Fat Bloom Formation and Characterization in Milk Chocolate Observed by Atomic Force Microscopy

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ABSTRACT: The surface microstructure and polymorphic behavior of milk chocolate subjected to multiple thermal cycles between 20 and 32, 33, or 34°C were examined using atomic force microscopy (AFM) and powder X-ray diffraction (XRD). The surface of unbloomed milk chocolate was smooth (surface roughness of 278 nm) and consisted of small, evenly distributed crystals. XRD results indicated the presence of mostly form V crystals and little or no form VI crystals. Cycling between 20 and 32°C resulted in little bloom formation and change in polymorphic behavior. Gradual bloom formation occurred as a result of cycling between 20 and 33°C, and was accompanied by the nascence of form VI crystals. Surface roughness increased gradually from 417 nm after one cycle to 476 and 521 nm after two and three cycles, respectively. Extensive bloom arose from cycling between 20 and 34°C. Surface roughness increased from 373 nm after one cycle to 603 and 736 nm after two and three cycles, respectively. This heavily bloomed chocolate consisted of jutting crystals and large raised, yet smooth areas that were haphazardly located within the chocolate matrix. In summary, a new perspective on the development of surface bloom due to thermal cycling is provided.

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KEY WORDS: AFM, chocolate, crystallization, fat bloom, morphology, polymorphism.

Milk chocolate consists largely of cocoa mass, milk solids, and sugar particles distributed within a continuous cocoa butter fat phase. The attributes of chocolate are strongly dependent on the size and distribution of these particles and on the polymorphic form and morphology of the fat phase. Cocoa butter is responsible for the snap, gloss, and sharp melting profile of chocolate at body temperature (1).

Fat bloom is the chief defect that afflicts chocolate and chocolate products. This physical imperfection makes the chocolate undesirable for consumers who expect a product to have a glossy surface and desired color. Instead, bloomed chocolate appears old and stale and is identified by a beige coating on the surface of the chocolate. Fat bloom is a result of improperly formed fat crystals larger than 5 μm located at the surface of the chocolate (2). With very small crystals (<5 μm) at the surface, chocolate appears glossy. Larger crystals can diffuse the reflection of light from the surface giving chocolate a dull appearance.

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Cocoa butter is polymorphic, consisting of six different crystal forms (I through VI) with each successive form exhibiting increased stability (3,4). The desirable polymorph in properly tempered chocolate is form V, as it has the most desirable melting and solidification properties compared to the other forms. However, it is not the most stable polymorph.

Chocolate bloom is believed to occur *via* any of three different scenarios. In one scenario, poor tempering of chocolate causes cocoa butter to crystallize in the form IV polymorph, which promptly changes to form V upon cooling and storage. The newly formed form V crystals located at the surface are visible as bloom. A second scenario involves chocolate containing a mixture of various types of TAG. In this case, the phase behavior of the TAG becomes disrupted and leads to the formation of large surface crystals (2,5). The third scenario involves chocolate that has been properly tempered to form V but is stored at elevated temperatures or is subjected to thermocycling, leading to the formation of form VI crystals (6).

Cocoa butter exhibits distinct m.p. for each polymorph. Heating the chocolate melts all but the most stable polymorphs. At sufficiently high temperatures, cocoa butter is found to be in a quasi-liquid state, and a few form V or VI crystallites with high m.p. remain intact (7). These high-melting crystals are composed of trisaturated TAG and may exist in liquid cocoa butter at temperatures as high as 38°C (8). Upon cooling, these crystallites act as a template upon which the cocoa butter can rapidly recrystallize as form VI polymorphs (7). The optimal temperature for crystallization of the form V phase is ~19°C, whereas the optimal temperature for form VI development is ~23°C (9).

A microscopy technique that has recently seen some use in food research is atomic force microscopy (AFM) (10–12). This technique "visualizes" the surface structure of substances by using a sharp vibrating probe with a tip size of <50 nm that scans a sample surface at a distance over which atomic forces act. The forces between the tip and sample cause the cantilever to deflect from its unimpeded range of oscillation. A photo detector measures this deflection, and from this information a map of the sample topography can be created. Quantitative surface analysis in the nanometer regime is possible in all three dimensions.

