

Impact of Storage on Dark Chocolate: Texture and Polymorphic Changes

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Abstract: Chocolate storage is critical to final product quality. Inadequate storage, especially with temperature fluctuations, may lead to rearrangement of triglycerides that make up the bulk of the chocolate matrix; this rearrangement may lead to fat bloom. Bloom is the main cause of quality loss in the chocolate industry. The effect of storage conditions leading to bloom formation on texture and flavor attributes by human and instrumental measures has yet to be reported. Therefore, the impact of storage conditions on the quality of dark chocolate by sensory and instrumental measurements was determined. Dark chocolate was kept under various conditions and analyzed at 0, 4, and 8 wk of storage. Ten members of a descriptive panel analyzed texture and flavor. Instrumental methods included texture analysis, color measurement, lipid polymorphism by X-ray diffraction and differential scanning calorimetry, triglyceride concentration by gas chromatography, and surface properties by atomic force microscopy. Results were treated by analysis of variance, cluster analysis, principal component analysis, and linear partial least squares regression analysis. Chocolate stored 8 wk at high temperature without fluctuations and 4 wk with fluctuations transitioned from form V to VI. Chocolates stored at high temperature with and without fluctuations were harder, more fracturable, more toothpacking, had longer melt time, were less sweet, and had less cream flavor. These samples had rougher surfaces, fewer but larger grains, and a heterogeneous surface. Overall, all stored dark chocolate experienced instrumental or perceptual changes attributed to storage condition. Chocolates stored at high temperature with and without fluctuations were most visually and texturally compromised.

Keywords: bloom, chocolate, descriptive analysis, polymorphism, storage

Practical Application: Many large chocolate companies do their own “in-house” unpublished research and smaller confectionery facilities do not have the means to conduct their own research. Therefore, this study relating sensory and instrumental data provides published evidence available for application throughout the confectionery industry.

Introduction

The unique chocolate matrix is a mixture of sugar and cocoa particles dispersed in a cocoa butter phase, yet its specific packing structure and particle interactions make chocolate an even more intriguing and complex substance. Chocolate texture is a combination of triglyceride packing structures (polymorphs), microstructural properties, dispersed particulates, particle size distribution, and solid fat content (SFC), the ratio of solid to liquid fat in a product.

Chocolate has a shelf life of approximately 12 to 24 mo; as chocolate is stored, structural changes occur (Bomba 1993; Subramanian 2000). With improper storage, these changes can be magnified, causing an increase in particle size, which is extremely important to mouthfeel (Morgan 1994). Minimal particle sizes detected by the human tongue are 20 to 30 μm ; control of particle size is essential for smooth mouthfeel, uniform melting, and proper volatile release (Rostagno 1969; Hoskin 1994). Various

storage conditions may lead to development of either fat bloom or sugar bloom, both of which compromise visual and textural quality. Bloom is the main cause of quality loss in the chocolate industry (Ziegler 1997). With total chocolate sales nearly \$15 billion annually in the United States, loss due to bloom formation may be substantial (Information Resources Inc. 2006). Market loss due to fat bloom is difficult to verify, since these changes arise many months after processing and often occur many steps down the distribution ladder. As bloom forms, particle size may also increase, but it is unclear whether microstructural and perceptual changes also occur. Very few studies have been conducted relating instrumental texture measurements to trained sensory panel results; also, data correlating sensory results with instrumental texture and flavor properties in stored chocolate are nonexistent.

The purpose of this study was to relate microstructural characteristics of stored dark chocolate to instrumental and sensory texture measurements. Sensory texture and flavor were determined by a trained panel and related to instrumental texture analysis. As chocolate blooms, color is significantly altered; therefore, color was also measured instrumentally throughout the study. Polymorphic transition from form V to VI may significantly impact the texture and flavor of chocolate and thus, was assessed by X-ray diffraction (XRD) and differential scanning calorimetry (DSC). Another more recently adapted instrumental technique that may give further insights to microstructural changes is atomic force

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microscopy (AFM). AFM is a technique used to illustrate nanoscale changes in chocolate microstructure during storage. Surface topography is visualized by measuring the force between the tip and the sample surface as detected by a deflection of the cantilever. A photo detector measures the deflection and a map of the surface topography is assembled. Triglyceride concentrations may be impacted by lipid polymorphic transitions or fat-bloom formation; therefore, concentrations of the major triglycerides were quantified throughout storage.

Materials and Methods

Materials

Dove® Dark Chocolate (Masterfoods Inc., Hackettstown, NJ, U.S.A.) was acquired from a local grocery store and stored immediately without wrappers under conditions stated in Table 1. Samples purchased at the same time were from the same lots. Sodium bromide salt was purchased from Leisure Time (Alpharetta, Ga., U.S.A.) and sodium chloride was obtained from Fisher Scientific Co. (Fair Lawn, NJ, U.S.A.) for use in relative humidity chambers. All solvents were purchased from Fisher Scientific Co. Triglyceride standards used for quantification included 1,3-dipalmitoyl-2-oleoyl-glycerol (POP; Sigma-Aldrich Co., St. Louis, Mo., U.S.A.), 1,3-distearoyl-2-oleoyl-glycerol (SOS; Sigma-Aldrich Co.), and 1-palmitoyl-2-oleoyl-3-stearoyl-glycerol (POS; Larodan Fine Chemicals, Malmö, Sweden).

