SCI LECTURE PAPERS SERIES

ANALYSIS OF VEGETABLE FATS

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ISSN 1353-114X
LPS 130/2003

Key words *chocolate, fats, CBE, analysis*

Introduction

The introduction of the Chocolate Directive 2000/36/EC has brought the need for a suitable method of analysis of non-cocoa fats (notably cocoa butter equivalents, CBE) in chocolate for manufacturing. There is a requirement for a methodology which can determine CBE in chocolate with suitable accuracy and precision at the 5% addition level. To understand the analytical approaches requires a knowledge of the composition of vegetable fats and the available methodologies.

The composition of oils and fats

Major components

The major components of all oils and fats are the triglycerides. These comprise a glycerol backbone esterified with three fatty acids. The major fatty acids in cocoa butter give triglycerides with total carbon numbers of C50, C52 and C54, which dominate the triglyceride profile. The triglyceride profiles of most CBE fats differ from that of cocoa butter making their analysis relevant to our requirement.

Minor components

Of the minor components of oils, sterols are the most useful in qualitative and quantitative analysis. They comprise desmethyl sterols, 4-methysterols and 4,4'-dimethysterols (Fig 1).

The 4-methysterols form an insignificant proportion of chocolate fats, and the desmethylsterols do not differ much in composition. Cocoa butter has a higher proportion of campesterol than CBE fats, and illipe a lower proportion than most other fats. Shea butter contains high quantities of 4,4'-dimethysterols which are useful indicators of this CBE fat.

Tocopherols are present in large quantities of palm and palm kernel fats but at inconsistent levels. They are substantially removed by refining. Refining also removes much of the sterol fraction by dehydration to steradienes (Fig 2).

Analytical methods
Analytical methods are based on physical determinations, chromatographic separations with varied detection systems, and mathematical treatment of data from each of these sources, individually or in combination.

Physical methods
Differential calorimetry (DSC) is widely used in the industry to study the melting behaviour of various polymorphic forms of cocoa butters. It has been used to distinguish between butters of different geographical origin but because of the similar melting properties of CB and CBEs it has been less useful in their differentiation. Infra-red spectroscopy over a range of wavelengths can show differences between fats but has not been applied successfully to cocoa butter.

Nuclear Magnetic Resonance techniques can compare the compositions of major and minor components of foods rapidly and without their separation. The methods are being applied more often to fats analysis but have yet to be proved in the cocoa butter field. Mass spectrometry is widely used as a sophisticated chromatography detection system but direct introduction of the pyrolysis products of foods is a useful technique. This has been used to differentiate between cocoa butters from different origins and applied to some examples of CB and CBE mixtures.

Chromatographic methods
The modern techniques of gas chromatography (GC) and high performance liquid chromatography (HPLC) are widely applied to the analysis of chocolate triglycerides. GC or combined HPLC-GC techniques are used for most minor component analyses.

Gas chromatography
Determination of the triglyceride, fatty acid and sterol composition is a classical technique the usefulness of which is being improved by developments in columns and introduction systems, and the building of larger databases for authentic oils and fats.

HPLC
HPLC gives similar or better separation of triglycerides with careful attention to the solvent system, and is the method of choice for the determination of tocopherols.

Mathematical treatments
Mathematical techniques are required for the processing of the large sets of data provided by virtually all of the spectroscopic methods. They can also be used to combine sets of data from several quite different types of analysis. Analysis of variance can determine and rank the effectiveness of individual parameters in a determination.

Applications

Steradiene determination
Steradienes can be measured with high sensitivity in the non-polar fraction of chocolate fat. Unlike CBE fats, cocoa butter is not bleached, therefore their presence in fat extracted from chocolate can confirm the addition of CBEs (Fig 3).

Triglyceride analysis
Triglyceride analysis by HPLC or more usually GC is the most useful method of determining the level of CBE in chocolate fat (Fig. 4).

Padley and Timms method
Plotting the proportions of C50 vs C54 triglycerides for cocoa butters gives a straight line of a limited length. Most CBE fats have different proportions of C50 or C54 and thus do not fall on the CB line. A line drawn from the position of a pure CBE fat through a CBE/CB mixture to the cocoa butter line can be used to calculate the percentage of that CBE in the mixture (Fig 5). Analysis of a range of pure CBEs (including fat mixtures but without CB) shows that they fall within a band, within which the location of the 100% point of an unknown CBE (X) can be estimated more closely.
These procedures are improved by knowledge of the identify of the CBE fat. This can be achieved in some cases by study of the composition of the glycerides or the steradienes using GCMS with canonical variant analysis of the resulting data.

Identification of CBE fats
Investigation of the steradiene composition of CBEs can be used to help identify the fat type and thus improve the performance of triglyceride based methods.

Difficulties and potential solutions
Some potential problems to be considered are the natural variations in the composition of CBEs and changes introduced by fractionation processes, and also the complexity of many chocolates. The reported methods have been applied mostly to simple chocolates without added components such as biscuit, nuts, milk, and coatings. These problems will be addressed by improvements to existing methodology (instrumentation and software), building databases of vegetable fat composition, by international testing and validation of methods, and by the use of standardised methods and analytical quality assurance procedures.

