chain triglycerides are presented in Table 1. These results confirm the great interest in the triad C₄₀, C₄₂, and C₄₄, with or without the small peaks between the main ones. It is preferable not to take into account these intermediate peaks, because

they slightly increase the SD% for pure MF. Also, there is the risk that they are not being integrated in 15:85 mixtures when MF is in the minority. The extended group of C₄₀ to C₄₆ can also be used, but has no advantage as compared to the triad. Finally, the group of C₄₂ to C₄₆ is useless because its SD% is too high. Therefore, we only shall take into consideration C₄₀, C₄₂, and C₄₄. Fractions can present problems but must not be neglected, especially stearins, which are often used; oleins are rarely used. The values of the C₄₀–C₄₄ triad for the representative fractions have a broader range: 21.3, 22.5, 22.45, 19.05, and 22.3% for stearins, and 22.0 and 20.0% for oleins. The SD% of the fractions is slightly higher due to variation when compared to pure MF.

By taking into account the results of these five stearins, which are representative of those currently used in Europe, we can assume that a method based on the triad C₄₀ to C₄₄ can be applied to them. Even in rare cases when oleins are present, the risk of error is minor with this method. The overall mean value of C₄₀–C₄₄ for the 45 analyses is 21.6%, which is close to values found in the literature (3,5).

Tables 2 and 3 show the results for 15 of the previous unfractionated MF samples, as well as the five stearin and two olein fractions. The tables give the percentages of C₄₀ to C₄₄ triglycerides, lauric acid, minor fatty acids between C₁₄ and C₁₆ (MINFA), myristic and palmitic acids. Table 4 (Ref. 10) shows the same indicative values, given in the literature, for MF, CB, coconut oil (COCO), palm oil, tallow, and hazelnut oil. The last four fats were chosen because they are representative of the vegetable fats that possibly could be used when allowed by legislation (palm oil and its fractions as CB extender) or because they can be found accidentally in chocolates and then present an analytical problem. Examination of Tables 2–4 leads to the following conclusions.

C₄₀ to C₄₄. Good SD% for samples 1–15 and higher SD%, which is correct for the group of 15 MF + 5 stearins + 2 oleins (Tables 2 and 3). Table 4 indicates that only lauric fats can

TABLE 1
Medium-Chain Triglyceride Contents of 38 Milk Fat (MF), 5 MF Stearins, and 2 MF Oleins

	C ₄₀ to C ₄₄	Idem + inter ^a	C ₄₀ to C ₄₆	Idem + inter ^a	C ₄₂ to C ₄₆	Idem + inter
MF (38 samples)						
Maximum	22.2	24.5	29.55	32.0	20.0	22.0
Minimum	20.25	21.15	25.75	27.05	16.25	16.6
Mean	21.6	23.15	27.65	30.0	17.95	19.85
SD	0.74	0.87	0.95	1.14	1.02	1.20
SD% of average	3.4	3.8	3.5	3.8	5.7	6.0
MF + 5 stearins (43 samples)						
Maximum	22.8	24.55	31.35	33.45	23.1	24.75
Minimum	19.05	19.4	25.75	27.05	16.25	16.6
Mean	21.6	23.1	27.8	30.1	18.25	20.15
SD	0.83	1.04	1.20	1.38	1.48	1.60
SD%	3.8	4.5	4.3	4.6	8.1	7.9
MF + 2 oleins (45 samples)						
Maximum	23.3	24.55	31.35	33.45	23.1	24.75
Minimum	19.05	19.4	25.75	27.05	16.25	16.6
Mean	21.6	23.1	27.8	30.1	18.25	20.1
SD	0.82	1.03	1.19	1.37	1.47	1.58
SD%	3.8	4.4	4.3	4.5	8.0	7.9

^aInter = intermediary small peaks present between main triglycerides with odd number of carbons; Idem = id or ditto.