We report here on the use of AFM to investigate the surface structure of unbloomed and bloomed milk chocolate complemented by analysis of cocoa butter polymorphic behavior and provide a new perspective on bloom formation and chocolate structure.

EXPERIMENTAL PROCEDURES

Milk chocolate wafers (38 × 38 × 1.5 mm) were purchased from a local department store. They were composed of 37% cocoa mass and 14% milk solids. Chocolate bloom was induced by cycling the chocolate from 20 to 32, 33, or 34°C and back to 20°C over a 24-h period using a Peltier temperature-controlled microscope stage (TS-60 stage, Instec Inc., Boulder, CO). The temperature during the heating portion of the cycle was ramped up at a constant rate over a period of 4 h. The temperature was then held at the target high temperature for 8 h before cooling, again at a constant rate over 4 h to the low temperature of 20°C. Samples were subjected to one, two, or three cycles.

A Bioscope atomic force microscope (AFM) with Nanoscope IIIa controller (Digital Instruments, Santa Barbara, CA) was used to image 50 × 50 µm areas of the chocolate wafer surface topography. The tips used had a cantilever spring constant of 40 N/m and were oscillated at approximately 350 kHz. The tips had an end point radius of 10 nm and a body angle of 30°. For soft samples, it is critical that the microscope tip not damage the surface being scanned but that it still contact the surface to obtain high-resolution measurements. To achieve this, the AFM was operated in tapping mode, which involves oscillating the tip to achieve a touch and release action. While the tip is touching, high-resolution surface height data are collected, and once released, the tip is able to move along the sample surface without causing damage. Roughness of the chocolate was determined using the software provided by the AFM manufacturer. The value is based on the root mean square (RMS) of the height deviations taken from the mean data plane and is expressed as:

$$\mathrm{RMS} = \sqrt{\frac{\sum Z_i^2}{N}} \qquad [1]$$

where Z and N are the individual height deviations from the mean data plane and the number of measurements, respectively.

A Rigaku Geigerflex (Danvers, MA) X-ray diffraction (XRD) unit (λ = 1.79 Å) was used to determine powder diffractograms of the polymorphic forms of the bloomed and unbloomed chocolate at 25°C. Scans from 3 to 30° 2θ were performed. To eliminate interference by sugar crystals in the chocolate during XRD, the method of Cebula and Ziegleder (2) was used. Samples were chopped with a knife and then sifted to obtain particle sizes less than 0.5 mm. This powder was then mixed with 500 mL of cold water, shaken, and allowed to stand at room temperature for 4 h. The insoluble material, including the cocoa butter fat, was recovered by vacuum filtration. Diffraction patterns taken from the original chocolate and from extracted samples were compared. No new peaks were produced as a result of the extraction process.

Thermal analyses were performed using a TA Instruments 2920 (New Castle, DE) differential scanning calorimeter. The instrument was calibrated at 10°C/min using a traceable indium reference standard (m.p. = 156.6°C and H_f = 28.71 J/g).

RESULTS AND DISCUSSION

Temperature cycling to 32°C. The surface of a properly tempered, unbloomed milk chocolate wafer was smooth and consisted of small, evenly distributed crystals measuring 1–3 µm in size (Fig. 1). Samples cycled at 32°C (Fig. 2) showed no visible bloom development after three cycles. However, roughness (RMS) values calculated based on the collected images after zero, one, two, and three cycles were 278, 372, 451, and 503 nm, respectively. X-ray powder diffractograms for the extracted samples showed no pattern change in the fingerprint 3 to 6 Å short spacings region from that of the control. Figure 3 illustrates the key differences between the XRD patterns of form V and form VI polymorphs.

Temperature cycling to 33°C. Cycling the chocolate wafers to 33ºC resulted in the gradual development of a small degree of visible surface bloom. AFM images show a gradual coarsening of the surface, with smaller features giving way to larger ones (Fig. 4). Image RMS roughness values increased through successive cycles from 417 nm for one cycle through to 476 and 521 nm for two and three cycles. XRD data show the gradual evolution of the form VI polymorph. The peak at 3.97 Å decreased in intensity relative to the neighboring peak at 3.86 Å; at the same time, the peaks at 3.66 and 3.75 Å

