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Using Differential Scanning Calorimetry To Explore the Phase Behavior of Chocolate

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Ⓢ *Supporting Information*

ABSTRACT: Chocolate is certainly one of the most familiar and commercially successful products made from plant resources. This success has been built on many decades of careful optimization and innovation in the procedures used to transform the complex mixture of chemical substances extracted from cocoa beans into commercial products. The favorable public image of chocolate has led to it being used both as a captivating aid to teaching chemistry and a convenient source of "real-world" samples for analytical chemistry classes. The objective of this experiment is to explore the melting behavior of different types of chocolate using differential scanning calorimetry. Students change the distribution of cocoa butter polymorphs by subjecting samples to specific thermal treatments. Interpretation of the thermograms of these samples helps students to understand the complexity and importance that low-melting phases have in real-world applications in cosmetics, pharmaceutics, and the food industry.

KEYWORDS: *Upper-Division Undergraduate, Analytical Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Instrumental Methods, Thermal Analysis, Food Science*

■ INTRODUCTION

Chocolate in various forms, as a wrapped confectionary tablet, as powdered flavouring, decoration or even as a drink, is certainly destined to continue its long history of commercial success. The description of the harvesting, preparation, and processing of the cocoa bean; the extraction of cocoa butter and the quite complex chemistry through which characteristic flavours are developed have been previously described in this *Journal*.[1] The popularity and commercial importance of chocolate have inspired the characterization of this material in Chemistry classes, using a variety of techniques.[1−3] More than 100 years of innovation in chocolate products has by no means exhausted the possibilities for improving production methods and product formulation. Not only are novel or more consistent commercial products being developed, but new ways of characterizing active components are continually being researched.[4−7]

In this experiment, Differential Scanning Calorimetry is used to evaluate the behavior of samples from different chocolate products and to demonstrate that differences in the source of raw materials, or the manufacturing process, are detectable using thermal analysis. Students gain practical experience with the instrument and apply specific software to extract relevant data from thermograms. The choice of chocolate as a sample provides students with the opportunity to observe the effect of sample storage on the thermograms acquired after different thermal treatments, as cocoa butter crystallizes and is transformed between various polymorphic forms. The annealing process applied by students causes a redistribution of phases that alters the physical and thermal properties of the sample.

The study of samples of chocolate provide students with a motivating "real-world" contact and introduces them to commercially important commodities where Natural Product Chemistry and Food Chemistry intersect.

■ THE FAT COMPONENT OF CHOCOLATE

Polymorphism is the ability of certain substances to exist in chemically identical but structurally different forms. The study of polymorphism is important because many different industrial products, including pharmaceuticals, agrochemicals, dyes, explosives, and food components, have been found to have one polymorph that has physical or chemical properties that are in some way better than the other(s). For commercial reasons, it is often important to produce the polymorph with the most desirable properties in highest possible yield. The study of cocoa butter is appropriate because it provides one of the most complex examples of polymorphism, and also one of the industrially most relevant systems.

In Food Science, the crystallization of edible fats has been extensively investigated. This research interest originates in the critical contribution of these fats in both "mouthfeel" and physical characteristics of a range of food products that include dairy products (butter, creams, and yoghurts), vegetable fat spreads (margarine and cheese), confectionery fillings and coatings, and, of course, chocolates.

The composition of cocoa butter is usually referred to in terms of fatty acids that include stearic, palmitic, oleic, and linoleic acids. These molecules are present as triglycerides (or triacylglycerols) and their incidence depends on the geographic origin of the cocoa beans.[3] The acyl components can be combined in various ways, and as the fat molecule packing is characteristic, they crystallize into different structures and melt at different temperatures. Cocoa butter has been extensively studied by chemists from both the oils and fat community and from the chocolate industry. These specialist communities have discussed the behavior of fat polymorphs using different

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designations, the oil chemists favoring the designation of polymorphs by greek letters, while chocolate industry researchers prefer to use roman numerals. Table 1 combines the designations used by these two industrial sectors and indicates the approximate temperature range of stability of the fat polymorphs in cocoa butter. The temperature ranges indicated in this table are not universally accepted. Phase stability limits in systems containing low-melting fats are very sensitive to the technique used for characterization, the origin and purity of the sample, and the nature of the impurities present.