TABLE 2
Analytical Results of 15 Pure Milkfat Samples^a

Number	40–44 (%)	C ₁₂ (%)	MINFA ^b (%)	Ratio ^c	C ₁₄ (%)	C ₁₆ (%)	Ratio ^d
1	22.55	4.05	3.4	1.19	11.85	30.3	0.39
2	22.8	4.5	3.65	1.23	13.65	35.75	0.38
3	21.85	4.1	3.45	1.19	13.4	36.9	0.36
4	22.75	4.15	3.6	1.15	11.75	27.9	0.42
5	22.3	4.35	3.7	1.18	12.3	28.15	0.44
6	21.05	3.75	3.85	0.97	12.1	30.4	0.40
7	21.05	3.25	3.7	0.88	11.8	35.6	0.32
8	22.1	3.7	3.8	0.97	11.55	27.55	0.42
9	21.2	3.85	3.8	1.01	12.1	31.45	0.39
10	20.8	3.45	3.85	0.90	10.95	24.9	0.44
11	21.3	3.65	3.7	0.99	12.25	31.55	0.39
12	22.65	3.7	3.45	1.07	12.1	33.85	0.36
13	21.65	3.3	3.45	0.96	11.55	33.25	0.35
14	22.1	4.45	3.9	1.14	13.1	32.45	0.40
15	22.5	3.95	3.5	1.13	12.15	33.25	0.37
Average	21.9	3.9	3.65	1.06	12.15	31.55	0.39
SD	0.69	0.39	0.17		0.72	3.4	
SD % of average	3.15	10.0	4.65		5.9	10.8	

^aAll results given in %, except ratios.

^bMINFA, minor fatty acids between C₁₄ and C₁₆, i.e., C₁₄:1, iso C₁₅, anteiso C₁₅, C₁₅:1, and iso C₁₆.

^cRatio = C₁₂/MINFA.

^dRatio = C₁₄/C₁₆.

impact the results. The content of C₄₀ to C₄₄ is practically identical for MF and for COCO; for example, one can be misled by 1% of lauric fat being taken for 1% of MF.

C₁₂. The SD% is too high to consider a determination of MF content. Palm oils have a small impact on the value, whereas lauric fats have a great impact. There is twelve times the amount of C₁₂ in COCO vs. MF (45.05:3.62).

MINFA. The SD% is reasonable. The use of this parameter appears suitable to directly determine the contents of MF. Only tallow can interfere; for example, 1% tallow in the chocolate would falsely calculate to 0.6% MF in the sample.

C₁₄/C₁₆. With an average ratio of 0.39 but a range from 0.32 to 0.44, a method based on this determination cannot be as precise as one based on triglycerides. Table 4 indicates that the other fats considered have all different ratios. Nevertheless, for hazelnut oil and the extender, whose C₁₄ contents are low, the incidence of these fats is almost nonexistent. For palm oil and especially for tallow, the influence can be more notable, and it is particularly strong for lauric fats with their high C₁₄ contents.

We can conclude from this discussion that the best way to proceed effectively is to measure the triad and the MINFA. Tables 5 and 6 present the results obtained by carrying out the same analyses on the 15:85 mixtures for the 22 previous MF samples and a standard CB. For the recalculation of MF contents, we did not use the percentages of C₄₀, C₄₄, C₁₂, etc., found in previous analyses, but redetermined them from the mixtures' analysis mean results. The condition of integration was different in this case because of the smaller size of the peaks. Therefore, the coefficient we used for C₄₀ to C₄₄ = 2.83/0.15 = 18.87%. In the same way, the coefficient for C₁₂

= 3.4%, and MINFA = 3.4%. For the ratio C₁₄/C₁₆, we used the formula of Hadorn and Zürcher (4):

$$\text{MF}\% = [(C_{14}/C_{16}) \cdot 100 - 0.225] / 0.40375 \quad [1]$$

Under normal conditions of determining an average MF content in a chocolate, the C₄₀ to C₄₄ triglyceride triad is confirmed to be the best adapted method. The necessary coefficient has been determined by carrying out one or several analyses of known mixtures under routine conditions. When there are no lauric fats, the Hadorn and Zürcher (4) method can be used as a secondary method, even if it gives results that are too high.