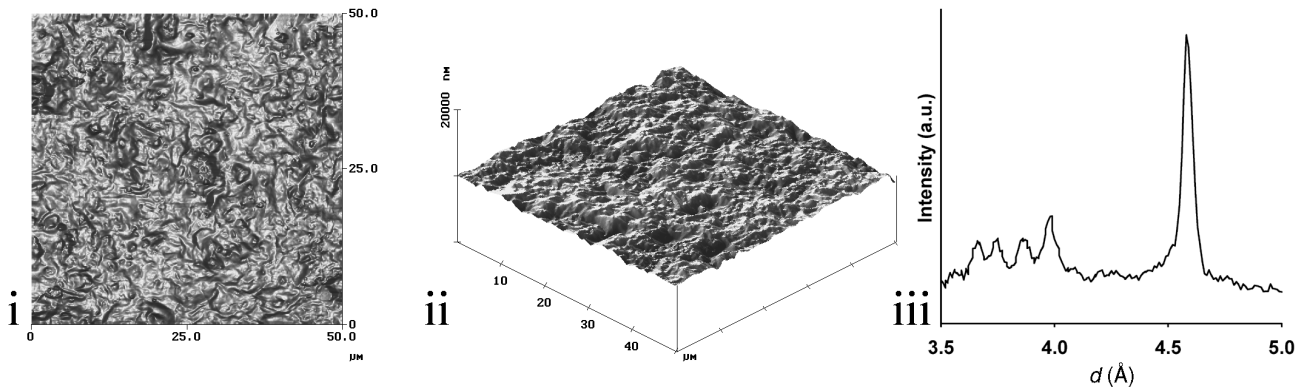

FIG. 1. Atomic force microscopy (AFM) images of control chocolate wafer surface showing fine textured surface in top view (i) and 3-D projection (ii). Frame iii shows the corresponding X-ray diffraction (XRD) spectrum.