Table 1. Temperature Stability Ranges for the Six Different Polymorphic Forms of Cocoa butter[a]

Form	Melting Range (°C)	Conditions of Formation of Polymorph
I or γ	−8 to 18	rapid cooling of melt
II or α	17−24	rapid cooling of melt and storage at 0 °C
III or β_1'	20−27	cooling and storage of melt at 5−10 °C
IV or β_2'	25−28	cooling and storage of melt at 16−21 °C
V or β_2	29−34	cooling and storage of melt at 30 °C
VI or β_1	34−36	several months of storage of melt at 30 °C

[a]Temperatures ranges show estimated phase stability range for cocoa butters with different authors and geographical provenance.[2]

Cocoa beans are harvested, fermented, dried, and roasted to permit the formation of specific components and develop the full characteristic flavor. The bean shell is removed and the kernel is ground to extract the cocoa butter and form a "chocolate liquor" which is then pressed to separate the cocoa butter from the dry "cake" or "cocoa mass". Chocolate confectionery products are all prepared using well-controlled specific formulations of cocoa mass and cocoa butter mixed with sugar, flavor enhancers, emulsifiers and milk fats. Some typical formulations are included in Table 2. The manufacturer's ingredients are blended to produce a homogeneous mixture with an appropriate content of cocoa butter, the key ingredient of the mixture.

This composition is now designated as the chocolate "crumb". The chocolate crumb is refined by rolling to produce the very small particle size necessary for a smooth "mouthfeel" in the finished product. The crumb, a paste-like substance, is then subjected to a process known as "conching". In this stage, a scraping/mixing motion is applied to the chocolate crumb and this contributes to flavor enhancement by promoting release of volatile components, reduction of water content, and the introduction of textural changes by further reducing particle size. At the end of this operation, emulsifiers are introduced and the cocoa butter content is adjusted to the manufacturer's specification before the product enters the final stage of "tempering". The objective of tempering is to produce sufficient crystals of the stable Form V to convert all the cocoa butter in the chocolate mass into this most desirable polymorph during in-mold cooling.

Table 2. Approximate % mass formulations for different chocolate products[a]

Component	Milk Chocolate (% mass)	Dark Chocolate (% mass)	Bittersweet Chocolate (% mass)
cocoa mass (solids and butter)	11.8	39.6	60.6
additional cocoa butter	20.0	11.8	2.6
sugar	48.6	48.1	36.3
soy lecithin (emulsifier)	0.4	0.4	0.3
flavoring (salt or vanillin)	0.1	0.1	0.2
whole milk powder	19.1	-	-
total fats in product	31.5	36.4	35.4

[a]Values represent typical manufacturer's formulations.[8]

In general, the polymorph with the highest melting temperature is the most stable, and thermodynamically, it is this form that usually dominates any mixture. Unfortunately, in the case of chocolate, the polymorph with the most convenient organoleptic and mechanical properties is Form V, not Form VI.

The characteristics that favor Form V include better flavor release, a glossy appearance, more appropriate mechanical properties (or "snap", the term used to describe the clean break-off-from-block behavior), texture (or "mouthfeel") and mold-release. The tempering process is applied so that the cocoa butter in the chocolate is present in this crystal form. The industrial procedure for tempering involves heating the chocolate mixture to about 45 °C to melt all the crystals present, then cooling the mixture to about 30 °C. Chocolate is a thixotropic fluid. This means that when subject to shearing forces during stirring, the formulation becomes less viscous and will flow more easily. Under these dynamic conditions, crystals of Forms IV and V are nucleated. The liquid is stirred to distribute these crystals and heated sufficiently to eliminate the Form IV crystals. The liquid chocolate is then poured into molds, cooled by passing through ventilated tunnels and demolded before wrapping or packing.

