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PHYSICAL PROPERTIES  
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## ANALYSIS

### Quality control of cocoa butter equivalents using differential scanning calorimetry

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#### Summary

*Quality control of cocoa butter equivalents (CBE) using the Jensen cooling curve is labour-intensive and operator-specific, while measurement of the melting properties of CBE by pulsed nuclear magnetic resonance (pNMR) takes two days to obtain a result. Therefore, differential scanning calorimetry (DSC) has been examined as an alternative method. The analysis of CBE using DSC takes less than 1 hour and it is less labour-intensive than current methods. A comparison with Jensen and pNMR shows that DSC has all the attributes necessary for a fast, accurate and discriminatory tool for quality control of CBE.*

#### Introduction

In August 2003, the European Union regulations covering the composition of chocolate changed. For the first time the use of vegetable fats (CBE) was permitted in all member states. However, there were a number of restrictions placed on this in the regulations. One of these constraints was on the physicochemical properties of the vegetable fat. The CBE used must be 'miscible and compatible' with cocoa butter in terms of its physical properties — both crystallization and melting (1).

Traditionally, the crystallization properties of CBE have been measured and monitored for quality control purposes by means of the Jensen cooling curve. This is a very specific method involving the measurement of temperature of the fat as it cools in a controlled way. The analysis is not only very labour-intensive but also subject to a degree of operator specificity.

The melting properties of CBE have been measured by means of pulsed nuclear magnetic resonance (pNMR) using method 2.150(b) of the International Union of Pure and Applied Chemistry (IUPAC). This involves a lengthy 40-hour stabilization at 26°C.

One method, therefore, is labour-intensive and operator-specific, while the other needs about two days to get a result. For these reasons we have begun to investigate the possibilities of using differential scanning calorimetry (DSC) as an alternative method. DSC is an instrumental method that measures the electrical power required to keep the temperature of a sample equal to that of an air reference. This power expressed as heat flow is then plotted against sample temperature. DSC has the advantages of being able to measure both crystallization and melting characteristics in the one instrument, is quick and is not subject to the same operator errors as are found with the Jensen cooling curve method.

**Table 1. Main contributors to principal components in analysis of cocoa butter equivalents.**

Evaluation(s)	PC 1	PC 2	PC 3
pNMR, Jensen	Jensen $T_{max}$ N30, N32.5	Jensen $t_{max}$ N20	Jensen $t_{max}$ N20, N25
DSC	C <sub>1</sub> PT, C <sub>1</sub> PO, C <sub>2</sub> PT, HPT	C <sub>1</sub> PH, CA, HA	C <sub>1</sub> PH, C <sub>2</sub> PH
PC 1 = principal component 1, etc. $T_{max}$ is the maximum temperature of the Jensen curve and $t_{max}$ is the time taken to reach that temperature. For a description of DSC abbreviations, see the text.			

Within the constraints imposed on CBE formulation there is scope for defining a range of distinctly different CBE compositions. Jensen cooling curve and stabilized pNMR distinguish between these types. The questions which this work addresses are:

- does DSC correlate with the existing methods?
- does DSC discriminate between different types of CBE as effectively as do the existing methods?

It has been shown (2) from previous work that DSC gives reproducibly distinct curve shapes and peak positions for each type of CBE. Not only that but different levels of minor components such as diacylglycerols and trisaturated triacylglycerols change the curve shape and peak position in reproducible ways. This is important because these minor components can have a significant effect on the physical performance of a CBE in chocolate.

### Statistical methods

To answer the two questions posed earlier we need to use a range of statistical discriminant and correlation techniques. The ones which have been used in this study are discriminant analysis (DA), principal component analysis (PCA) and stepwise multiple linear regression analysis (SMLR).

Discriminant analysis indicates how well the quality control evaluations separate different fat types. It plots each sample in  $n$ -dimensional space where  $n$  is the number of parameters (each N-value, Jensen parameter etc) describing a sample. Having plotted all the samples, DA then considers each individual sample and attempts to assign it one particular group (i.e. CBE type) by calculating the distance to each group and identifying the closest group. The degree to which it is successful in classifying samples into the correct group is a measure of the discriminating power of the evaluation(s) concerned.