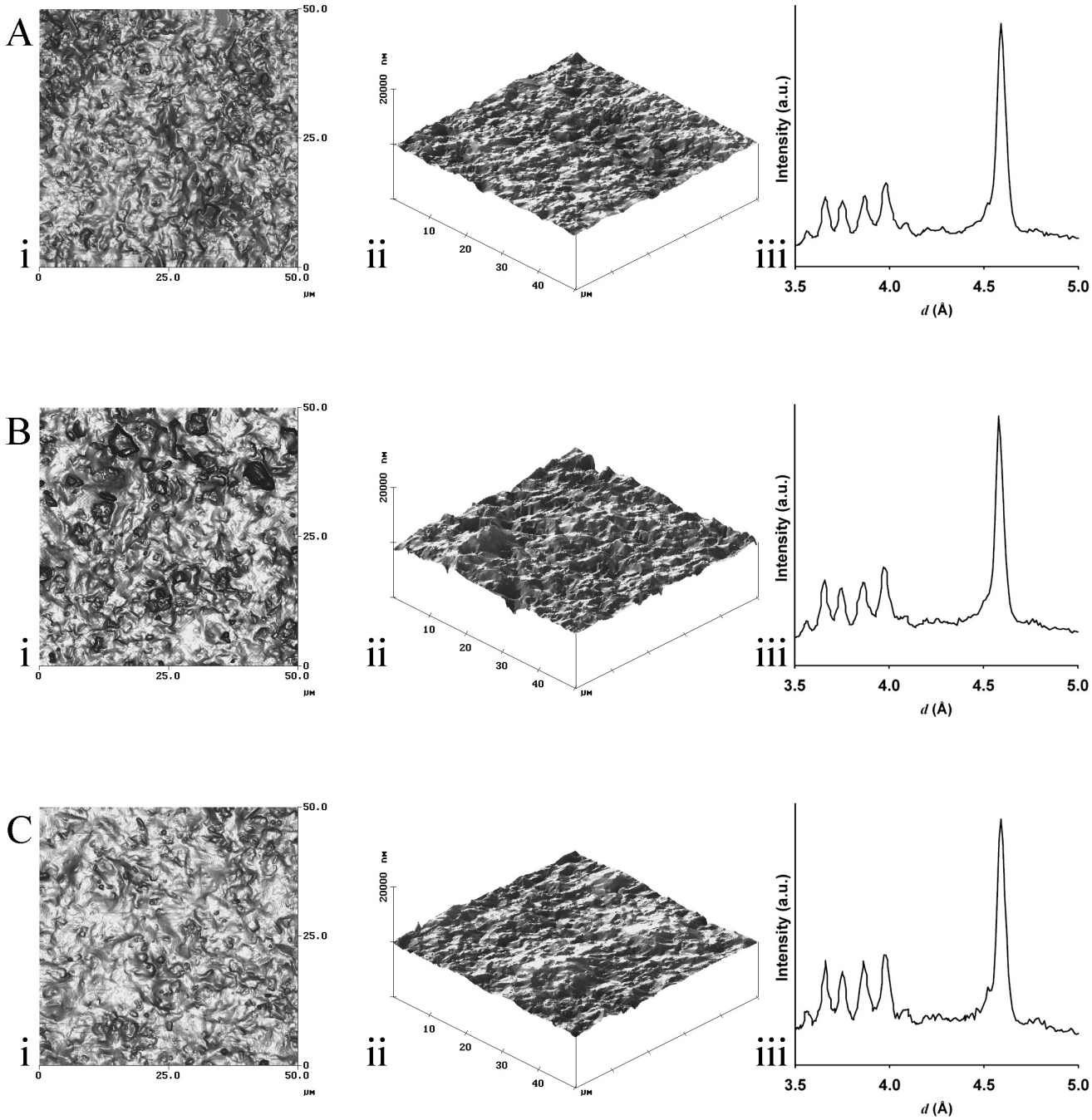

FIG. 2. AFM images and corresponding XRD patterns for chocolate wafers cycled between 20 and 32°C for one, two, and three cycles (A, B, and C). For abbreviations see Figure 1.

diminished and a new peak emerged between them at 3.69 Å. This change in diffraction pattern was consistent with the evolution of form V to form VI cocoa butter polymorphs (13).

Temperature cycling to 34°C. Surface microstructure and polymorphic behavior of milk chocolate were substantially affected by temperature cycling between 20 and 34°C. A visible coating of bloom was formed on the chocolate wafers after only one cycle. Further cycles increased its visual presence. A single cycle resulted in a visibly bloomed surface that consisted of a less-ordered surface structure with an increased roughness (RMS = 373 nm). As the number of cycles increased, smaller structural features were lost at the expense of large elements (Fig. 5). There was increased protrusion of crystals in the bloomed chocolate, which became more noticeable as the bloom became more pronounced. After two cycles from 20 to 34°C, the RMS surface roughness had increased to 603 nm. Following three cycles, surface roughness had increased up to 736 nm. This heavily bloomed chocolate consisted of jutting crystals and large raised, yet smooth areas that were haphazardly situated on the sample surface.

The unbloomed chocolate consisted mostly of form V crystals and little or no form VI crystals, based on XRD

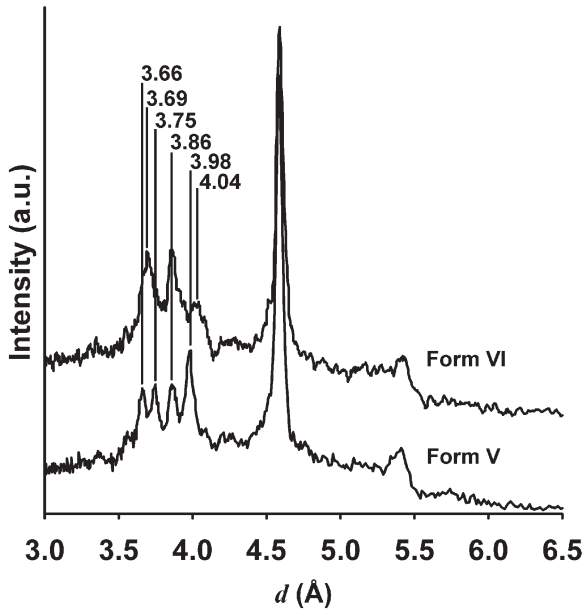

FIG. 3. Comparison of XRD short-spacing patterns of form V and form VI of extracted chocolate solids. For abbreviation see Figure 1.

results. As a result of temperature cycling between 20 and 34°C, the strong spacing at 3.98 Å weakened, the medium-intensity peak at 3.86 Å became stronger, and the doublet (at 3.75 and 3.66 Å) converged into a median peak at 3.69 Å (Fig. 5). Bloom formation did not affect the location or intensity of the very strong peak at 4.6 Å. There were only minor changes in the short spacings and no changes in the long spacings. The change from form V to VI only involved small changes in cocoa butter structure, yet there was extensive change in the surface morphology. DSC results showed a shift toward higher-melting crystals (Fig. 6). There was also early evidence of the development of a second m.p. at approximately 35°C corresponding to the form VI melting points observed in cocoa butter studies by Hicklin *et al.* (34.6°C) (14) and Wille and Lutton (36.3°C) (3).