Texture analysis

Sample texture was examined in duplicate with a TA-XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, N.Y., U.S.A.) and Texture Expert Software version 1.11. A 35-mm acrylic cylinder probe (TA-11) was used for the 2-bite compression test (20% compression). Test settings were as follows: pretest speed of 2 mm/s, test speed of 5 mm/s, posttest speed of 5 mm/s, 20% deformation, relaxation time of 5 s, and force of 20 g. Chocolate bar dimensions were 25 mm × 25 mm × 10 mm (l × w × h). Sample orientation was kept constant in all texture analyzer tests. Parameters measured included hardness (maximum force required during the first compression), cohesiveness (ratio of the area under the curves for the two-bite compression test), adhesiveness (measurement of the attractive forces of the probe to the chocolate), springiness (height that the food regains following the first bite), gumminess (product of hardness and cohesiveness), and chewiness (product of gumminess and springiness). These values represent standard parameters and calculations as devised by Bourne (1982).

Color evaluation

Color change during storage was monitored using a Minolta ChromaMeter Cr-400/410 (Konica Minolta, Mahwah, N.J.,

U.S.A.). Color was evaluated in triplicate on full chocolate samples. Numbers generated by the colorimeter represent an average lightness compared with darkness for 3 distinct color spectra (L : 100 = white, 0 = black; a : 100 = red, -100 = green; b : 100 = yellow, -100 = blue). Whiteness index (WI) is an overall measurement of the color change associated with all 3 spectra and has been used to determine bloom formation (Bricknell and Hartel 1998). WI was calculated based on the following formula (Briones and Aguilera 2005): $WI = 100 - [(100 - L)^2 + a^2 + b^2]^{0.5}$.

Differential scanning calorimetry

Melting profiles of stored chocolate were conducted in duplicate using a Thermal Advantage 2920 DSC system (TA Instruments, New Castle, Del., U.S.A.). The instrument was calibrated with indium (m.p. 156.59 °C), as well as with an empty cell to correct for baseline shifts. Melting points were determined for finely chopped chocolate samples of 1 to 2 mg, hermetically sealed in aluminum pans. Small sample sizes (<2 mg) are a more accurate representation of the sample; while larger samples (>2 mg) may mask small details (Manning and Dimick 1983). Samples were heated in the range of -20 to 60 °C at a rate of 10 °C/min. A rapid heating rate was necessary to prevent annealing (transition of lower stability polymorphs to more stable forms during heating). Melting points were reported as the temperature at which the maximum energy was absorbed by the sample, rather than onset temperature because of peak overlapping (Loisel and others 1998; Kinta and Hartel 2010).

Powder X-ray diffraction

Chocolate polymorphs were identified using XRD as described by Cebula and Ziegler (1993). Sugar was removed so it did not interfere with the diffraction pattern. In short, finely chopped chocolate (approximately 5 g) was mixed with 500 mL cold double-deionized water and allowed to set at room temp for at least 4 h to remove the sugar. Following extraction, the chocolate-water mixture was filtered under vacuum, using Whatman 1 filter paper, and dried overnight. Once dry, the sugarless chocolate was again finely chopped and stored in amber bottles until analysis.

Powder XRD patterns were recorded in duplicate at room temperature (23 °C) on a Rigaku D/Max-b X-ray diffractometer (Rigaku Corp., Tokyo, Japan). XRD is measured by Bragg's law: $n\lambda = 2d \sin \theta$; where n is a positive whole number, λ is the X-ray wavelength, d = space between crystal planes, and θ is the angle of incidence. Short d spacings are used in the 2θ range of 16 to 30° to identify polymorphs. Form V will have 4 distinct d spacings; while form VI will have only 3, thus demonstrating a shift in crystal lattice orientation (Schenk and Peschar 2004).

A sample volume of $5 \times 5 \times 0.1 \text{ cm}^3$ was pressed into a glass cell and mounted vertically in the machine. Copper radiation (Cu K_{α}) with an average wavelength of 1.5418 Å set at 45 kV and 20 mA and a 1° divergence slit was used. A 2θ scan from 18° to 26°, step of 0.008, and a scan rate of 0.2 d/min were also utilized.

Atomic force microscopy

Chocolate samples were fixed onto a gently heated glass slide. Tapping-mode AFM (Dimension 3100 AFM with Nanoscope IIIa controller; Digital Instruments, Santa Barbara, Calif., U.S.A.) was used to image a $50 \times 50 \mu\text{m}$ area of the chocolate samples. An etched silicon probe coated with aluminum (BS-300Al; Innovative Solutions Bulgaria, Sofia, Bulgaria) was used to analyze height and phase differences in duplicate samples. Sample roughness, number of grains, and average grain sizes were determined using the

Table 1—Chocolate storage conditions.

Condition	Temperature (°F/°C)	Relative humidity (%)
Ambient (storage room)	77.0/23.0	45.4 ^b
Freezer	-17.0/-27.2	40.9 ^b
Temperature fluctuations	87.0 ± 3/30.5 ± 1.7 ^a	77.0 ^b
High temperature	87.0/30.5	44.1 ^b
High relative humidity 1	77.0/23.0	57.6 ^c
High relative humidity 2	77.0/23.0	75.3 ^c

^aIncubator temperatures fluctuated every 3 h.

^bValues were obtained using a Thermo Hygrometer Humidity Measuring Stick (Cole-Parmer Instrument Co., Vernon Hills, Ill., U.S.A.) and may be approximate.

^cSalt solutions were used to attain these RH conditions.

software. Sample roughness was based on the root mean square (RMS) of height deviations of the sample, as discussed by Hodge and Rousseau (2002). Phase imaging is receptive to compositional variations, tip-sample adhesion, and viscoelasticity (Rousseau and Sonwai 2008). Surface topography and phase imaging may be combined to allow a more complete description of surface characteristics. Phase imaging provides qualitative information regarding microstructural properties not gained through topographical imaging (García and others 2007; Rousseau and Sonwai 2008).

