The Use of Atomic Force Microscopy to Measure the Formation and Development of Chocolate Bloom in Pralines

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ABSTRACT: Atomic force microscopy (AFM) has been used to study the surface of chocolate as well as the progress of chocolate bloom over time. Fresh chocolate was found to be relatively smooth but with deep holes. These could be pipes leading deep down into the body of the chocolate, perhaps reaching the filling. After storage for a few weeks, we observed the growth of small drops around these holes. With increasing time, these drops became larger and more structured. After further storage, a crystalline structure and bloom were revealed. These results suggest that bloom growth in pralines is a two-phase process, with drops initially forming on the surface and then bloom crystals nucleating and growing from them. Further, we deduced pipes leading down into the center of the chocolate through which the migration of filling fats can preferentially occur.

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The crystal structure of cocoa butter is somewhat complex, as it is composed of a mixture of many different TG (1). The composition of cocoa butter varies from source to source (2) but always contains high concentrations of palmitic-oleic-palmitic, stearic-oleic-stearic, and palmitic-oleic-stearic TG. The unsaturated chain is in the 2-position of the glycerides, which is relatively unusual for natural fats. This gives the cocoa butter its sharp melt in the mouth, a desired attribute of chocolate.

Traditionally, cocoa butter has been assigned six different polymorphic forms, with m.p. from 17.3 to 36.3°C. The different forms have been identified by X-ray diffraction analysis and DSC. Various nomenclature has been proposed, but the most common has been that of Wille and Lutton (3), who described the forms from I to VI, where I is a γ form, II is α, III and IV are β′ and V and VI are β. More recently, Van Malssen *et al.* (4) have suggested that forms III and IV are not really distinct. Instead, there is a gradual transition of the β′ structure as the crystallization temperature varies. Thus, an extremely large number of physically distinct β′ forms exist.

Cocoa butter is the basis for the manufacture of chocolate. Chocolate consists of a dispersion of sucrose crystals, cocoa powder, and milk powder in a matrix of cocoa butter. The preferred form of cocoa butter for edible consumption is form V, which is a β form. However, it is not the most stable form of cocoa butter crystals; this is form VI, another subphase of the β polymorph. Uncontrolled recrystallization of form V to form VI on the surface of chocolate leads to the development of unsightly gray crystals of chocolate bloom. This is the basis of fat bloom. Transformation from form V to form VI is relatively easy, as the structural differences and barriers to transformation are not so great. Transformation is aided by the presence of other oils in the system (5), which presents a problem during the storage of filled pralines. In this case, bloom can occur in a well-stored product over its normal shelf life of a few months. For a well-stored solid tablet of chocolate, the process takes several years. However, bloom formation may be encouraged in a chocolate block by warming it to near the m.p. and/or by temperature cycling.

Bloom also can be caused by transformations in the sugar crystals (6) due to excess moisture. This is an unrelated phenomenon that also leads to product damage.

The prevention or delay of bloom formation is an important consideration for the chocolate manufacturer. Various products on the market are sold as ingredients for bloom prevention, and the control of processing and storage is important. Current understanding of the mechanisms behind bloom formation is complicated by the nature of the process. The interaction between the different TG and subsequent crystalline transformation has complicated the development of novel techniques.

Atomic force microscopy (AFM), also referred to as scanning force microscopy, a technique invented in 1986 (7), enables the high-quality, detailed investigation of surfaces. Many applications have been found, and several good reviews are available on its application in organic and biological material (8–11). The technique involves measuring the interaction between a sharp tip and a surface. Essentially, the tip is moved across the surface and its position is noted. By using a laser reflection technique, the position of the tip in the *z*-direction can be determined with a very high degree of accuracy. Indeed, resolution down to the angstrom scale is possible. Because the tip movement in the *x*- and *y*-directions can be extremely well controlled, it is possible to develop maps of the surface. Surface topography as well as other properties such as viscoelasticity and friction can be monitored simultaneously. Use of the tapping mode (tapping the tip on the surface) allows the researcher to probe the mechanical behavior at a particular point. This

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gives a so-called phase image of the surface and allows regions of different phases on perfectly smooth surfaces to be determined. The response of the tip on the surface depends on the attractive and repulsive force fields of the sample during vibration of the tip. Thus, different materials can give different responses and thus be differentiated, even if the surface is perfectly flat. Another possible measurement is of the friction of a surface.

Problems may arise with the study of soft surfaces. The tip may damage the surface, sticking may occur, or excessive local heating may arise. This has often been a limiting factor when studying food and biological surfaces. However, by using the tapping mode, contact times between the tip and the surface are very short and physical damage can be eliminated. This enables many more sensitive systems to be studied.