Surface roughness and bloom formation. The RMS roughness of the milk chocolate wafers increased as they were cycled at 32, 33, and 34°C, with the greatest increases in roughness being observed at 34°C. It was initially expected that bloom formation would be well correlated with an increase in the surface roughness of the chocolate. However, as previously mentioned, no obvious bloom formation was observed on the milk chocolate ramped to 32°C after one, two, and three cycles. Still, RMS roughness using this thermal cycling regime was 372, 451, and 503 nm after one, two, and three cycles, respectively, up from an RMS roughness of 278 nm in the properly tempered milk chocolate. By comparison, the heavily bloomed chocolate, cycled three times to 34°C, had an RMS roughness of 736 nm. Therefore, it appears that surface roughness in milk chocolate may not be as strong an indicator of visible bloom formation in milk chocolate as initially thought. However, the increase in roughness does provide thought-provoking evidence that bloom formation in milk chocolate necessitates a complex structural rearrangement whereby the visible aspect of bloom is only the final stage.

Mechanism of bloom formation. The cocoa butter in the control milk chocolate consisted of numerous small form V crystals. Through gradual temperature cycling, resulting in bloom formation, larger form VI crystals on the chocolate surface replaced small form V crystals.

The AFM images show the development of large, jagged features on the surface of the milk chocolate surface as bloom formation ensues. We have not yet observed what is happening within the chocolate matrix; however, on the surface it is apparent that there is a growth in the size of surface features that is related to the visual appearance of bloom. Presumably these growing features are composed of cocoa butter and not sugar, cocoa solids, or nonfat milk solids, which are insoluble as they are in the fat phase and are unaffected by the temperature regime employed here. Although temperature cycling will generally result in an increase in the average size of crystals, the growth of the crystals on the surface may be enhanced by a mechanism whereby cocoa butter migrates from the body of the chocolate to the surface. This mechanism has been described as a "pumping action" whereby the higher-melting TAG responsible for bloom dissolve in and migrate through the molten lower-melting fractions toward the surface (13). We will now elaborate on this mechanism based on our results.

Using mercury porosimetry, Loisel *et al.* (15) reported that chocolate has a porous structure. Overtempered chocolate containing 31.9% cocoa butter had a porosity of 4%, whereas well-tempered chocolate had cavities accounting for 1% of the chocolate volume. Warming of chocolate causes some of the cocoa butter to melt, and in doing so the net volume of this component increases due to dilation. This expansion and growth in the liquid phase volume then cause cocoa butter to be pushed to the surface of the chocolate. Crystals of more stable polymorphs and higher-melting TAG remain intact. These crystals can dissolve in fluid cocoa butter consisting of lower-melting TAG and together can well up to the surface.

Subsequent gradual recooling will result in progressive recrystallization. By their nature, the more stable polymorphs, such as forms V and VI, take longer to form, as their crystal lattices are more compact and it takes more time for the molecules to arrange themselves into these increasingly stable configurations. Conversely, less stable polymorphs generally crystallize first as a result of their less compact crystal lattices. Forms I through IV form autogenically from the melt but are less stable and over time transform into the more stable configurations (16). Heating the sample to 34°C melts these less stable forms. Were the sample to be cooled quickly, the less stable forms would be preferentially generated, as they are capable of autogenic nucleation. Forms V and VI have not been observed to appear spontaneously but rather evolve out of the less stable forms (7). However, through gradual cooling, higher-melting TAG will crystallize in form V or form VI crystals provided there are existing crystals that can act as templates. As mentioned earlier, crystallites of form VI may remain in a mostly melted cocoa butter phase, up to