If the normal food handling and storage precautions are observed, the chocolate arrives at the point-of-sale in optimal condition for consumption. Unfortunately, often handling and storage precautions are not respected, and sometimes it is the consumer who exposes the chocolate to warmer or cooler temperatures or to a humid atmosphere. The temperature cycling that results from exposure to ambient temperatures and fridge storage promotes the conversion of cocoa butter Form V to Form VI with degradation of taste characteristics; the chocolate appears dull and becomes harder to break. Worst of all, the transformation often takes place with "fat blooming" where a white fatty deposit is formed on the surface. This unattractive deposit looks like mold and frequently results in the chocolate being returned to the manufacturer as unfit for consumption.

In warmer weather, after prolonged exposure to temperatures over about 33 °C, cocoa butter will convert to Form VI, to adopt a more densely packed structure and often a thin layer of a white fat deposit will appear on the surface. Blooming may also be caused by an increase of humidity at the chocolate surface during refrigerator storage. Chocolate readily adsorbs moisture from its environment and the presence of even a small amount of water on the surface causes sugar migration. When the chocolate returns to room temperature, the sugar solution evaporates to leave a powdery white solid designated as "sugar bloom". Although the chocolate is still fit for consumption, it has departed from the optimal condition; it has lost its attractive visual aspect.

EXPERIMENTAL PROCEDURE

Sample vials, each with about 0.5 g of an unidentified chocolate, were distributed to students working in pairs. The chocolate sample was cut using a scalpel and a mass of about 5−10 mg was transferred to each of three aluminum 40 μL DSC pans. A Mettler DSC 351e heat-flux DSC equipped with a LabPlant intercooler was used to analyze the samples. The lids of the pans were pierced to allow evolved gas to be released during the thermal analysis experiment and the pans were closed using a mechanical press.

At the beginning of class, the sample pan closing procedure and the use of the DSC instrument were demonstrated to students by the laboratory facilitator. The three pans with samples were subjected to different thermal procedures. The first pan was located in the DSC furnace and heated from −10 to 50 °C at 5 °C min^{-1}, and the resulting thermogram was recorded, analyzed, and printed. The second sample was placed in a 60 °C oven for 30 min, then transferred to a safe storage area in the laboratory at between 18 and 22 °C for a further 30 min. This sample was then located in the DSC furnace and heated from −10 to 50 °C at 5 °C min^{-1}. The third sample was stored at 30 °C for 24 h before being subjected to the same thermal analysis program as the other samples.

The students of each group were asked to interpret the three thermograms they obtained with their samples and explain the differences based on their knowledge of the phase behavior of cocoa butter in chocolate.

HAZARDS

The samples characterized during this laboratory activity were recovered from certified commercial foodstuffs and as such represent no significant hazard to students. Nevertheless, to minimize contamination of samples, all operations should be carried out using gloves, safety spectacles, and lab coats. Due care should be taken when operating instruments with high temperature ovens and during the manipulation of the scalpel blades used to cut small fragments from the chocolate samples.

RESULTS AND DISCUSSION

Thermograms obtained with examples of white, milk, dark (or bitter) and culinary chocolate are illustrated in Figure 1. These chocolate samples were purchased in Europe. American chocolate generally has a lower cocoa solids and higher sugar content, but this does not affect the procedures, or significantly alter the thermal behavior. The thermograms presented in this figure were acquired between −10 and 50 °C, using a 5 °C min^{-1} temperature program after preliminary experiments demonstrated that only marginal benefit was obtained from slower heating rates. Exploratory experiments also confirmed that samples removed from different parts of the same bar, from different bars from the same production lot, and from different lots, all produced very reproducible behavior (see Supporting Information). This is an expected consequence of the application of modern production procedures and efficient process control in the food industry and confirms that a commercial product of uniform composition results.

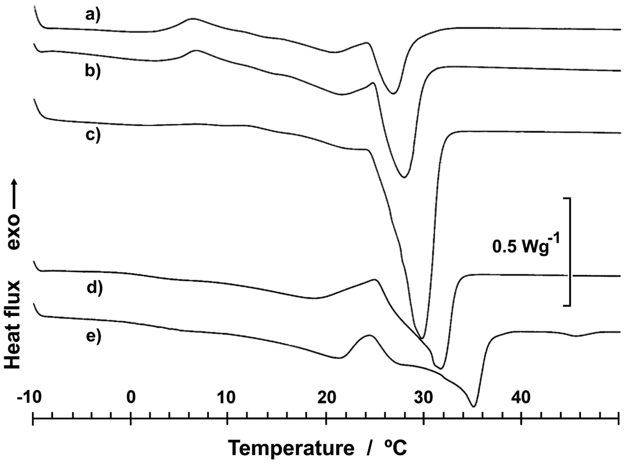

Figure 1. Thermograms of selected commercial chocolates: (a) dark culinary Portuguese; (b) milk Swiss; (c) white Belgian; (d) milk chocolate English; (e) dark bitter Swiss.