Some study of TG crystals with AFM has been reported. Doyle and Adams (12) studied the structure of large single β crystals of tripalmitin. Molecular layers on the surface were clearly visible, as were defects. On more practical systems, Hodge and Rousseau (13) found a clear increase in roughness with time for the surface of chocolate as bloom developed. This was to be expected, as the uncontrolled recrystallization that occurs during bloom growth will lead to a rougher surface. This roughness will lead to light scattering and so give a characteristic gray bloomed surface.

The aim of this work was to study the structure of chocolate using AFM and to produce detailed images of the surface of fresh chocolate and of chocolate in which bloom was developing.

EXPERIMENTAL PROCEDURES

Materials. Handmade and hand-tempered chocolate pralines were received from Karlshamns AB (Karlshamn, Sweden). A series of pralines with three different fillings, both dark and milk chocolate, were studied (six systems in all). The chocolates were relatively thin (approximately 1 cm thickness) to allow them to be studied at the AFM stage. We also received pure chocolate tablets from Karlshamns AB for initial testing, observation, and calibration.

Methods. The handmade and hand-tempered chocolate pralines were carefully separated from their molds and stored in a temperature- and humidity-controlled environment at 23°C and 50% RH. The samples were removed for study by AFM and then returned to the storage room for continued storage. Samples were studied on arrival and then after 4, 8, 12, and 20 wk. In all procedures, care was taken to ensure that the samples were not touched by hand.

A Nanoscope IIIa Multimode atomic force microscope (Digital Instruments, Santa Barbara, CA) in tapping mode was used for all the observations. Various points on the surfaces were randomly selected for study, and these were studied over scales of differing lengths. The sample temperature was held at 16°C to prevent melting and heat damage to the sample under investigation. There was a risk of melting due to the heat evolved in contact between the microscope tip and the sample. Indeed, when initial measurements were performed with no temperature control, significant melting was caused by contact between the tip and the chocolate. This was immediately apparent as the surface became soft and featureless. By controlling the temperature, it was possible to determine the experimental conditions under which melting did not occur. Therefore, good temperature control was always required. Measurements were performed in the tapping mode, and both topographic and phase images were taken.

RESULTS AND DISCUSSION

The initial chocolate surface is shown in Figure 1. This image shows a relatively flat surface with some fat crystals protruding. The overall variation in surface area of 20×20 μm is given as 800 nm per division. The surface also shows various pits. It was not possible to see the bottom of these pits with the microscope, and we were unable to determine the depths of the bottoms of the pits, as they were beyond the range of the microscope tip. In reality, these pits or pipes penetrated deep into the chocolate, with a radius that varied from around 1 to 4 μm.

After 4 wk of storage, the chocolate still appeared glossy to the naked eye. However, the AFM instrument revealed that the structure of the chocolate surface had changed somewhat (Fig. 2). Irregular features of up to 10 μm in size had appeared, mainly concentrated in the vicinity of the observed holes or pipes. In all cases, measurements were performed at many (at least 20) sites over the surface of the chocolate, and the number, size, and density of features were measured accordingly.

After an additional 4 wk of storage, the numerical density of these structures had increased (Fig. 3), and they appeared to cover a significant proportion of the sample surface. At this point, the sample was no longer completely glossy, but no bloom crystals were visible to the naked eye. Some structure appeared in the drops, and we observed some evidence of crystal planes within the amorphous, unstructured drops. After 8 wk of storage, the sample roughness had increased greatly, to 3,000 nm per division, because of the increase in droplet size. Unfortunately, because the sample area was so small, it was not possible to measure the same locations every time. Therefore, we performed many scans at different magnifications and at different locations.

On 12 wk of storage, even more structure appeared to have formed in the amorphous drops, but with no overall change in behavior. After 20 wk of storage, the samples were visibly bloomed and AFM images showed large, relatively hard structures (Fig. 4). These were presumed to be bloom crystals growing out of the surface.

The density of the amorphous structures on the sample surfaces were measured after 4 and 8 wk. The samples were also rated for degree of blooming at the conclusion of the experiment, with 1 being the heaviest bloomed sample and 6 being the lightest bloomed. These results are illustrated in Table 1.