Detection of lauric fat in chocolates. According to Table 4, we can confirm that only pure, fractionated, and/or hydrogenated lauric fats have an influence on MF gross content determined by C₄₀ to C₄₄ triglycerides, and that the MINFA content is not changed. So, each time the ratio C₁₂/MINFA is higher than in MF, the presence of lauric fat may be suspected. For the MF samples analyzed, this ratio is on average 1.06, with a range from 0.8 to 1.24. In 15:85 mixtures MF/CB, the ratio is 0.99, with a range from 0.73 to 1.15, which is equivalent to pure MF.

To evaluate the repeatability and linearity of the ratio C₁₂/MINFA, we started with one MF and one standard CB and prepared five MF/CB mixtures from 9:91 to 21:79; this range covers the major types of chocolates and milk chocolates. The methyl esters were analyzed by GLC, and the 15:85 mixture was injected several times at the same concentration, and later at various concentrations (Table 7). We seized the opportunity with these injections to test the repeatability and the linearity of the Hadorn and Zürcher (4) calculation, based

TABLE 3
Analytical Results of 5 MF Stearins, 2 MF Oleins, Alone and in Combination with the 15 MF Samples of Table 2

	40-44	C ₁₂	MINFA	Ratio ^a	C ₁₄	C ₁₆	Ratio ^b
Stearins (n = 5)							
S1	21.3	3.75	3.3	1.14	11.9	31.2	0.38
S2	22.5	4.35	3.6	1.21	13.95	38.7	0.36
S3	22.45	4.05	3.65	1.11	12.45	33.3	0.37
S4	19.05	3.2	4.1	0.78	12.15	32.35	0.38
S5	22.3	3.7	3.55	1.04	12.75	37.7	0.34
Mean	21.5	3.9	3.65	1.06	12.65	34.65	0.37
SD	1.46	0.39	0.43		0.80	3.35	
SD% of mean	6.8	10.0	11.7		6.3	9.65	
1 to 15 MF from Table 2 + 5 stearins (n = 20)							
Mean	21.8	3.85	3.65	1.06	12.3	32.35	0.38
SD	0.91	0.39	0.20		0.75	3.6	
SD% of mean	4.2	10.1	5.4		6.1	11.1	
Oleins (n = 2)							
Ol.1	22.0	3.5	3.55	1.24	12.9	33.55	0.38
Ol.2	20.0	3.7	4.0	0.80	12.05	27.1	0.44
1 to 15 from Table 2 plus 5 stearins plus 2 oleins (n = 22)							
Mean	21.7	3.85	3.65	1.06	12.3	32.15	0.39
SD	0.95	0.38	0.20		0.73	3.6	
SD% of mean	4.4	9.9	5.8		5.9	11.2	

^aRatio = C₁₂/MINFA. Abbreviations as in Tables 1 and 2.

^bRatio = C₁₄/C₁₆.

TABLE 4
Examples of Compositions of Various Fats^a

	MF ^b	CB	COCO	PALM	TALLOW	Hazelnut	Extender
Fatty acids							
C ₁₂	3.62	0.01	45.05	0.65	0.09	trace	0.06
C ₁₄	11.24	0.11	17.80	1.54	2.80	0.22	0.67
C ₁₆	29.15	25.43	9.47	46.82	23.37	6.17	42.21
C ₁₄ /C ₁₆	0.39	<0.01	1.88	0.03	0.12	0.04	0.02
MINFA	3.58	0.03	0.04	0.07	0.18	0.02	0.04
Triglycerides							
C ₄₀ -C ₄₄	21.6	trace	22.7	trace	0.55	trace	<0.20

^aAccording to Precht (Ref. 10) and Pontillon (unpublished results).