In principal component analysis the evaluation parameters are combined to give 'principal components' (PC). These are calculated such that the first PC is the single best parameter for showing differences between the samples, and so on. Each PC

is a linear combination of the original parameters. The first three PC would ideally separate samples in the plot better than any three of the original variables. Plotting each sample against the first three PC can help in visualising the data, as it is considered by DA.

Stepwise multiple linear regression analysis attempts to formulate an equation relating, in this case, DSC parameters to those from pNMR and Jensen cooling curve. The relationship between the DSC parameter which most closely correlates with a particular Jensen or pNMR parameter is defined in the form  $y = m_1x_1 + c$ . Next, the DSC parameter that best correlates with the remaining differences between the actual and predicted values is defined to give the best two-parameter equation  $y = m_1x_1 + m_2x_2 + c$ . These steps are repeated until all the parameters are involved.

### Methodology

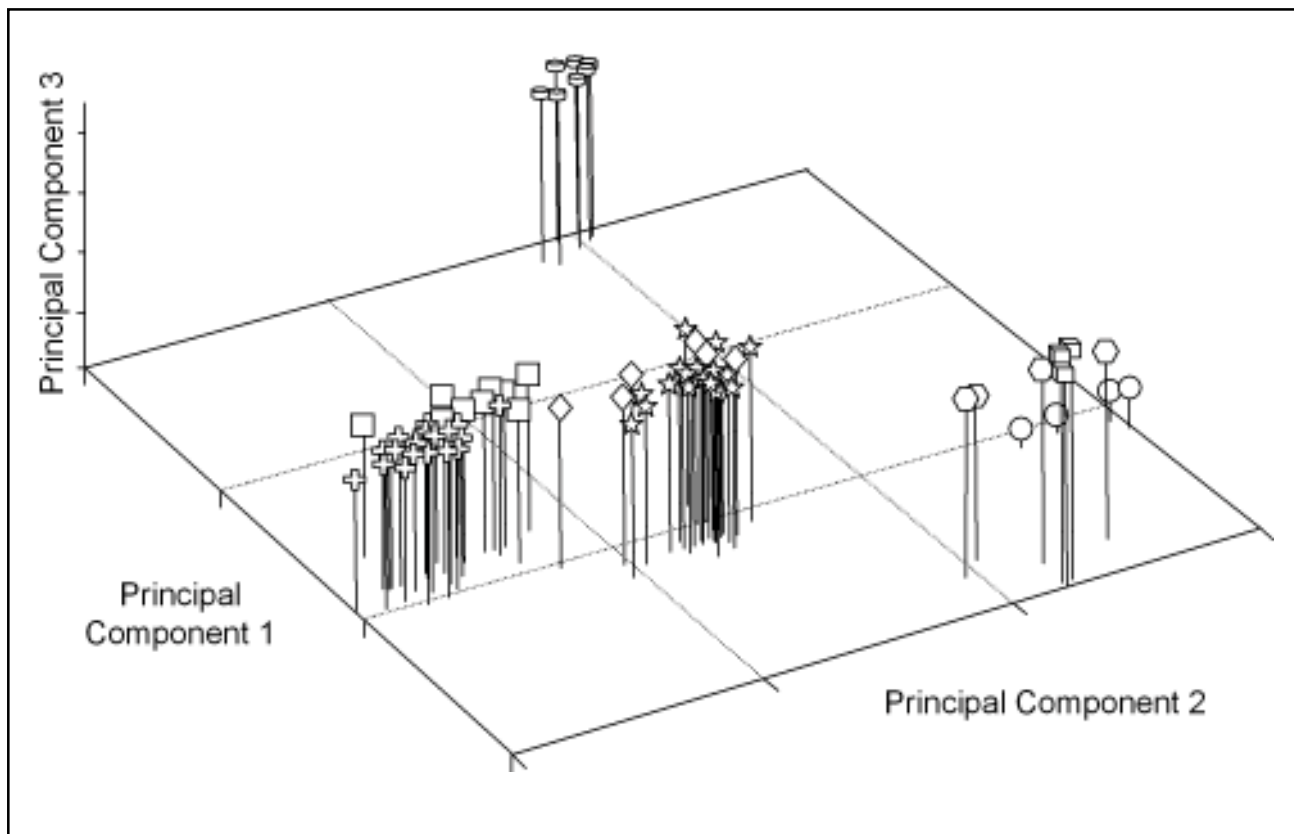
Two groups of CBE were evaluated. The first group contained a range of 69 samples of four different types of CBE produced by Loders Croklaan and a further 18 samples of fractions of palm, shea and sal oils used in some of the CBE.