Fresh chocolate surface. The surface of fresh chocolate is relatively flat and featureless, giving a glossy appearance. A film of liquid fat seems to cover most of the structural elements,

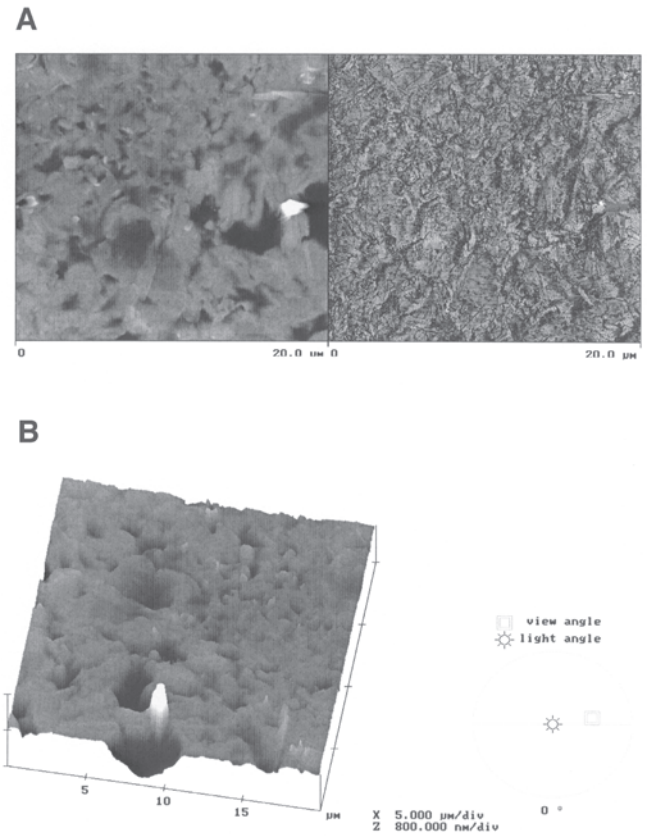

FIG. 1. Image of a fresh chocolate surface. (A) Topographic and phase images; (B) a 3-D representation of the topographic image. 20 × 20 µm; z = 800 nm per division.

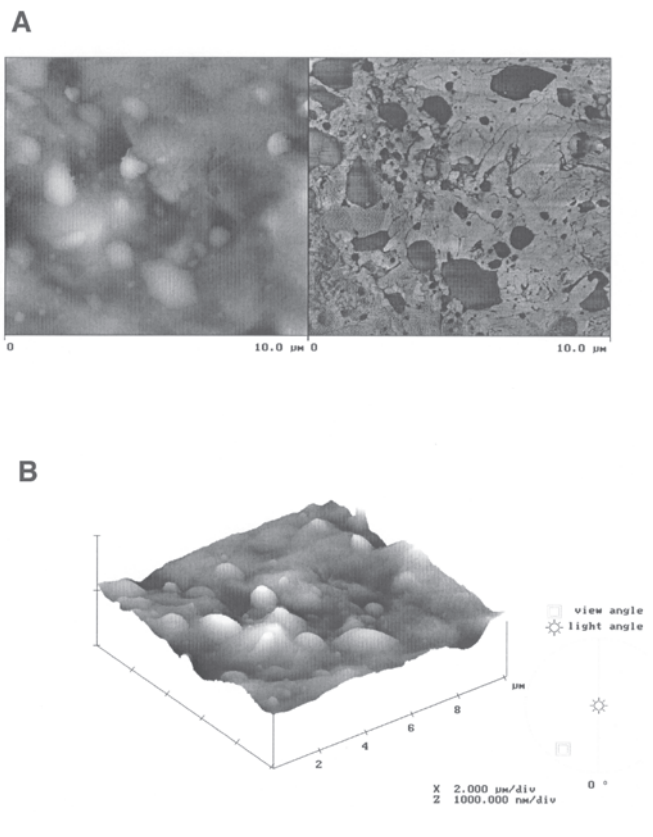

FIG. 2. Images of chocolate after 4 wk of storage. (A) Topographic and phase images; (b) a 3-D representation of the topographic image. 10 × 10 µm; z = 1,000 nm per division.

with the exception of a few fat crystals that protrude from the surface. However, the surface has many small, deep holes.

The formation of stable chocolate is a complex thermal process that involves a great deal of temperature variation. A sample must be cooled to form seed crystals, warmed to ensure melting of undesirable polymorphic forms, and then cooled again for crystallization. Subsequent tempering is then necessary. Thus, it is conceivable that the chocolate will have many defects, akin to the casting defects that arise during metallurgical processing (14). The formation of pipes that extend deep into the body of the chocolate, such as those we observed in the fresh chocolate, is thus highly likely. These pipes could conceivably reach down into the filling, providing convenient routes for migration of the filling oil through the chocolate matrix and onto the surface. Here, interaction with cocoa butter crystals would lead to visible bloom.

This hypothesis was supported by the fact that the initial transformations appeared to occur primarily around the entrances of these pipe or hole features. Although our research results do not provide enough evidence to fully substantiate this hypothesis, they are in agreement with those obtained for mercury porosimetry on chocolate (15). These authors suggested that 1–4% of the volume of chocolate (depending on the degree of tempering) does in fact consist of voids, pipes, and air bubbles.