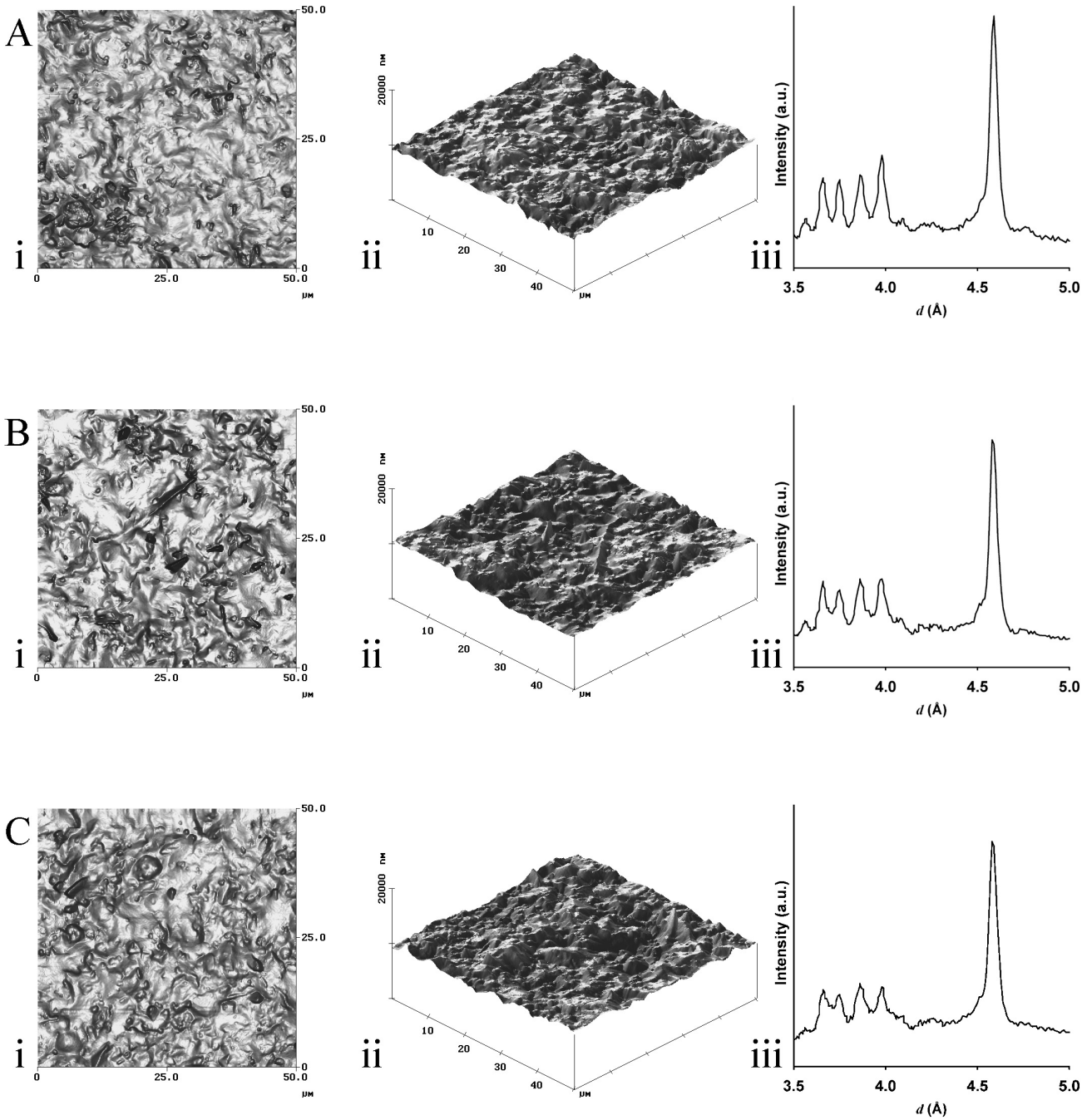

FIG. 4. Result of temperature cycling between 20 and 33°C for one, two, and three cycles (A, B, and C). AFM images of chocolate wafer surface and corresponding XRD patterns of washed samples show concurrent evolution of surface roughness and gradual polymorphic transition of form V to form VI. For abbreviations see Figure 1.

