# An isothermal calorimetric study of cocoa butter polymorphism

**Instruments to which this note applies**: Biocal 2000, I-Cal Flex/Ultra

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**Target use**: Food science, crystallization of food ingredients (in this case, cocoa butter)

## Introduction

The quality of chocolate is mainly a function of its cocoa butter content. Solidifying the cocoa butter into the desired polymorph (crystal form) gives a glossy and firm chocolate with desired characteristics. In past studies on cocoa butter crystallization, various techniques such as differential scanning calorimetry (DSC) and X-ray diffraction (XRD) have been used. In this work we use of a combination of isothermal calorimetry and DSC. With the isothermal calorimetry we followed the kinetics of the slow recrystallization of pure cocoa butter, and with the DSC we could at different times assess which crystal forms we had (DSC results are not further discussed here).

Cocoa butter and, in turn chocolate, is made from the fat obtained from the kernels in the fruit of the Cocoa tree, *Theobroma cacao*. The raw cocoa beans are fermented and dried before arrival at the factory. On arrival, they are cleaned, mixed into a desired blend, fragmented and stripped of their husks. The inner kernels or nibs are roasted and ground into a liquid cocoa mass from which the fat, cocoa butter, is pressed out under high pressure. In order to remove any solid cocoa remnants, the butter is finally filtered.

The main constituents of fats are triacylglycerols (TAGs). TAGs are esters of glycerol and fatty acids. This consists of a glycerol backbone to which three fatty acids are esterified. Cocoa butter mainly consists of a homogeneous blend of palmitic acid (P); stearic acid (St) and oleic acid (O). The three dominating TAGs are POP, POSt and StOSt, which comprise 80% of the TAGs in cocoa butter.

Cocoa butter has a complex crystallization behavior and six identified polymorphs (crystal forms, Table 1). Each of these polymorphs have characteristic melting points and distinct crystal patterns associated with them. To manufacture good quality chocolate, it is essential to induce the formation and stabilization of the $\beta_2$ polymorph, and this requires controlled crystallization during production. Under-tempered chocolate shows a characteristic $\beta_1'$ polymorph and when the crystals transform from $\beta_1'$ to $\beta_2'$, a transformation polymorph $\beta_1$ occurs, which is associated with fat bloom. The metastable $\gamma$ and $\alpha$ phases and the more stable $\beta'$ phases can crystallize directly from the melt, but the desired $\beta_2$ form can only be obtained by phase transforming the $\beta'$ form.

*Table 1 - Cocoa butter polymorphs and their approximate melting temperatures [2].*

| Cocoa butter polymorph | | Melting temperature / °C |
|---|---|---|
| **Lipid science nomenclature** | **Chocolate industry nomenclature** | |
| $\beta_1$ | I | 34-36 |
| $\beta_2$ | II | 32-34 |
| $\beta_1'$ | III | 26-28 |
| $\beta_2'$ | IV | 24-26 |
| $\alpha$ | V | 22-24 |
| $\gamma$ | VI | 16-18 |

## Materials and methods

*Cocoa butter*

Three kilograms cocoa butter from Bühler AG was melted in an oven at 80 °C and was held at that temperature for 15 min to homogenize it and erase crystal memory [3]. The butter was then kept agitated by a Sorval Omni-Mixer (Omni International, Georgia, United States) while 10 g samples were weighed into 125 ml calorimetric plastic vials and immediately placed into 4 °C for cooling.

*Seeding*

Cocoa butter was recrystallized by dissolving it in pentane and rapidly cooling it to -20 °C (with a calcium chloride coolant) to create small $\beta_1$ crystals. The sample was shaken at intervals during crystallization. Once precipitated, the sample was filtered in a cold room and the crystals were dried in a fume hood to evaporate the pentane. The sample was then bottled and stored at 4 °C. The melting point of the seeds were measured by DSC to ensure they were in the required

polymorphic form ($\beta_1$ or $\beta_2$, both these forms will induce $\beta_2$ crystallization in the cocoa butter).

The calcium chloride coolant was prepared by dissolving, 77.6 g of calcium chloride (Sigma) in 150 ml of water to make a saturated solution of calcium chloride. This solution was frozen at -20 °C. On freezing, it formed a gel-like liquid which was used as a coolant for seeding.