Bloom formation. After a short time, obvious features developed on the surface of the chocolate. The phase image revealed the clear formation of droplike features that were homogenous and relatively softer than the surrounding chocolate. Thus, they appeared to be drops of proto-bloom. There was some indication that they had formed around the holes seen on the fresh surfaces. Again, this supports the hypothesis that these are pipes through which migration can occur. These drops are softer than the

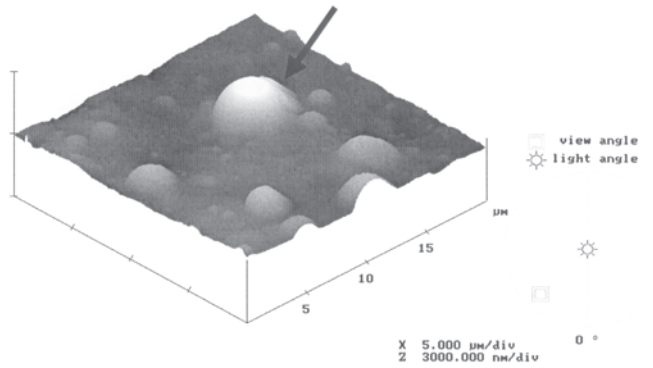

FIG. 3. A 3-D topographic image of chocolate after 8 wk of storage. The arrow marks the initial crystalline area. 20 × 20 µm; z = 2,000 nm per division.

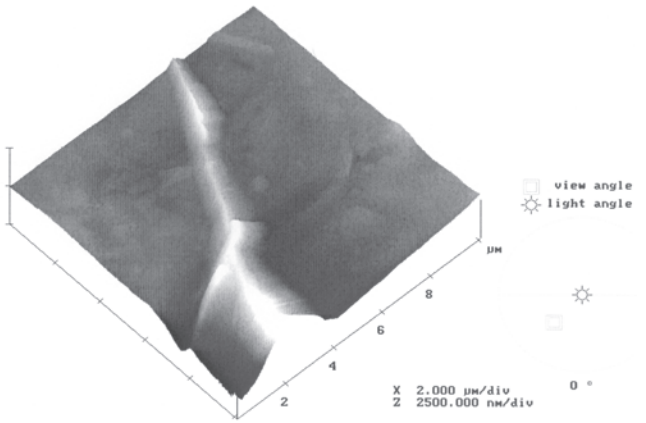

FIG. 4. A 3-D topographic image of chocolate after 20 wk of storage. 10 × 10 µm; z = 2,500 nm per division.

chocolate surface and could conceivably be drops of liquid or semiliquid oil that contain a very high proportion of the filling fat. We observed large numbers of drops across the chocolate surfaces, and although the number density of the drops varied from sample to sample, there was no variation in structure.

With time, the size of the proto-bloom drops increased. After 8 wk, they were clearly larger than after 4 wk of storage. Along with the increase in size, differences in the state of formation of the structure were indicated. Thus, crystallization of the bloom appeared to occur within the proto-bloom droplets.

At the end of the study period (20 wk of storage), the samples appeared bloomed to the naked eye. The AFM measurements showed large, hard ridgelike bloom crystals growing from the surface. This structure was in agreement with results on the optical microscopy of bloom (16).

We postulated that the proto-bloom drops seen were the forerunners of actual bloom crystals. To determine the likelihood of bloom crystals appearing, we determined the number density of drops on chocolate surfaces after 4 and 12 wk. The degree of blooming of the samples at the end of the study also was determined, as shown in Table 1. The most heavily bloomed sample was ranked 1, through to the least bloomed (rank 6). There was a clear correlation between the number density of the proto-bloom droplets and the degree of development of bloom.

AFM is thus a useful technique for investigating the structures on chocolate, as it indicates the mechanisms that may be occurring. Clear evidence for the migration of filling fats to the surface, possibly through pipes in the chocolate, was revealed, as were the formation of proto-bloom drops and their subsequent transformation into bloom crystals.

TABLE 1
Comparison of Drop Densities of Samples After 4 and 12 wk, and Ranking of Bloom Development[a]

	Drops/mm^2		
Sample	After 4 wk	After 12 wk	Rank
1	0.14	0.20	4
2	0.15	0.25	1
3	0.07	0.06	6
4	0.12	0.14	3
5	0.07	0.10	5
6	0.09	0.13	2

[a]1 represents the most heavily bloomed sample and 6 the least bloomed sample, as determined by visual inspection. All samples were hand manufactured and hand tempered. Samples 1, 3, and 5 are dark chocolate and samples 2, 4, and 6 are milk chocolate. There are three distinct fillings: Samples 1 and 2 have the first filling, samples 3 and 4 the second, and samples 5 and 6 the third.

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