Triglyceride monitoring by gas chromatography (GC)

Triglyceride analysis by GC was conducted using a modified method of Guyon and others (2004). Chocolate samples were diluted in iso-octane (0.25 g/10 mL) and 3 μ L was injected in triplicate. Retention times of POP, POS, and SOS (P = palmitic, O = oleic, and S = stearic acid) standards diluted in iso-octane were used to identify triglycerides in chocolate and cocoa butter. Triglycerides were quantified by constructing standard curves with pure POP, POS, and SOS.

An HP 5890 GC (Hewlett-Packard Co., Avondale, Pa., U.S.A.) coupled with a flame ionization detector (GC-FID) and Rtx-1 column (dimethyl polysiloxane, 10 m \times 0.25 m \times 0.1 m; Restek Corp., Bellefonte, Pa., U.S.A.) was used. Injector and detector temperatures were 380 and 383 $^{\circ}$ C, respectively. The oven temperature was increased from 80 to 320 $^{\circ}$ C at 30 $^{\circ}$ C/min, 320 to 380 $^{\circ}$ C at 10 $^{\circ}$ C/min, and held at 380 $^{\circ}$ C for 20 min. Helium was the carrier gas at a flow rate of 3.2 mL/min. Chromatograms were analyzed using PeakSimple version 3.21 (SRI Instruments, Torrance, Calif., U.S.A.).

Sensory evaluation

Total of 10 panelists (3 male and 7 female, aged 18 to 39) were recruited and trained in the technique of descriptive analysis. Judges were trained for 20 h over 4 wk. Panelists were trained to analyze chocolate texture and flavor using 15-cm line scales with word anchors. Time-intensity measurements were used for melting time. Rinsing protocol, references, and terms were generated by the panel. Texture attributes generated by the panel included hardness, cohesiveness, chewiness, fatty mouthcoating, dry mouthfeel, and toothpacking. For flavor, terms established by

the panel included sweetness, bitterness, chocolate flavor, cream flavor, and roasted aftertaste. Panelists analyzed all samples in duplicate, using 5 to 6 samples/session. Compusense[®] Five 4.2 software (Compusense Inc., Ontario, Canada) was used for data collection. Samples were served in 2-oz plastic cups with lids and labeled with random 3-digit numbers, evaluated under black light and ambient temperature and relative humidity. Time-intensity measurements of chocolate melting time were analyzed by placing a previously measured sample (0.25 to 0.30 g) between the tongue and roof of the mouth and rating over 2 min or until the sample was completely melted.

Statistical analysis

Data were analyzed using Statistical Analysis Software (SAS) version 9.1 (SAS Institute Inc., Cary, N.C., U.S.A.) to determine the analysis of variance (ANOVA) and Fisher's least significant difference (LSD) for all results. Mean ratings of significant attributes ($P < 0.05$) were further analyzed using covariance matrices for principal component analysis (PCA) in SAS. Linear partial least squares regression analysis (PLS) was conducted to relate the instrumental and sensory measurements using Unscrambler[®] software (CAMO Technologies Inc., Woodbridge, N.J., U.S.A.). PLS2 was used to correlate all statistically significant instrumental and sensory attributes, while relationships between instrumental texture data and a single sensory attribute were evaluated by PLS1.

Results

Texture analysis

Storage condition significantly impacted instrumental hardness, cohesiveness, adhesiveness, springiness, gumminess, and chewiness (Figure 1). Chocolate samples stored in the freezer were the hardest, most cohesive, gummy, and chewy. Samples stored at high relative humidity were the most adhesive by instrumental measures.

Color evaluation

Storage at various temperature and relative humidity conditions significantly altered all color spectra (L^* , a^* , b^* , and WI; $P < 0.05$). Samples stored at high temperature with fluctuations

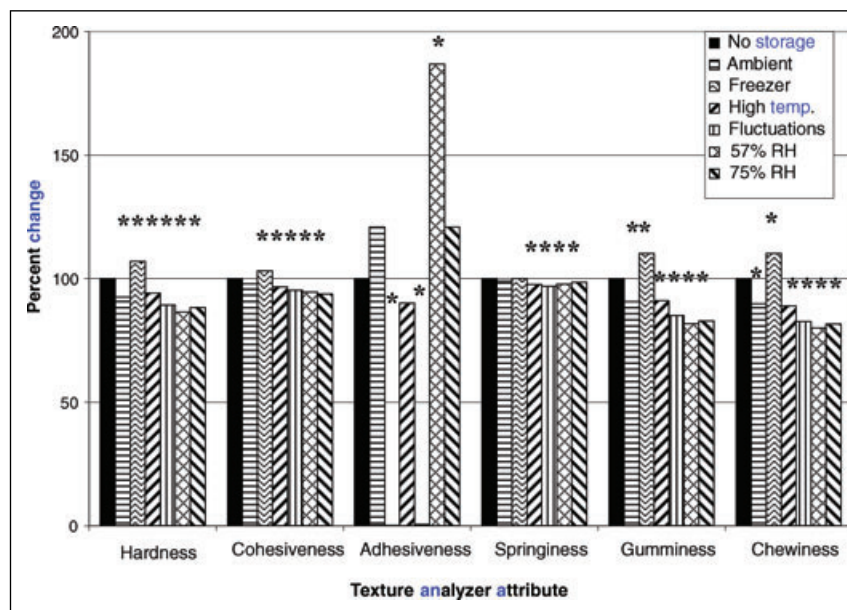


Figure 1—Percent change in texture analyzer results after 8 wk of storage (0 wk = 100%). Texture attributes were significantly impacted by storage ($P \leq 0.05$).

became significantly lighter in color and more yellow and red (Figure 2). According to the WI calculation, chocolate samples stored at high temperature with fluctuations were significantly lighter in color, as apparent by a 70% increase in WI following 8 wk of storage at these conditions. Chocolate stored 4 wk in the freezer had a 4% increase in WI, while a 6% increase was found following 8 wk of storage at high temperature without fluctuations. Chocolate stored at high relative humidity actually darkened in color, as noted by the negative numbers associated with percent change in WI.