The second group comprised 103 samples of the same four types of CBE produced by Loders Croklaan. This group of samples did not contain any of the basic CBE components.

The pNMR data measured on these samples consisted of the solid fat contents measured at 20, 25, 30, 32.5 and 35°C following the IUPAC 2.150(b) method. These measurements were denoted as N20, N25, N30, N32.5 and N35 respectively.

The Jensen cooling curve parameters used were the minimum temperature achieved ( $T_{min}$ ), the maximum temperature following this ( $T_{max}$ ) and the time ( $t_{max}$ ) taken in minutes to reach this maximum from the starting point of 32°C.

The DSC parameters used were the peak position, height and onset for the first two peaks in the cooling trace (C<sub>1</sub>PT, C<sub>1</sub>PH, C<sub>1</sub>PO, C<sub>2</sub>PT, C<sub>2</sub>PH and C<sub>2</sub>PO, respectively) and the major peak in the heating trace



**Figure 1. Principal component plot using data from DSC. Square: CBE-1, cross: CBE-2, star: CBE-3, diamond: CBE-4, cube: palm fraction A, hexagon: palm fraction B, circle: sal fraction, cylinder: shea fraction. The plot shows that all of the CBE fall within one area of 3-D space with CBE-1 and CBE-2 being close together but clearly separated and CBE-3 and CBE-4 also being close together but, in general, separated. The four components also form four distinct groupings with, not surprisingly, the two types of palm fraction being close together.**

(HPT, HPH and HPO). The overall peak areas for each trace (CA and HA) were also used.

The four types of CBE studied (CBE-1, CBE-2, CBE-3 and CBE-4) differed mainly in the ratios of palm fraction to shea or sal fraction. CBE-1 contained the least palm fraction; CBE-4 contained the most palm fraction. Each has a specification describing the minimum and maximum values of each pNMR and Jensen cooling curve parameter. In some cases, the minimum values of one CBE may be close to, or even overlap, the maximum values of the next CBE. This is an important point to bear in mind, particularly with regard to discriminant analysis.

Discriminant analysis and principal component analysis were performed on the first data set only, i.e. the data set containing both CBE and components. Stepwise multiple linear regression was carried out on the second data set containing only CBE.

### Discriminant analysis results

Two groups of evaluations were considered: (i) pNMR and Jensen together (i.e. the conventional means of QC) and (ii) DSC alone. DA defines whether a CBE of one group has characteristics appropriate to

that group or whether it has been misclassified and, statistically, belongs to another group.

All the misclassifications, of which there were only a few, were between CBE-3 and CBE-4. The conventional quality control method of Jensen cooling curve and pNMR gave a total of 6 misclassifications out of 87 samples. DSC alone, however, gave only 2 misclassifications showing that this is certainly no worse than current quality control tests and could actually be better in terms of identifying product groupings.

### Principal component analysis results

The data were again evaluated in the same two groups of physical methods. The parameters which contribute most to the first three principal components are shown in **Table 1**. All the parameters listed have approximately equal contributions. The positions of each sample in relation to the first three principal components for the DSC data are shown in **Figure 1**. This graph shows very well the relative positions of each of the product groups. It gives a good visual confirmation of the separation of the groups as determined by DA.

