Development of a Rapid Method for the Detection of Cocoa Butter Equivalents in Mixtures with Cocoa Butter

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A simple and rapid gas chromatographic (GC) method was developed for the detection of cocoa butter equivalents (CBEs) in cocoa butter (CB). It is based on the use of a 5 m nonpolar capillary column for the separation of the main triglycerides of CB according to their acyl/carbon numbers. The GC procedure was optimized to avoid thermal degradation of the triglycerides. By computing the ratio C54/C50 and (C54/C50) × C52 and by 2-dimensional plotting of these values, authentic CB samples were clearly distinguished from samples containing various CBEs. The detection of little as 1% CBE in CB (corresponding to about 0.3% CBE in chocolate) in a model system was shown to be possible. Under real conditions, for a wide range of CBs, about 2.5% CBEs in CB were detected. With this method, quantitation was possible at a concentration of 5% CBEs in CB mixtures, which corresponds to around 1% in chocolate; this value is far below the maximum level of 5% CBEs allowed to be added to chocolate.

The new European Chocolate Directive 2000/36/EEC (1) allows the addition of up to 5% of vegetable fats, the so-called cocoa butter equivalents (CBEs), other than cocoa butter (CB) in chocolate products. The fats to be used have been specified in the directive and are the following: palm oil, illipé (borneo tallow or tengkawang), sal, shea, kokum gurgi, and mango kernel. In addition to the mandatory labeling for added vegetable fats other than CB, labeling indicating that those fats have not been added is also allowed.

There is a perceived need within official control laboratories for precise methods to quantitate such vegetable fats in chocolate in order to implement the new directive.

The composition of CB and alternative fats as well as the analytical approaches for identification and determination were reviewed recently (2, 3). The chemical composition and physical properties of CBEs resemble those of CB very closely, and CBEs are mixable with CB in any proportion. Because the major triglycerides in CB and CBEs are the same, the detection and quantitation of added CBEs are difficult. The uncertainty in predicting the CBE level in chocolate is mainly caused by 2 factors. One factor is the large variety of CBs derived from different geographical locations all over the world that could lead to the presence of CBs of nonaverage composition; the other factor is the possible addition of illipé fat that has been shown to be the most difficult fat to detect/determine in chocolate (4, 5).

We recently reported the performance of 4 chromatographic methods in combination with multivariate statistical data analysis for the major components of fat in chocolate, i.e., triglycerides and fatty acids (6). Minor components (e.g., tocopherols, trienols, and sterenes), however, have been found to be of limited use for quantitative purposes, but could be additional indicators for the presence of other vegetable fats in chocolate (7, 8).

An extensive study of a large variety of the most suitable approaches revealed that the best method for quantitation should be based on the analysis of the major components, the triglycerides (4). Triglyceride profiling of CB/CBE mixtures by high resolution capillary gas chromatography (GC) has been shown to be the method of choice for the quantitation of CBEs in chocolate, resulting in an accuracy of approximately 2%, i.e., 2 g CBE/100 g chocolate, provided that the following assumptions are fulfilled: (1) the permitted level of CBE addition is 5 g/100 g chocolate, and (2) the fat content of the chocolate is about 20%. The value of 2% is the uncertainty of the worst-case scenario, i.e., samples including rather unusual soft and hard CBs and illipé-containing CBEs. If the data set is restricted to commercially used CB blends or to CBEs that contain no illipé fat, the maximum prediction error decreases to about 1% (4).

A certain benefit in terms of lower error of prediction can be achieved by combining several methods; however, it may be outweighed by the increased costs of the necessary analytical efforts. Recently a combined method was described (9), based on the identification of CBEs by analysis of sterol and triterpene alcohol degradation products formed during the processing of the CBEs (10, 11) and the determination of 3 main classes of triglycerides: C50, C52, and C54 (12).

In this study we describe a simplified and rapid approach for triglyceride determination that does not use triglyceride profiling; however, it involves the 3 triglyceride classes (C50,
Figure 1. Chromatogram of a commercial CB.

Figure 2. Plot of R1 and X1 values, showing different levels of CBEs added to a CB standard.
Figure 3. Plot of R1 and X1 values for various CBs and mixtures of CBs with CBEs at a concentration of 2.5%.