Differential scanning calorimetry

As chocolate evolved from form V to form VI, melting point increased due to tighter chain packing. The average melting temperature for all chocolate samples is presented in Table 2. Fresh chocolate samples had an average melting point of 33.5 °C. Storage treatments significantly impacted the melting temperature of chocolate ($P < 0.05$). More specifically, melting points for chocolate stored at high temperature with fluctuations were significantly higher (36.3 and 36.1 °C for 4 and 8 wk of storage, respectively) than all other samples. Storage at high temperature without fluctuations led to a melting temperature of 35.4 and 35.8 °C for 4 and 8 wk of storage, respectively. The increase in melting point indicated that chocolate stored 8 wk at high temperature without fluctuations and 4 wk at high temperature with fluctuations may

have transitioned to form VI. Confirmation of the transition was determined by XRD patterns of the samples.

Powder X-ray diffraction

Although DSC thermograms may demonstrate an increase in melting point and the colorimeter may illustrate an increase in lightness or WI due to bloom, polymorphs can only be identified

Table 2—Average chocolate melting point determined by DSC and polymorphs by XRD.

Storage condition	Melting point (°C) ^a	Polymorph
0 wk	33.5 ± 0.5 ^{ef}	V
4 amb	34.0 ± 0.0 ^{de}	V
8 amb	33.7 ± 0.2 ^{ef}	V
4 fre	34.4 ± 0.3 ^d	V
8 fre	33.4 ± 0.1 ^f	V
4 wk 87 F	35.4 ± 0.0 ^c	V
8 wk 87F	35.8 ± 0.2 ^{bc}	VI
4 wk 87 ± 3 F	36.3 ± 0.1 ^b	VI
8 wk 87 ± 3 F	36.1 ± 0.4 ^b	VI
4 wk 57 %RH	33.7 ± 0.2 ^{ef}	V
8 wk 57 %RH	33.5 ± 0.2 ^f	V
4 wk 75 %RH	33.5 ± 0.2 ^{ef}	V
8 wk 75 %RH	33.9 ± 0.3 ^{def}	V

^aResults stated as mean ± SD.

^bto^f Numbers with same letters were not significantly different at $P \leq 0.05$.

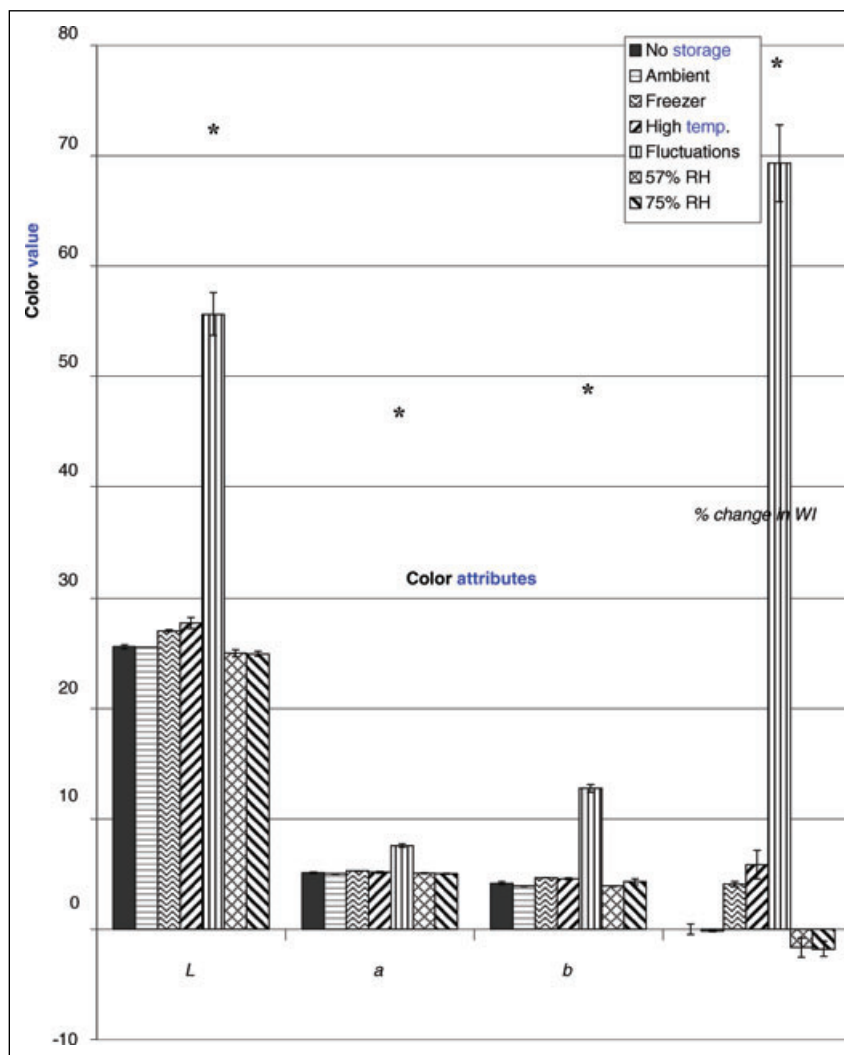


Figure 2—Instrumental color data following 8 wk of storage (mean ± SEM). Color attributes were significantly impacted by storage ($P \leq 0.05$). L: 100 = white, 0 = black; a: 100 = red, -100 = green; b: 100 = yellow, -100 = blue. WI = whiteness index.

by their *d* spacings through XRD. A typical form V diffraction pattern contains 4 distinct peaks in the scan range of 22 to 24°, while form VI contains only 3. In the transition from V to VI, the first peak becomes less pronounced and the third and fourth peaks combine to form a single peak, signifying a rearrangement in crystal packing.