^bMF, milk fat; CB, cocoa butter; COCO, coconut oil; PALM, palm oil. Other abbreviation as in Table 2.

on the ratio C₁₂/C₁₆; the results are in the last columns of Table 7. We confirm that this method presents acceptable repeatability and may be used for the whole range of mixtures studied. It also is valid for injection of concentrations from half to twice the normal concentration of 200 mg/mL.

The C₁₂/MINFA ratio is also constant enough to be used as a basis for the calculation of the possible correction. The advantage of using a ratio of percentages instead of percentages alone is that it is not necessary to have the entire chromatogram. By selecting conditions that neglect short-chain acids as well as those above C₂₀, we save time.

For the detection of a lauric fat adjunction, the following is pertinent. The average C₁₂/MINFA ratio for pure mixtures was around 1.0. The ratio limit of 1.0 cannot be used because 9 out of 22 of our mixtures would falsely seem to contain a little lauric fat. It is necessary to take the highest value of the range as a reference, which is 1.25. Thus qualitatively, any mixture of MF with other fats in the domain of

chocolate products is supposed to contain lauric fat if the ratio R = C₁₂/MINFA is higher than 1.25.

Estimation of the contents of lauric fat. Starting from R, the possible excess of lauric acid, EC₁₂, is given by:

$$EC_{12\%} = (R - 1.25) \cdot \text{MINFA} \quad [2]$$

The problem is to derive from this value the actual quantity of lauric fat. Kuksis (11) gives C₁₂ contents for raw coconut oil and palm kernel oil as 43.7 ± 3.1 and 46.4 ± 3.8, respectively. Rognon and Wuidart (12) give as values 39-54 and 44-51, respectively. For chocolate and coating products, it is preferable to refer to the compositions given for the most common lauric fats used in these industries, which are pure or lightly hydrogenated coconut oils, palm kernel stearins that are totally hydrogenated, and palm kernel oleins that are partially hydrogenated. Their respective average contents of C₁₂ are 48, 58, and 43%. When the analyst has no information concerning the quality of the lauric fat present, it is necessary to use

TABLE 5
Analytical Results for 15:85 Mixtures of Normal MF/CB, and Recalculation of MF Content from the Average Values^a

	40-44	C ₁₂	MINFA	Ratio ^b	C ₁₄	C ₁₆	Ratio ^c	% Milkfat calculated from			
								40-44	C ₁₂	MINFA	C ₁₄ /C ₁₆
1	2.90	0.52	0.47	1.11	1.67	26.5	6.30	15.35	15.3	13.8	14.85
2	2.88	0.57	0.51	1.12	1.79	26.85	6.67	15.3	16.75	15.0	15.15
3	2.84	0.49	0.54	0.91	1.69	27.2	6.21	15.05	14.4	15.9	14.1
4	2.81	0.50	0.50	1.00	1.60	25.95	6.17	14.9	14.7	14.7	14.0
5	2.86	0.44	0.44	1.00	1.65	26.3	6.27	15.15	12.95	12.95	14.2
6	2.69	0.55	0.55	1.00	1.72	26.75	6.43	14.3	16.2	16.2	14.6
7	2.76	0.53	0.56	0.95	1.85	27.9	6.63	14.6	15.6	16.45	15.05
8	2.78	0.40	0.50	0.80	1.52	24.5	6.20	14.75	11.75	14.7	14.05
9	2.74	0.46	0.51	0.90	1.97	28.6	6.89	14.5	13.55	15.0	15.7
10	2.64	0.41	0.56	0.73	1.65	25.9	6.37	14.0	12.05	16.45	14.45
11	2.78	0.55	0.53	1.04	1.80	24.9	7.23	14.75	16.2	15.6	16.5
12	2.87	0.52	0.44	1.18	1.95	27.95	6.98	15.2	15.3	12.95	15.9
13	2.83	0.56	0.53	1.06	1.93	28.2	7.06	15.0	16.45	15.6	16.1
14	2.96	0.50	0.52	0.96	1.75	25.85	6.77	15.7	14.7	15.3	15.4
15	3.05	0.61	0.53	1.15	1.87	27.45	6.81	16.15	17.95	15.6	15.5
Mean	2.83	0.51	0.51	0.99	1.76	26.7	6.60	15.0	14.9	15.1	15.05
SD	0.10	0.06	0.04		0.14	1.19		0.54	1.76	1.12	0.80
SD%	3.65	11.8	7.4		8.0	4.45		3.6	11.8	7.4	5.35