38°C. At elevated temperatures, the only polymorphic forms that will survive are the more stable ones, such as form V and VI. As the fat crystallizes within the chocolate, there is a reduction in volume that will lead to a combination of drawing liquid fat and smaller crystals back into the chocolate matrix and, when this mobility is restricted, increasing the porosity of the chocolate. The size to which crystals develop on the surface is unrestricted. In a partial-melt/recrystallization scenario, polymorphs sufficiently stable to survive the high temperature "extreme" of the melt cycle will grow in size upon cooling as they act as a template for recrystallizing TAG. The development of large surface crystals or bloom will be most evident and rapid when the maximum temperature encountered in the cycling regime is just below the melting point of the stable form VI polymorphs. The growth of form VI crystals is accelerated at conditions of higher temperatures. Under these conditions, these growing crystals are surrounded by liquid oil, and thus they may crystallize in their preferred

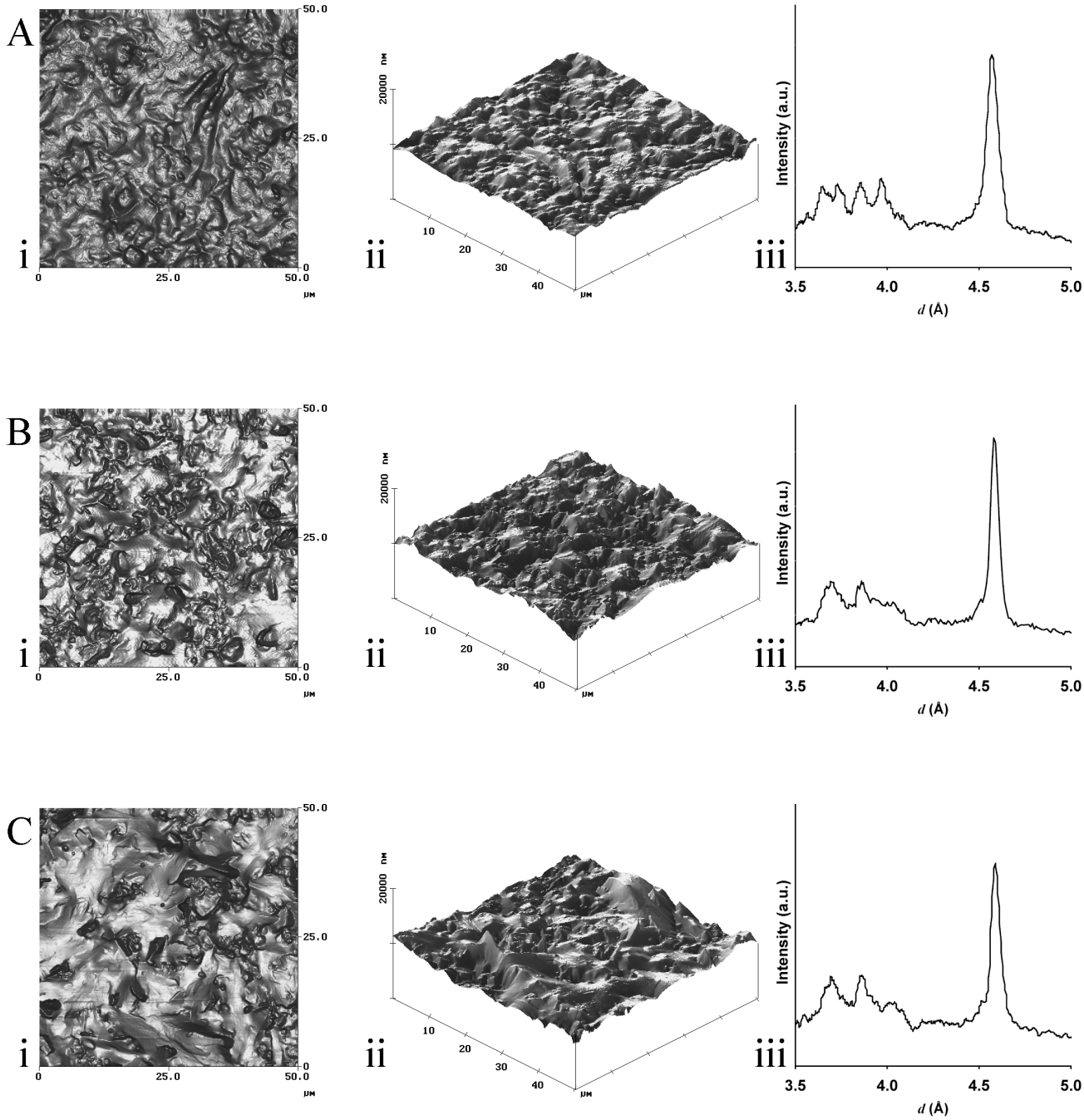

FIG. 5. Result of temperature cycling between 20 and 34°C for one, two, and three cycles (A, B, and C). AFM images of chocolate wafer surface and corresponding XRD patterns of washed samples show concurrent evolution of surface roughness and polymorphic transition of form V to form VI. For abbreviations see Figure 1.