Chocolate polymorphs for each storage condition are listed in Table 2. Chocolate stored 8 wk at high temperature without fluctuations and at least 4 wk with fluctuations had transitioned to form VI. Chocolate stored 4 wk at high temperature without fluctuations contained a mixture of forms V and VI, as illustrated by the 3rd and 4th peaks beginning to merge in the diffraction pattern.

Atomic force microscopy

Surface topography determination by AFM is a tool used to visualize the impact of storage on the sample surface at the nanoscale level. Furthermore, AFM software can be used to calculate surface roughness, particle number, and particle size. Three-dimensional AFM images are presented in Figure 3. The 3D and phase images of fresh chocolate (Figure 3A and 3B) illustrate a uniform, homogeneous surface with many small particles. Samples stored at ambient conditions experienced a gradual decrease in uniform crystal arrangement with increased storage time (Figure 3C to 3F). Freezer storage slightly exacerbated the increase in crystal size as shown in Figure 3G to 3J. High-temperature storage without fluctuations had a much different surface topography and phase distribution than observed in samples stored with fluctuations. Samples kept at high temperatures without fluctuations had a relatively smooth surface with several jagged crystals (Figures 3K to N). Phase imaging of these samples illustrated an initial phase change in a few crystals formed (Figure 3L). Further storage at high temperature without fluctuations showed a definite phase change in the large, jagged crystals that had formed (Figure 3N). Chocolate stored at high temperature with fluctuations caused a surface covered with relatively few, but large crystals (Figure 3O to 3R). These crystals are not nearly as tall or jagged as those found on the high-temperature samples, possibly due to the melting, recrystallization, and/or crystal aggregation indicative of temperature fluctuations during storage. Phase imaging of these structures illustrated an evident change in phases that were exacerbated with further storage (Figure 3P and 3R). Storage at high temperatures demonstrated a definite shift from a homogeneous to a heterogeneous sample surface. Storage at high relative humidity had little effect on the surface topography (Figure 3S to 3Z), except chocolate stored 8 wk at 75% relative humidity (Figure 3Y) showed the growth of several larger crystals, which upon investigation revealed a contrast in phase of these crystals with the surroundings (Figure 3Z).

Surface roughness and grain size, number, diameter, and height were calculated via Nanoscope III software on 2D images of the samples shown in Figure 3. All chocolate surface measurements were significantly ($P < 0.05$) impacted by storage in various conditions. According to Figure 4, the RMS roughness significantly increased from fresh samples (approximately 120 nm) to ambient (approximately 175 to 260 nm), frozen (approximately 440 to 550 nm), high temperature (approximately 350 to 675 nm), temperature fluctuations (approximately 610 to 650 nm), and high relative humidity (approximately 160 to 290 nm). Also, according to this plot, the 3 conditions leading to form VI transition all had a surface roughness >600 nm. During storage, grain number significantly decreased and grain size dramatically increased

(Figure 5) in almost all conditions. Storage at ambient conditions and high relative humidity had the least impact on grain size and number. Dark chocolate stored at conditions leading to form VI transition were characterized by having <37 grains in an area of $50 \times 50 \mu\text{m}$ and an average particle size $>38 \mu\text{m}^2$. According to Ros-tagno (1969) and Hoskin (1994), food products with a particle size $>20 \mu\text{m}$ may be detected as grainy by the human tongue; therefore, chocolate stored at frozen and high-temperature conditions may have an altered texture profile due to increased crystal size.

Triglyceride monitoring by gas chromatography (GC)

As lipid polymorphs transitioned from form V to form VI, it was anticipated that triglyceride concentrations may vary. Both POS and SOS were significantly ($P < 0.05$) impacted by storage, yet no specific trends were found between triglyceride concentrations and storage condition (Figure 6). Unstored chocolate was significantly higher in POS than all other conditions, while SOS was higher in unstored chocolate and chocolate stored 8 wk in ambient conditions.