^aAbbreviations as in Tables 2 and 4.

^bRatio = C₁₂/MINFA.

^cRatio = (C₁₄/C₁₆) × 100.

TABLE 6
Analytical Results for 15:85 Mixtures of Five MF Stearins and Two MF Oleins with Cocoa Butter^a

	40-44	C ₁₂	MINFA	Ratio ^b	C ₁₄	C ₁₆	Ratio ^c	% Milk fat calculated from			
								40-44	C ₁₂	MINFA	C ₁₄ /C ₁₆
Stearins alone at 15:85 ratio of stearins to CB (n = 5)											
ST1	2.59	0.44	0.47	0.94	1.65	25.8	6.40	13.75	12.95	13.8	14.55
ST2	2.97	0.49	0.46	1.07	1.78	26.95	6.60	15.75	14.4	13.55	15.0
ST3	2.86	0.51	0.51	1.00	1.74	26.4	6.59	15.15	15.0	15.0	14.95
ST4	2.36	0.48	0.61	0.79	1.89	27.75	6.81	12.5	14.1	17.95	15.5
ST5	3.02	0.55	0.50	1.10	1.90	26.9	7.06	15.0	16.0	14.7	16.1
Average	2.76	0.49	0.51	0.98	1.79	26.75	6.69	14.65	14.55	15.0	15.2
SD	0.28	0.04	0.06	0.11	0.7	1.48	1.49	1.76	0.60		
SD%	10.1	8.15	11.7	5.85	2.7	10.1	8.2	11.7	3.9		
1 to 15 MF + 5 stearins, all at 15:85 ratio of fat to CB (n = 20)											
Average	2.81	0.50	0.51	0.99	1.77	26.75	6.62	14.9	14.8	15.05	15.1
SD	0.16	0.05	0.04	0.13	1.1	0.84	1.61	1.25	0.75		
SD%	5.6	10.9	8.3	7.35	4.0	5.6	10.9	8.35	4.95		
Oleins alone at 15:85 ratio of oleins to CB (n = 2)											
OL1	2.75	0.62	0.52	1.19	1.83	29.9	6.12	14.6	18.25	15.3	13.85
OL2	2.59	0.40	0.53	0.75	1.49	27.5	5.42	13.7	11.75	15.6	12.2
All MF, stearins, oleins at 15:85 ratio of fat to CB (n = 22)											
Average	2.80	0.50	0.51	0.99	1.76	26.9	6.55	14.85	14.85	15.1	14.9
SD	0.16	0.06	0.04	0.1	1.25	0.84	1.84	1.2	0.97		
SD%	5.65	12.3	7.95	7.9	4.6	5.65	12.35	7.95	6.5		

^aAbbreviations as in Tables 2 and 4.

^bRatio = C₁₂/MINFA.