Various examples of white, milk, dark, and culinary chocolates were evaluated. Although slight variations in peak onset or offset temperatures, and peak intensity, were recorded due to specific differences in manufacturer's formulations and component sources, results broadly similar to those represented in Figure 1 were obtained with all the samples. The variation in thermal behavior of chocolate, both in terms of phase distribution and crystallinity, is clear in this figure. It is also clear that the formulation of chocolate used by manufacturers, the inclusion of milk fats, additional non-cocoa fats, sugar and emulsifiers and specific production routines, all contribute to a shift of the melting ranges found in cocoa fat. The samples included in this study were obtained from manufacturers in different countries, and therefore, some differences are also expected as a result of the use of raw materials with different geographical origin.

Results of heating/annealing experiments with a commercial milk chocolate are shown in Figure 2. Curve a was recorded with a sample removed directly from a commercial chocolate. The point-of-sale sample probably retains some amorphous fraction in the formulation. As the sample is heated, molecules in this fraction rearrange and crystallize into Forms II, III, and IV, giving rise to an exothermic event. These crystal forms melt, and this process is observable as irregularities imposed on a broad peak due to Form IV melting and finally as a peak due to Form V.

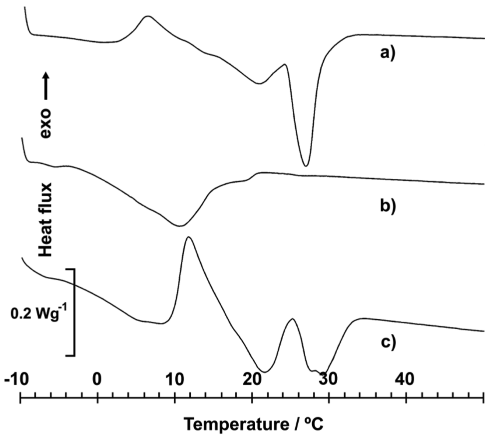

Figure 2. Thermal behavior of commercial milk chocolate subjected to different thermal treatments: (a) as supplied at point-of-sale; (b) 30 min after cooling from 60 °C to room temperature; (c) after cooling to room temperature from a period of 24 h at 30 °C. Further examples of thermograms supplied in Supporting Information.

Curve b was recorded after subjecting an unstirred chocolate sample to 60 °C for 30 min, causing complete fusion of all fat polymorphs. During the first 30 min of storage at 20 °C, nucleation of the low-melting fat crystals of Form I takes place with conversion to Form II and from Form II to III within the first few minutes.[7] The heating curve of this sample shows poorly resolved peaks corresponding to the first three fat forms. There are no thermal events that suggest the presence of Forms IV, V, or VI in the samples melted and recrystallized at 20 °C. The sample of milk chocolate has a phase structure that will change with time and evolve, over a period that is determined by components present in the chocolate formulation, to a distribution of polymorphs similar to that observed in the point-of-sale thermogram (curve a).

The third sample in this figure (curve c), stored at 30 °C for 24 h, was found to show the onset of fusion of Form I polymorph at temperatures close to −5 °C. This polymorph is formed from the amorphous fraction of the fat component during cooling from the annealing temperature. The structures of low-melting Forms I, II, and III cannot survive during storage at 30 °C, but they are crystallized rapidly from the liquid fat fraction as the sample is transferred from the storage location to the cold DSC furnace. As the temperature increases, the fat molecules align to crystallize into Forms II, III, and IV, and this process is observed as an exothermic peak. Form V and Form VI polymorphs do not crystallize directly from the melt but transform from less stable fat structures.[7,9] The annealing conditions were chosen to promote formation of Form VI and melting curve c has less Form V polymorph than the untreated chocolate (curve a) and a significant amount of the Form VI polymorph, seen as a peak with an intensity comparable to that of Form V but with a higher melting range.