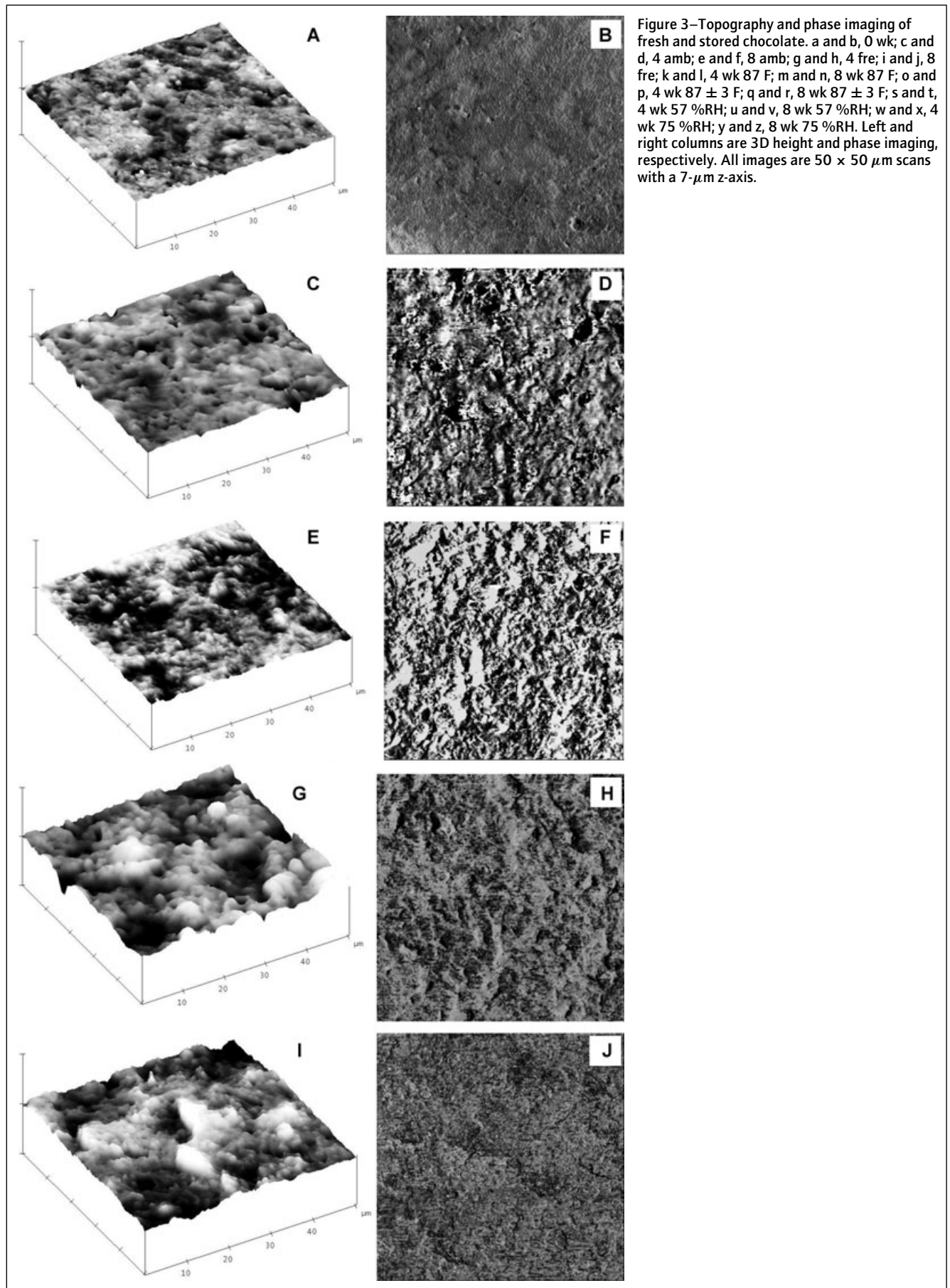
Sensory evaluation

Descriptive panelists determined that 8 of the 12 attributes assessed were significantly altered ($P < 0.05$) during chocolate storage (Table 3). Judges were a significant source of variation in 10 of the 12 attributes. This is typical of descriptive analysis panels, where panelists may either not be using the entire scale or the panelists may be using different parts of the scale to rate samples. Chocolate storage condition (treatment) was a significant source of variation in all attributes, except fatty mouthcoating, bitter taste, and chocolate and roasted flavor. Replication was a significant source of variation for hardness, fatty mouthcoating, melting time, sweet taste, and chocolate and cream flavor, signifying the panel was not reproducible over replications for these attributes. Judge by treatment interaction was a significant source of variation for chewiness, dry mouthfeel, and bitter taste, indicating the panelists did not agree on the intensity differences for these attributes across the chocolate samples. Judge-replication interaction was also a significant source of variation for all attributes; signifying panelists did not rate samples in the same order upon replications for these attributes. Treatment (storage condition) by replication interaction was only significant for hardness, chewiness, dry mouthfeel, and toothpacking; indicating that storage conditions caused uniform changes in the chocolate samples, yielding reproducible results across most attributes.

All significant attributes assessed by the panel, including T-I measurements, were evaluated by PCA as depicted in Figure 7. Overall, samples were separated into 2 groups, high-temperature storage and the remainder of the samples. Toothpacking, hardness, and melting time were negatively correlated with chewy, cohesive, dry mouthfeel, sweet taste, and cream flavor. Chocolate stored at high temperature with and without fluctuations was harder, more toothpacking, and took longer to melt. Samples stored without fluctuations were more toothpacking than chocolate with temperature fluctuations. Fresh chocolate and chocolate stored at ambient, frozen, and high relative humidity was more cohesive, chewier, sweeter, and had higher intensities of dry mouthfeel and cream flavor.

Sensory and instrumental correlation

Instrumental and sensory correlation is difficult and complex, especially with the multitudes of instrumental techniques employed in this experiment. Overall, storage treatment caused 8