^cRatio = (C₁₄/C₁₅) × 100.

the average value: 50%. So, when R > 1.25, the quantity of lauric fat (LF%) is given by:

$$LF\% = (R - 1.25) \cdot \text{MINFA} \cdot (100/50) \quad [3]$$

Correction of the gross content of MF in the presence of lauric fat. The MF content is calculated from triglycerides C₄₀ to C₄₄; so, in the presence of lauric fat, a correction has to be applied to these triglycerides, too. The three types of lauric fats mentioned above have an average content of C₄₀

TABLE 7
Effects of MF/CB Ratios and Injection Concentration on the Repeatability and Linearity of C_{12} /MINFA and C_{14}/C_{16} Ratios^a

Study variable	C_{12}	MINFA	Ratio ^b	C_{14}	C_{16}	Ratio ^c	Reference 4 method estimated values		
							MF% by ratio ^c	Diff. ^d	Diff.% ^e
MF/CB Ratio									
9.07–90.93	0.35	0.31	1.14	1.10	26.07	4.23	9.92	0.85	9.37
11.86–88.14	0.45	0.40	1.13	1.43	26.48	5.41	12.85	0.99	8.35
14.97–85.03	0.57	0.50	1.16	1.77	26.70	6.62	15.84	0.87	5.81
17.66–82.34	0.68	0.58	1.17	1.08	26.71	7.79	18.74	1.14	6.46
21.05–78.95	0.86	0.70	1.23	2.54	27.28	9.31	22.49	1.44	6.84
Repeatability									
14.97–85.03	0.56	0.48	1.16	1.74	26.38	6.58	15.73	0.76	5.08
17.66–82.34	0.57	0.31	1.14	1.79	26.83	6.67	15.95	0.98	6.55
21.05–78.95	0.60	0.51	1.17	1.87	27.22	6.79	16.25	1.28	8.55
Concentration (14.97–85.03 ratio)									
Double conc. (2C)	0.57	0.50	1.15	1.79	26.72	6.68	15.99	1.07	6.81
Normal conc. (C)	0.58	0.31	1.16	1.82	26.97	6.73	16.11	1.14	7.61
(C/2)	0.62	0.53	1.17	1.91	27.79	6.88	16.49	1.57	10.10
(C/3)	0.56	0.47	1.19	1.77	26.87	6.60	15.79	0.82	5.92

^aAbbreviations as in Tables 2 and 4.

^bRatio = C_{12} /MINFA.

^cRatio = $(C_{14}/C_{16}) \times 100$.

^dDifference = MF% estimated–actual.

^eDifference% = $100 \times (\text{estimated} - \text{actual}) / \text{actual}$.

to C_{44} —22.7, 26.7, and 21.9%, respectively, compared to 21.7% for MF.

The quantity, Q , to deduct from the gross content of MF is given by:

$$Q\% = EC12 \cdot (\text{coefficient from lauric acid to lauric fat}) \cdot (\text{coefficient from lauric fat to milk fat}) \quad [4]$$

If we go back to the average values for the three types of abovementioned lauric fats, $Q\%$ values are, respectively:

$$EC12\% \cdot (100/48) \cdot (28.7/21.7) = 2.18 \text{ EC12\%} \quad [5]$$

$$EC12\% \cdot (100/58) \cdot (26.7/21.7) = 2.12 \text{ EC12\%} \quad [6]$$

$$EC12\% \cdot (100/43) \cdot (21.9/21.7) = 2.35 \text{ EC12\%} \quad [7]$$

with an average of 2.2 (EC12%). Therefore, the final formula becomes

$$Q\% = 2.2 (R - 1.25) \text{ MINFA} \quad [8]$$

In conclusion, we recommend determining the triglyceride triad, C_{40} , C_{42} , and C_{44} , by direct gas chromatography of the fat extracted from chocolate, followed by determining the ratio between lauric acid (C_{12}) and the minor fatty acids between C_{14} and C_{16} via the analysis of its methyl esters of fatty acids. Using the proposed formulas, one can calculate the milk fat content of the chocolate—even when a LF is present. In addition, one can quantitate the amount of LF present. This last point is particularly interesting in light of the following situations: when there is broader acceptance of vegetable fats

in chocolate as reflected in the regulation, when the fat migrates from the biscuit or filling into the enrobed chocolate, or in the case of accidental mixing in pipes, conches, and enrobing machines.

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