**Table 2. Parameters found for models fitted using stepwise multiple linear regression, relating DSC to traditional methods for CBE-1.**

	Fitted parameters <sup>a</sup>	95% Limits <sup>b</sup>
<b>pNMR Parameter</b>		
Solid at 20°C	C <sub>1</sub> PT, C <sub>2</sub> PT, C <sub>2</sub> PH, CA	±2.5
Solid at 25°C	C <sub>1</sub> PT, C <sub>1</sub> PO, C <sub>1</sub> PH, C <sub>2</sub> PT	±2.3
Solid at 30°C	C <sub>1</sub> PT, C <sub>2</sub> PO, C <sub>2</sub> PH, CA	±3.4
Solid at 32.5°C	C <sub>1</sub> PT, C <sub>1</sub> PO, C <sub>2</sub> PT, C <sub>2</sub> PO	±2.2
Solid at 35°C	C <sub>1</sub> PT, C <sub>1</sub> PO, C <sub>2</sub> PT, CA	±1.0
<b>Jensen Parameter<sup>c</sup></b>		
T <sub>min</sub>	C <sub>1</sub> PT, C <sub>1</sub> PO, C <sub>1</sub> PH, CA	±0.56
T <sub>max</sub>	C <sub>2</sub> PT, C <sub>2</sub> PO, C <sub>2</sub> PH, CA	±0.71
t <sub>max</sub>	C <sub>1</sub> PT, C <sub>2</sub> PT, C <sub>2</sub> PH, CA	±7.1
<sup>a</sup> For a description of DSC abbreviations, see the text. <sup>b</sup> 95% of the calculated values will fall within the quoted figure of the actual value of the measurement. <sup>c</sup> T <sub>min</sub> is the minimum temperature of the Jensen curve, T <sub>max</sub> is the maximum temperature of the Jensen curve and t <sub>max</sub> is the time taken to reach that temperature.		

In the evaluation of DSC data the peak heights and temperatures of the first two peaks in the cooling trace and the onset of the first peak are particularly important parameters. Previous work (2) has shown that the relative peak positions vary with CBE type and that the levels of diacylglycerols and trisaturated triacylglycerols affect the width (i.e. the difference between peak onset and peak position) of the first cooling peak. This agrees well with the data analysed in this study.

### Stepwise multiple linear regression results

SMLR was carried out only on the second data set (CBE only). Although the best results were obtained using both DSC cooling and heating trace parameters, the results obtained using the cooling data alone were only slightly worse. This would suggest that for CBE at least, only the DSC cooling curve would be needed for quality control analysis. In general a maximum of four DSC parameters were all that was needed to get a good correlation. Most correlations were significant at 99% or greater.

As an example, the data fitted to the DSC cooling parameters for CBE-1 are shown in **Table 2**. Models fitted for the other CBE were similar. In this table, the 95% limits are shown. In most cases these limits are not too far away from the standard deviation of the measurements themselves. Indeed, much of the difference between the calculated and actual values could be due to errors present in the Jensen and pNMR

measurements themselves. All of this indicates that DSC is certainly no worse than the current quality control methods.

There is also a good deal of logic between which parameters are important in the two types of cooling curve—Jensen and DSC. In calculating the Jensen T<sub>min</sub>, parameters from the first cooling peak are the most important. This is logical since it is the early stages of crystallization which define the positions of both T<sub>min</sub> and the first DSC cooling peak. Similarly, the Jensen T<sub>max</sub> value is best described using the second DSC cooling peak. This too is logical since both of these parameters are defined by the period of maximum crystallization of the CBE. The Jensen time (t<sub>max</sub>) is defined by both first and second cooling peak

DSC parameters. Again, this is logical since the Jensen time covers both the initial and main crystallization times of the CBE.

### Conclusions

What conclusions can we then draw from this work?

- In terms of its ability to discriminate between different types of CBE, DSC is certainly no worse and, arguably, may be better than Jensen and pNMR.
- It is sufficient to measure the DSC cooling trace to have a good measure of the quality of CBE (and possibly even of CBE components)
- It is possible to predict Jensen and pNMR parameters from the DSC cooling parameters — to within an error which would be expected from the standard deviation of the Jensen and pNMR measurements.
- The analysis time of DSC — less than 1 hour for CBE — is much less than that needed for the current methods. The DSC method is also less labour-intensive than current methods.

Therefore, taking everything into account, DSC has all the attributes necessary for a fast, accurate and discriminatory tool for quality control of CBE.

### References

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