It is quite clear from these results that chocolate samples undergo characteristic transformations at onset temperatures and with peak intensities that are determined by the manufacturer's formulation. Fusion and storage at close to room temperature establishes a new thermal history and fat crystallization takes place in the liquid chocolate under conditions that are quite different from those imposed by the manufacturer during production. The distribution of polymorphs that results is determined by the amount of cocoa fats, cocoa solids, non-cocoa fats, emulsifier, and flavor additives. Samples of dark, bitter, white, and culinary chocolate from various manufacturers, produced in different countries, were studied in preliminary experiments, and the results suggest that any of these commercial products would be suitable for use in the experiments described. This experiment has potential for development into a laboratory project in which students might compare the performance of different chocolate samples. Although it is hard to imagine an appropriate crime scene, a chocolate bar could almost be identified by the thermal behavior of a very small sample fragment.

One of the difficulties that must be addressed in this experiment is that in teaching laboratories it is normal to have access to a single thermal analysis instrument and sample preparation press. This experiment is therefore best included as one of a series of activities in an analytical instruments practical class. With a total sample preparation and analysis time of about 1 or 2 h per group, and typical classes of 3 h duration, it is necessary to provide students with supporting activities to occupy the interval between sample preparation and their instrument contact time. For this reason, examples of inlaboratory questions are provided to make better use of these intervals.

Further experimental details for students and examples of chocolate behavior have been included in the Supporting Information as support for practical aspects, and additional content of postlaboratory questions is also available.

■ CONCLUSION

Thermal Analysis is a subdomain of Analytical Chemistry that has developed to accompany advances and respond to the analytical requirements of a wide variety of products that includes polymers, textiles, pharmaceuticals, and food components. Students have been motivated by experiments that describe the study of samples from dogbones,[10] PET preforms from beverage containers,[11] textile fibers,[12] automobile lenses,[10] and pharmaceutical products.[13] The experiment described in this paper continues the strategy of engaging students by studying samples recovered from domains where commercial products have clear chemical relevance.

The application of thermal analysis to the study of samples of chocolate as described in this paper has been very well accepted by students. We have found that our students are more interested and motivated by the study of chocolates than by traditional thermal anaylsis of polymer or inorganic samples. Students respond enthusiastically to the postlaboratory questions and invest more unsupervised time in researching the topic and preparing the laboratory report. The level of student satisfaction with this laboratory activity, appraised by their response in the end-of-term assessment, was very high. The relevance of phase behavior of cocoa butter components in other areas of Food Chemistry is evident and the experience provides benefits in other Physical Chemistry course units.

The study of commercial chocolates represents a more complex challenge to students than “text-book” samples included in traditional instrumental courses. In this laboratory activity, the samples are formulations optimized for commercial purposes and not the usual pure samples often presented in academic environments. This new scenario is much more representative of a real-world industrial environment.

The interactions between added fats, cocoa solids, emulsifiers, and flavor components alter the behavior of the cocoa fats and it is important for future chemists to understand that these interactions may introduce significant alterations in the behavior of key components. The manner in which multiphase systems respond to isothermal annealing is rarely discussed with students, although it is of great relevance in many domains. This example, discovered in Food Chemistry, helps broaden student experience and may provide the support necessary to interpret observations in many other areas of Chemistry.

The learning objectives of this experiment include providing students with hands-on experience with thermal analysis instruments and use of specific software to practice data extraction. Students consult the chemical literature to find the explanation for differences between commercial samples and rationalize the thermograms recorded during their study. Contact with commercial samples containing low-melting fats leads them to an appreciation of the importance of these natural products in industrial markets including cosmetics, pharmaceutics, and food. Finally, the process of isothermal annealing and the consequences for the distribution of components between the phases present in semicrystalline samples is an important aspect that provides useful insight into the relationship between structure and behavior of complex systems.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00284.

> Notes for instructors, step-by-step procedures and in-lab and postlab questions for students and suggestions for further studies (PDF, DOCX)

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Notes

The authors declare no competing financial interest.

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