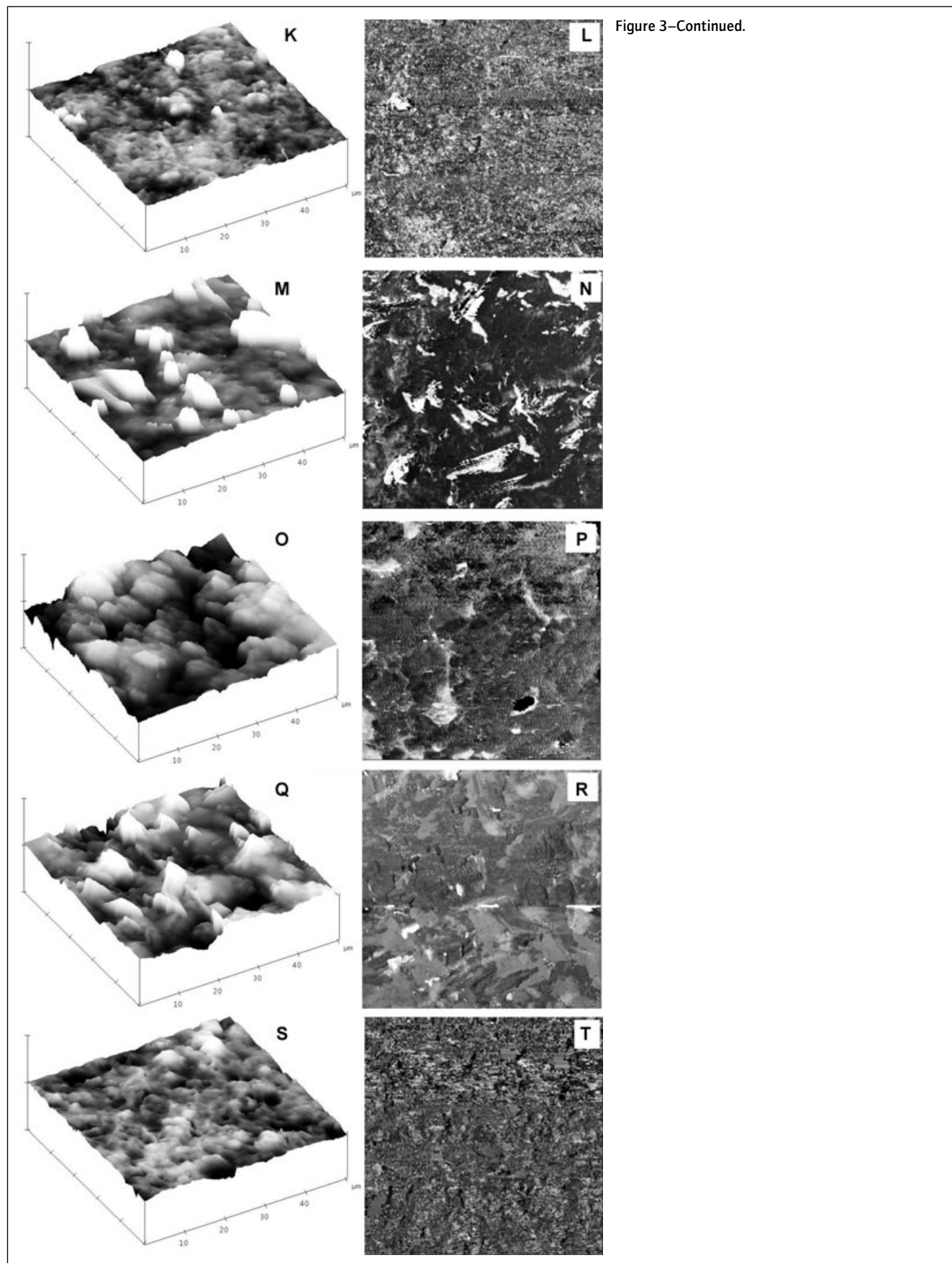


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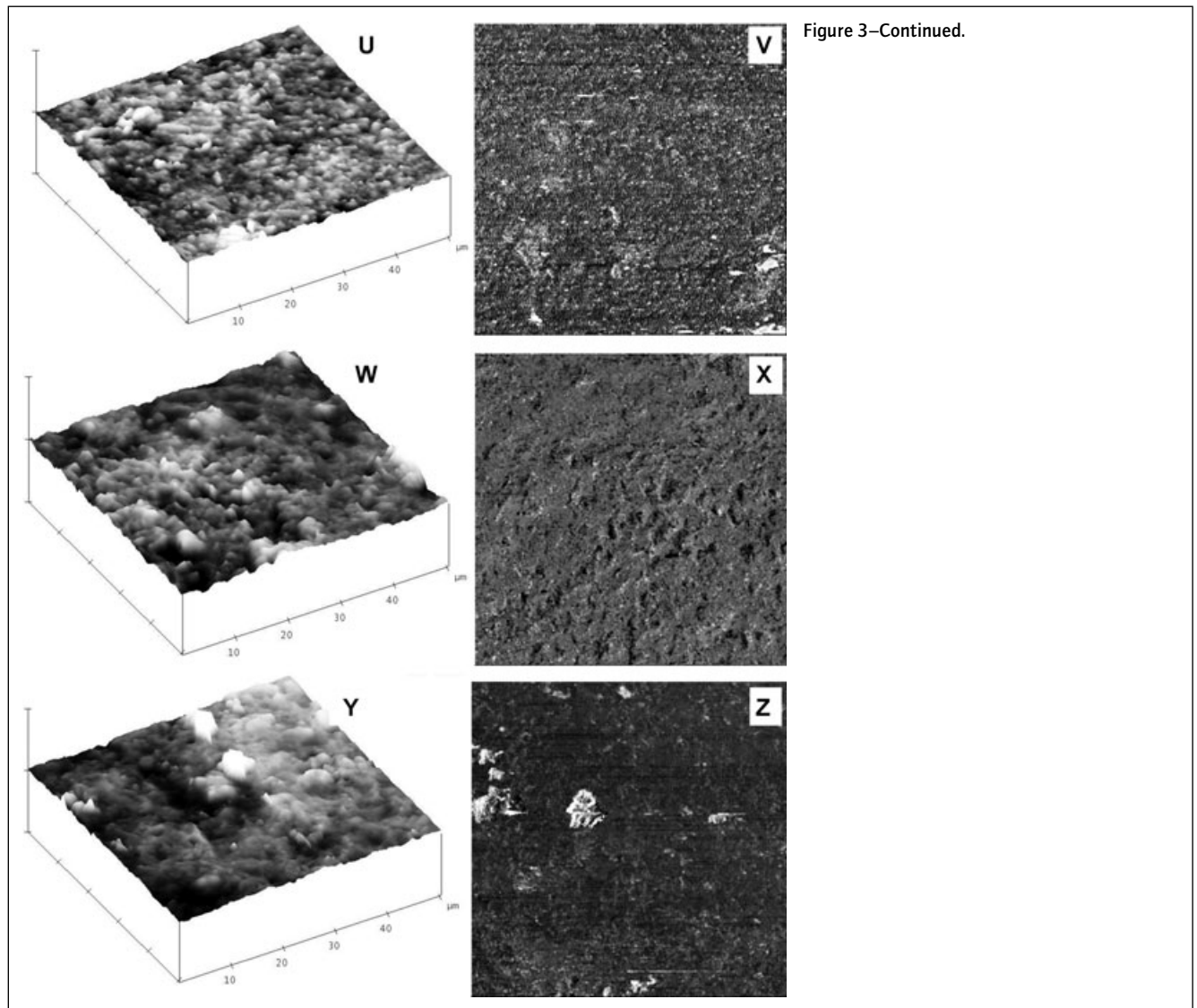