morphology, i.e., a flat needle-like habit (17). Through successive cycles, there will be an evolution of melting behavior whereupon the amount of lower-melting fraction decreases and the amount of higher melting fraction increases. This was shown in the DSC results for samples cycled to 34°C (Fig. 6). Samples cycled through 33°C showed a similar combination of events, resulting ultimately in bloom development. The XRD patterns began to shift slightly from a typical form V to a form VI pattern. DSC results showed a similar progression toward a greater proportion of higher-melting compounds and the development of increased surface roughness, but not to the same extent as was observed at 34°C.

Based on AFM observations of surface structure and roughness and the accompanying changes in polymorphic behavior, bloom formation in milk chocolate apparently is a complex multistep process involving melting and then (re)crystallization of TAG accompanied by a significant rearrangement of the existing microstructure. It is proposed that

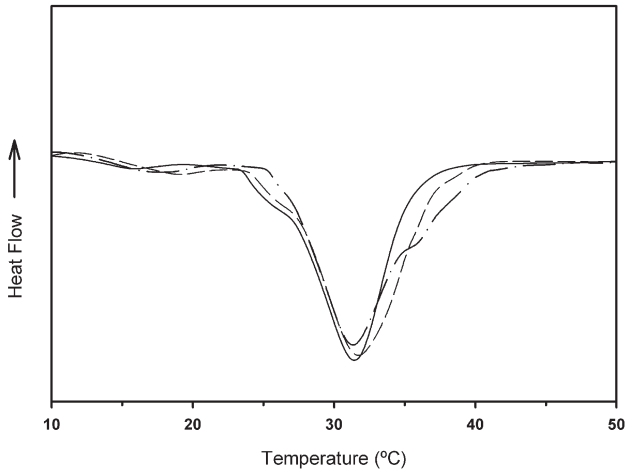

FIG. 6. DSC melting curves at 10°C/min of chocolate cycled from 20 to 34°C for one cycle (—), two cycles (– – –), and three cycles (— · —).

one of the mechanisms responsible for this structural rearrangement, termed the "pumping action" mechanism, involves the following events. Upon increasing the temperature from room temperature to a temperature below the melting point of form V and/or VI crystals, these steps likely occur: (i) the melting of lower-melting TAG; (ii) an increase in the fluidity of the milk chocolate, thereby allowing unrestricted movement of the milk chocolate components (still-intact cocoa butter crystals, milk solids, sugar, cocoa solids, etc.) throughout the chocolate mass, on a micrometer scale (based on multiple AFM images); and (iii) disruption of the ordered network structure developed through proper tempering, as evidenced by the increased roughness at 32°C (where no bloom formation was observed).

At temperatures slightly below the m.p. of form V and/or VI crystals, migration and aggregation of components will occur quite freely. At this point, there has been no formation of larger crystals responsible for cocoa butter bloom. Upon cooling, the following steps occur: (iv) Higher-melting TAG will (re)crystallize in form V or form VI crystals provided there are existing crystals that can act as templates; (v) as a result of solidification, a reduction in volume leads to a combination of drawing liquid fat and smaller crystals back into the chocolate matrix; (vi) a substantial increase in continuous phase viscosity is observed "locking-in" smaller crystals within the chocolate matrix; and (vii) larger crystals remain near the surface of the milk chocolate resulting in the visible bloom.

By using AFM, we imaged chocolate bloom caused by thermal fluctuations from stages of its early development through to a complete manifestation in a commercial milk chocolate product. Combining this visual evidence of changes in surface morphology with corresponding data on polymorphic form and melting behavior, the "pumping action" mechanism of chocolate bloom development could be elaborated. This technique holds promise as a tool for studying more of the causative factors in chocolate surface bloom under various scenarios of inducement.

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