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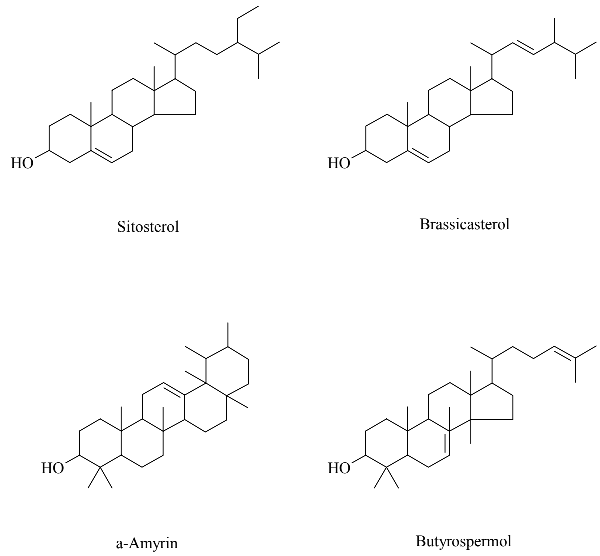

Figure 1: Sterols in CBE fats

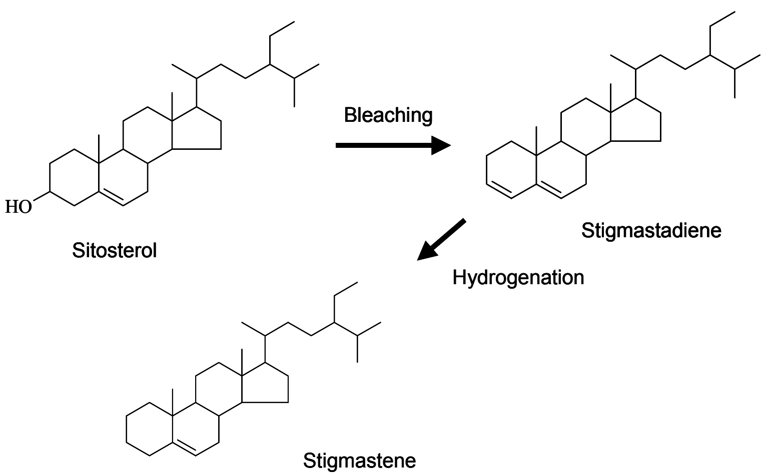

Figure2: Sterol degradation products

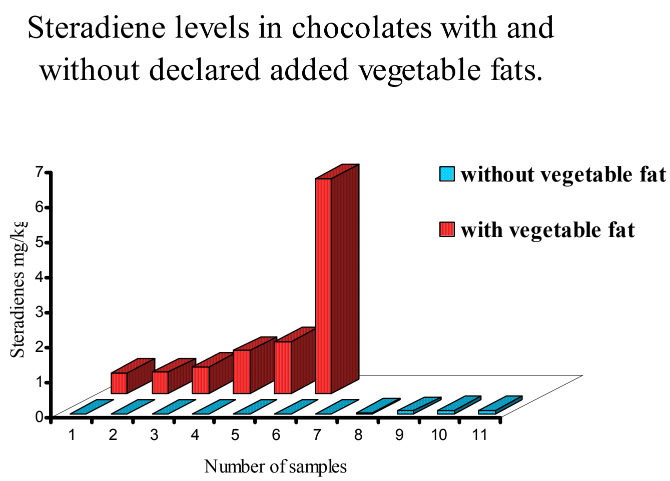

Figure 3: Detection of CBEs in chocolate

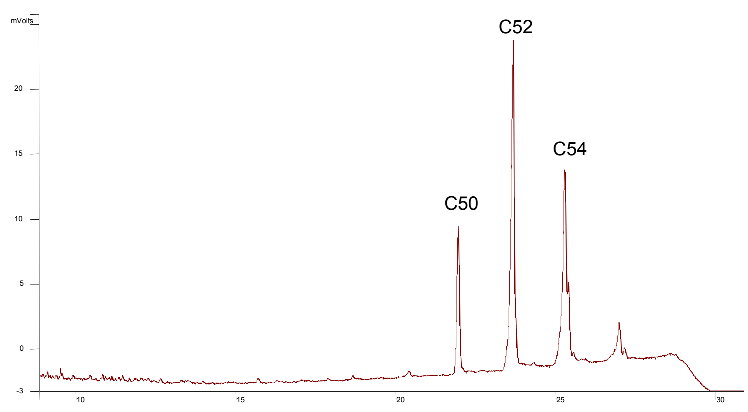

Figure 4: GC chromatogram of cocoa butter triglycerides

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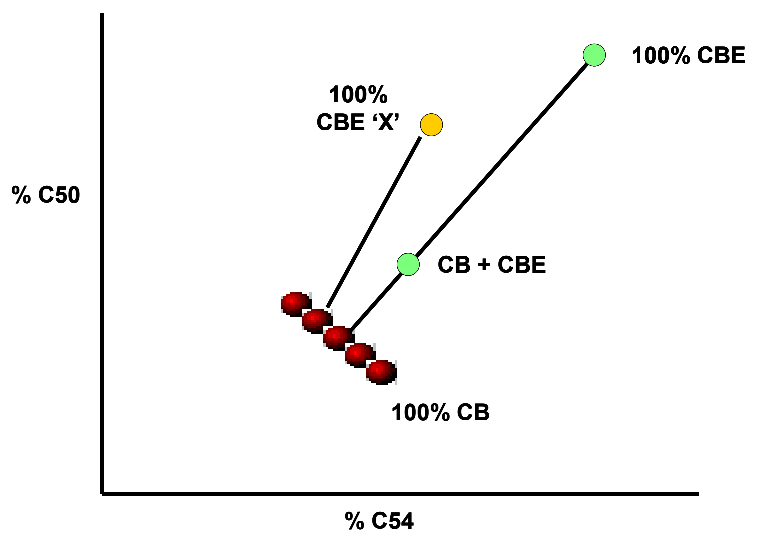

Figure 5: Quantification of CBE in chocolate (Padley & Timms)