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EU CHOCOLATE ANALYSIS PROJECT – CURRENT STATUS AND FUTURE WORK

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Introduction

Chocolate is one of the most popular foods all over the world. Its principle ingredients, cocoa mass and cocoa butter (CB), are obtained from cocoa beans. Triggered by the volatility in cocoa bean prices and poor quality of individual harvests, substitutes to cocoa products were sought, which led to the development of the so-called cocoa butter alternatives. They may be used for a partial or even total replacement of CB in confectionery and chocolate without impacting the functionality of the fat phase. To be of value for chocolate manufacturers, added vegetable fats must not interfere with the crystallization process, otherwise textural and visual defects (fat bloom) develop. Palm oil fractions and other vegetable fats of tropical origin (shea, sal, illipé, kokum) form the basis for manufacturing of cocoa butter equivalents (CBEs).

Analytical Methods

According to the Directive 2000/36/EC ("Chocolate Directive"), a number of specified vegetable fats other than CB may be added to a maximum of 5 % of the total weight of the finished product. If such CBEs are added during manufacturing of confectionery products, consumers have to be informed about their presence by appropriate labelling. Unfortunately, the Directive does not cover aspects regarding methods of analysis for law enforcement. Due to their similarity to genuine CB, CBEs are difficult to detect when added to chocolate and even more difficult to quantify. In order to provide methods to check for compliance with this threshold, the former Food Products Unit of the Institute for Health and Consumer Protection, JRC Ispra (now the Food Safety and Quality Unit, JRC Geel) co-ordinated an investigation into analytical methods to quantify the addition of other vegetable fats to chocolate. In collaboration with 6 partners from 5 EU Member States the following approaches were critically re-appraised[1]:

- triglyceride pattern analysis by high-resolution (high-temperature) capillary gas-liquid chromatography (GLC), by high-performance liquid chromatography (HPLC) coupled to an evaporative light scattering detector (ELSD) and by packed-column gas chromatography (so-called CAOBISCO method)

- fatty acid methyl ester pattern analysis and analysis of trans-fatty acids by GLC
- determination of trace elements by inductively coupled plasma mass spectrometry (ICP-MS)
- analysis of sterol and sterol degradation products by GLC
- determination of tocopherols (Vitamin E) and tocotrienols by HPLC
- pattern analysis of thermal degradation products by pyrolysis-mass spectrometry (Py-MS)
- melting behaviour by differential scanning calorimetry (DSC).

In addition, the potential of Fourier-transform infrared spectroscopy (FT-IR) and analysis of triglycerides by means of mass spectrometry (TD-MS-MS) were explored.

Results

A careful evaluation of all results revealed that only triglyceride pattern analysis by high-resolution (high-temperature) capillary gas chromatography seemed to be the most reliable approach for the quantification of CBEs in mixtures with CB. As a further outcome of the study, the urgent need for a certified reference material to validate methodologies for the quantification of the triglyceride profile of CB was recognised.

The idea was taken up and a feasibility study (intercomparison of methods) with 15 participating laboratories showed that[2]:

- HPLC and capillary GLC were equally efficient techniques for separating individual triglyceride fractions of CB
- Both techniques were equivalent in quantitative terms
- Calibration of the analytical system with a mixture of 5 major CB fractions (POP, POO, POS, SOS and SOO) was sufficient to achieve an acceptable agreement between results submitted by different laboratories using different analytical techniques (different column types, injection techniques, calibrants)
- The contents of the major triglyceride fractions of CB can be certified with sufficiently low values for uncertainty.

Reference Materials and principle of detection of CBEs

Based on the very encouraging outcome of the feasibility study a CB candidate reference material was developed by the EC-DG-JRC's Institute for Reference Materials and Measurements (IRMM) in co-operation with laboratories from industry, academia and food authorities. The CRM (IRMM-801) will be available very soon from the IRMM (Table 1).

For purity control purposes of CB, a method jointly developed by Padley and Timms[3], and by Fincke [4] in the late 1970s, has found wide application in industry and food control. However, appropriate validation data have not been made available up to now.

Table 1. Certified triglyceride fractions of IRMM-801

Triglyceride species		Mass fraction in g TG/100 g total TG	
		Certified value	Uncertainty
POP	1,3-dipalmitoyl-2-oleyl-glycerol	18.14	0.26
POS	1-palmitoyl-2-oleoyl-3-stearoyl-glycerol	44.68	0.30
POO	1,2-dioleoyl-3-palmitoyl-glycerol	2.26	0.16
SOS	1,3-distearoyl-2-oleoyl-glycerol	31.63	0.29
SOO	1,2-dioleoyl-3-stearoyl-glycerol	3.29	0.17

In co-operation with partner laboratories from industry, law enforcement agencies and academia, the JRC has developed and validated an integrated approach to detect CBEs in CB and confectionery products down to a level of 3 %, related to the fat phase without producing false-positive or false-negative results. The approach is based on the principles developed by Padley and Timms, but instead of the triglyceride fractions with acyl-C-numbers 50, 52 and54, the POP, POS and SOS fractions separated by capillary GLC are taken (Figure 1).