Figure 4. Plot of R1 and X1 values for various CBs and mixtures of CBs with CBEs at a concentration of 5%.
C52, and C54) as already obtained with developed methods applied in the past (12–20). However, the GC method described here was modified in such a way that a fast separation of the 3 groups was obtained at the lowest applicable temperature to avoid degradation. In addition, a new mathematical approach was applied to distinguish between CBEs and CBs and to make a quantitative assessment.

**Experimental**

**Reagents and Solutions**

CBs and CBEs were donated by commercial suppliers. Samples consisted of 12 CB commercial mixtures, 1 CB reference standard (also a commercial mixture, in the certification process), 5 pure vegetable fats [kokum, illipé, palm mid fraction (PMF), shea, and sal], and 4 commercial mixtures of CBEs from various producers.

Mixtures of the CB standard with CBEs (pure CBEs) at 1, 2.5, and 5% were prepared (corresponding to ca 0.3–1% CBEs in chocolate) to establish the detection limit. The 4 commercial CBE mixtures were tested at the 5% level only.

All samples were prepared at a 10% (w/v) concentration in isoctane, and the solutions were further diluted with isoctane to a mean concentration of 0.4 mg/mL.

**Gas Chromatography**

Triglyceride analysis was performed by using an HP 6890 gas chromatograph equipped with an on-column injector, autosampler, and flame ionization detector (Hewlett-Packard, Milano, Italy). HP Chemstation software (Hewlett-Packard) was used for data analysis.

A 5 m × 0.32 mm DB 1 column with a film thickness of 0.2 μm was used with hydrogen as the carrier gas at a linear speed of 2 m/s at a constant flow rate.

A volume of 1 μL was injected on-column at 110°C. The oven temperature was programmed to increase at 35°C/min to 330°C and then at 2°C/s to 345°C.

**Results and Discussion**

With this method, the triglyceride groups were eluted in <10 min, as shown by the gas chromatogram of a commercial CB (Figure 1). With the conditions described above, baseline separation is obtained. However, only the triglyceride classes C50, C52, C54, and C56 are separated instead of the individual triglycerides.

The ratio of the percentages of the triglyceride classes had already been used for the detection of CBEs in CB in developed triglyceride methods (11, 16–18), in which the sum of the C50, C52, and C54 triglycerides was set to 100%, and the percentage of C50 was plotted versus the percentage of C54. With this approach, the data points of authentic CB should follow a straight line relationship, whereas the addition of CBEs to pure CB should result in a deflection of the points from the line (12, 13). However, our recent study of a very large number of CBs, CBEs, and their mixtures, including a wide compositional variation of CBs, demonstrated that discrimination between authentic CBs and CB/CBE blends was not always possible (6). Therefore, another mathematical approach was developed in this study to improve the determination of the addition of CBEs to CB. Two different ratios of the triglyceride groups were established: the first, R1, is the ratio of the peak areas (%) of C54 and C50, i.e., C54/C50, and the second, X1, is derived by multiplying R1 by the peak area (%) of C52, i.e., X1 = (C54/C50) × C52. By computing the ratios R1 and X1 and by 2-dimensional plotting of these values, authentic CB samples were clearly separated from samples containing CBEs even if those mixtures contained low amounts of CBEs (1–5% in CBs, corresponding to around 0.3–1.2% in chocolate). This is demonstrated in Figure 2.

Because it is also important for control purposes to know the minimum amount of CBEs added to chocolate (in order to comply with possible labeling indicating the absence of CBEs), mixtures containing <0.5% CBEs in CBs were analyzed by this method. A standard CB (currently under certification) was chosen to represent a commercial CB mixture used by chocolate manufacturers.

It was found that the detection of as little as 1% CBE in CB (corresponding to about 0.3% CBE in chocolate) was possible. However, this is only achievable if the composition of the CB is known. In addition, the detection limits were observed to be different for the various CBEs. The addition of the very CB-like illipé fat was detectable at levels of 1%; for some other CBEs such as kokum or PMF, such levels were even lower. Further studies will investigate this effect in detail. Under real conditions, in analyses of a wide range of CBs of dif-

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**Table 1. X1 values obtained for 2 different injections of various fat types**