*Isothermal calorimetry*

An isothermal calorimeter (BioCal 2000) was used to follow the crystallization. This instrument uses 125 ml polyethylene vials. The mass of cocoa butter used in each measurement was 10 g and the measurements were made at 26 °C. For the isothermal measurements, a homogenized sample was melted in an oven at 80 °C and was held at that temperature for 15 min. The sample was then cooled on a shaker plate at 300 rpm at 4 K/min to 29 °C. 0.1 g of the created seeds were added to the agitated samples and further cooled to 26 °C before loading the sample in the isothermal calorimeter at 26 °C.

*Fig. 1 - Visually similar grainy texture in two samples after homogenizing and subjecting it to the isothermal experiment at 26 °C with seeding*

**Results and discussion**

The cocoa butter was semi-solid at the time it was placed in the calorimeter, but clearly re-crystallized during the 20 h measurement, and was solid at the end of the measurement. The seeded and homogenized samples yielded reproducible results in the isothermal calorimetry measurements (measurements on non-homogenized or non-seeded samples were not reproducible and results of such measurements are not shown here). The isothermal calorimetric results showed a clear two-stage process (Fig. 2), with one process peaking after 2-3 h, while the main peak comes after about 6.5 h. These test results can be sued in a number of ways, such as to the modeling of complex crystallization behavior, using for example, the two-stage model by Foubert et al. [3].

**Conclusions**

Isothermal calorimetry is a convenient and useful technique to follow long term crystallization processes in chocolate products. Once a suitable protocol has been established, the test procedure, sample preparation and calorimetry test itself are uncomplicated to perfrom. The measurements in this application note were done on a 24-hour timescale, but it is possible to continue measuring for longer times, given the high thermal powers from the crystallization reaction, even for small samples.

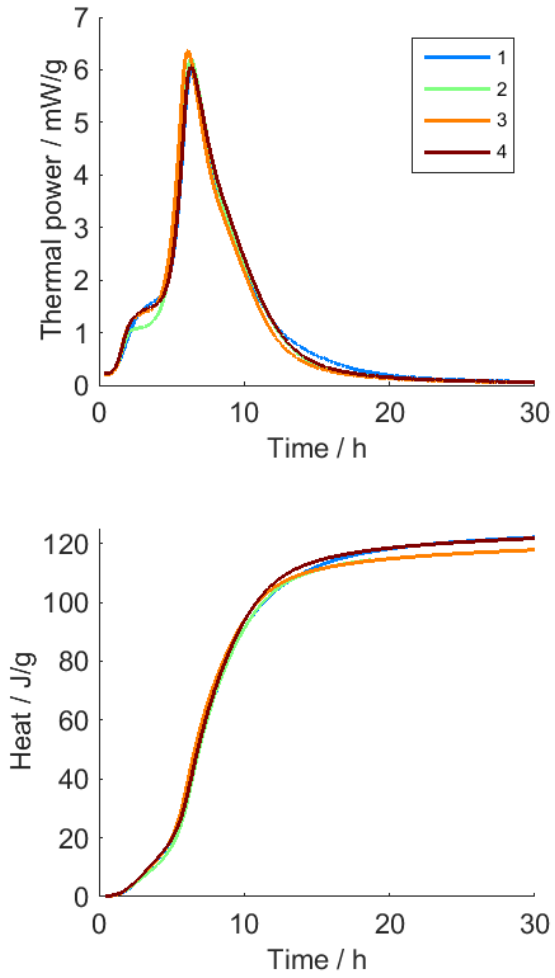


*Fig. 2 - Examples of highly repeatable thermal power (left) and heat (right) results for four replicate samples.*

**References**

1. Shetty, R.S., *Isothermal calorimetric studies of cocoa butter polymorphism*, in *Department of Food Technology and Nutrition*2014, Lund University, Sweden.
2. Tannenbaum, G., Chocolate: a marvelous natural product of chemistry. J. Chem. Educ., 2004 81 8 1131-1135.
3. Foubert, I., K. Dewettinck, and P.A. Vanrolleghem, Modelling of the crystallization kinetics of fats. Trends Food Sci. Technol., 2003 14 79-92.