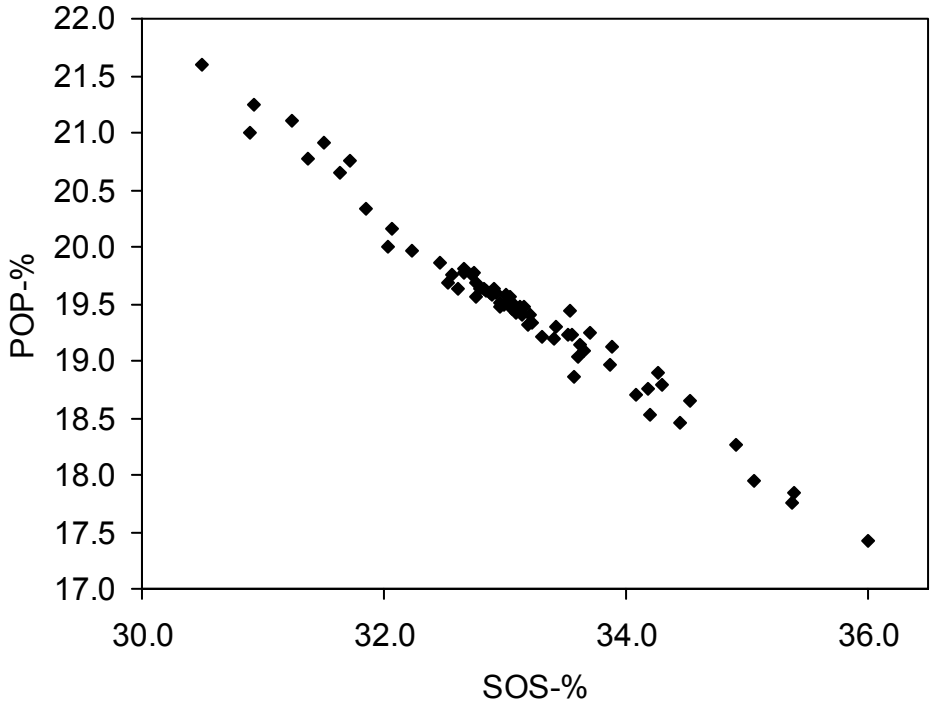

Figure 1. Modified Padley and Timms approach to detect CBEs in CB. The triglyceride profile was determined by high-resolution GLC using a 25 m CP-TAP capillary column.

The end user of the described approach has just to calibrate the GLC system using the certified reference material IRMM-801, separate the sample in question and submit the results to statistical analysis by a simple spreadsheet macro (MS Excel) to authenticate a CB sample. A comprehensive database covering the TG composition of a wide range of authentic CB as well as CBEs as used by the industry was used to elaborate the mathematical expression, which allows the user to determine whether CBEs are present with a given level of statistical confidence. By using the certified reference material for calibration purposes a traceability chain of the actual analysis to the database is established; this ensures excellent data comparability.

Furthermore, the JRC has developed and validated algorithms to enable a reliable quantification of CBEs in confectionery. The same analytical data set as used for the qualitative detection of CBEs was subjected to multivariate regression analysis and partial-least squares regression analysis. The deviations (bias) of the known from the values found by the participants of the validation study are given in Table 2. The accuracy of the method was estimated to be 0.5 % (absolute).

Table 2. CBE content in chocolate determined by partial least square regression. CB/CBE blends were used in the validation study and the results expressed for a chocolate with an assumed fat content of 30 %.

True value	Found by participants (overall mean and range of laboratory means)	Bias
4.44	4.54 (4.37-4.68)	-0.10
7.48	7.44 (7.20-7.62)	0.04
9.00	8.87 (8.57-9.01)	0.13
4.53	4.99 (4.89-5.06)	0.46
7.47	7.76 (7.54-7.93)	-0.29
9.00	9.21 (9.01-9.42)	-0.21
4.49	4.70 (4.55-4.84)	-0.21
7.49	7.25 (7.04-7.39)	0.24
8.99	8.56 (8.33-8.68)	0.43
Unknown sample	3.68 (3.35-3.96)	

Conclusion

To conclude, simple and reliable methodology is currently at hand to detect added CBEs. Due to its similarity illipé may pass undetected at low levels of addition. However, as illipé supplies are limited, substitution of larger amounts of CB by illipé is not regarded as an economic incentive. The accuracy of

quantification of CBEs in chocolate is dictated by the availability of additional information about the product. The more prior knowledge regarding the type and composition of the fats used for blend formulation is at hand, the more accurate is the estimate.

References

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