<table>
<thead>
<tr>
<th>Fat type</th>
<th>X1</th>
<th>X1 Re-injection</th>
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<tbody>
<tr>
<td>Illipé, 5%</td>
<td>99.17</td>
<td>99.88</td>
</tr>
<tr>
<td>Illipé, 2.5%</td>
<td>97.40</td>
<td>97.35</td>
</tr>
<tr>
<td>Illipé, 1%</td>
<td>96.45</td>
<td>96.50</td>
</tr>
<tr>
<td>Palm oil fraction, 5%</td>
<td>67.77</td>
<td>67.78</td>
</tr>
<tr>
<td>Palm oil fraction, 2.5%</td>
<td>76.86</td>
<td>76.81</td>
</tr>
<tr>
<td>Palm oil fraction, 1%</td>
<td>82.10</td>
<td>81.97</td>
</tr>
<tr>
<td>Shea, 5%</td>
<td>78.92</td>
<td>78.85</td>
</tr>
<tr>
<td>Shea, 2.5%</td>
<td>82.68</td>
<td>82.47</td>
</tr>
<tr>
<td>Shea, 1%</td>
<td>84.66</td>
<td>84.49</td>
</tr>
<tr>
<td>Sal, 5%</td>
<td>77.83</td>
<td>77.70</td>
</tr>
<tr>
<td>Sal, 2.5%</td>
<td>82.04</td>
<td>81.60</td>
</tr>
<tr>
<td>Sal, 1%</td>
<td>84.24</td>
<td>84.18</td>
</tr>
<tr>
<td>Kokum, 5%</td>
<td>93.97</td>
<td>93.30</td>
</tr>
<tr>
<td>Kokum, 2.5%</td>
<td>89.48</td>
<td>89.23</td>
</tr>
<tr>
<td>Kokum, 1%</td>
<td>87.37</td>
<td>87.00</td>
</tr>
<tr>
<td>CB standard 1</td>
<td>85.90</td>
<td>85.66</td>
</tr>
<tr>
<td>CB standard 2</td>
<td>85.86</td>
<td>85.83</td>
</tr>
<tr>
<td>CB standard 3</td>
<td>85.86</td>
<td>85.83</td>
</tr>
</tbody>
</table>
Different geographical origins, and therefore of different compositions that included illipé, about 2.5% CBEs in CB could be detected (Figure 3). Quantitation of the amount of CBEs is possible at concentrations of 5% CBEs in CB mixtures, which corresponds to around 1% of CBEs in chocolate (Figure 4). This value is far below the maximum level of 5% CBEs allowed to be added to chocolate. The ellipse indicating the area of all possible CBs in Figures 3 and 4 is an indicative area only (not based on statistical analysis).

The above conclusions are based on experiments with mean CBs (commercial CB mixtures used for chocolate production). In the presence of a CB with an extreme triglyceride composition (e.g., a CB derived from Brazil with a greater amount of C54), a shift towards the area of illipé could be expected and would make the differentiation more difficult.

Because the aim of this study was to obtain a robust method, the thermal degradation of triglycerides during the GC separation was evaluated by verifying the R1 ratio under various GC conditions: columns of various lengths, from 30 to 5 m, and different diameters (0.32 and 0.25 mm), with various stationary phases of different polarities. In addition, the gas flow rate was varied. The thermal degradation observed with the use of long columns and slow flow rates was demonstrated to be nonexistent when the method described here was used (short column, high gas flow rate, and hydrogen as the carrier gas). The ratio R1 was constant over many GC runs and over many days at about 1.93–1.94, whereas the ratio X1 was also stable between 84.5 and 85.5. Therefore, even the most difficult-to-detect CBE illipé fat was clearly separated from the CB group. The thermal degradation of illipé on long columns is assumed to be one of the reasons for its difficult detection in CBs when the triglyceride profiling methods are used.

Instrumental precision was measured by reinjection of the same sample on 2 different days and calculation of X1, which incorporates the sum of errors derived from measurement of the triglyceride groups C50, C52, and C54. Table 1 shows the values obtained for a series of CB/CBE mixtures that were found to be very similar, although not identical.

**Conclusions**

In-house validation of the method described here demonstrated that it is a robust and rapid alternative to other triglyceride methods based on triglyceride profiling. The use of short, nonpolar capillary columns to avoid the thermal degradation of CBs and CBEs, especially illipé fat, makes possible the detection of CBEs at a level of around 2.5% and their quantitation at a concentration of 5% in CBs, corresponding to concentrations of 0.5 and 1%, respectively, in chocolate. The method is therefore appropriate for assessment of compliance with the labeling requirements for CBEs and is ready for validation in a collaborative study.

**References**