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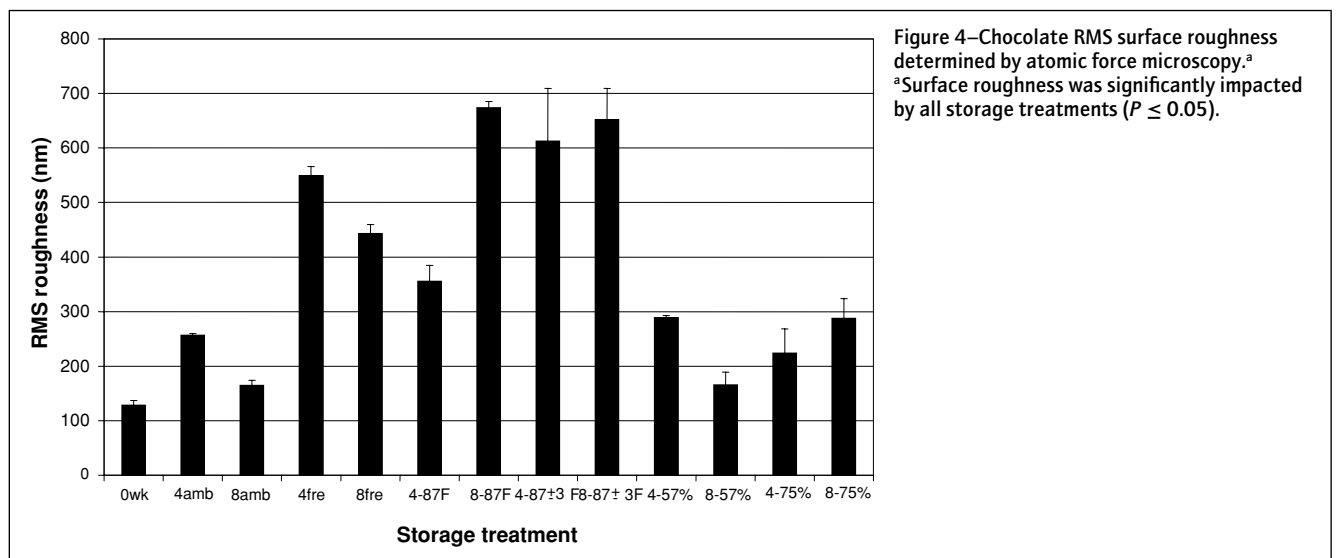


Figure 4-Chocolate RMS surface roughness determined by atomic force microscopy.^a ^aSurface roughness was significantly impacted by all storage treatments ($P \leq 0.05$).

sensory attributes and 20 instrumental variables to be significantly altered. Correlations of sensory and instrumental results were determined by cluster analysis, principal component analysis, and partial least squares linear regression. For ease of discussion, only PCA biplots will be illustrated.

Color, AFM measurements, DSC melting point, and the triglyceride SOS were highly correlated with sensory hardness, melting time, and toothpicking (Figure 8). High-temperature storage caused samples to be lighter in color, as shown by an increase in WI; these samples were also harder and took longer to melt by both sensory and DSC measures, had rougher surface area, and larger grain size, diameter, and height. Grain number was negatively correlated with previously mentioned instrumental assays, but positively correlated with sensory cohesiveness, chewiness, sweet taste, and cream flavor of chocolate. Unstored chocolate, room temperature, frozen, and high relative humidity storage were associated with cohesive and chewy texture.

Linear PLS, and more specifically PLS2, for multivariate analysis is commonly used to evaluate relationships between sensory and instrumental matrices (Hough and others 1996; Blazques and others 2006; Gonzalez Viñas and others 2007; López-Feria and others 2008). A PLS2 biplot of sensory and all instrumental results explained 67% of instrumental data and 72% of sensory data. Instrumental texture and sensory results explained 96% of texture analyzer data, but only 53% of sensory data. Color and sensory results explained 92% of color data and 56% of sensory data; while a plot of melting temperature and sensory results showed that 100% of DSC measurements and 68% of sensory results were explained by the plot. A biplot of AFM and sensory results explained 94% of AFM data and 54% of sensory results; while 99% of TAG results and only 21% of sensory data were explained in a plot of TAG and sensory results. Therefore, sensory results were best explained by DSC measurements, while a combination of all instrumental results also gave adequate results.

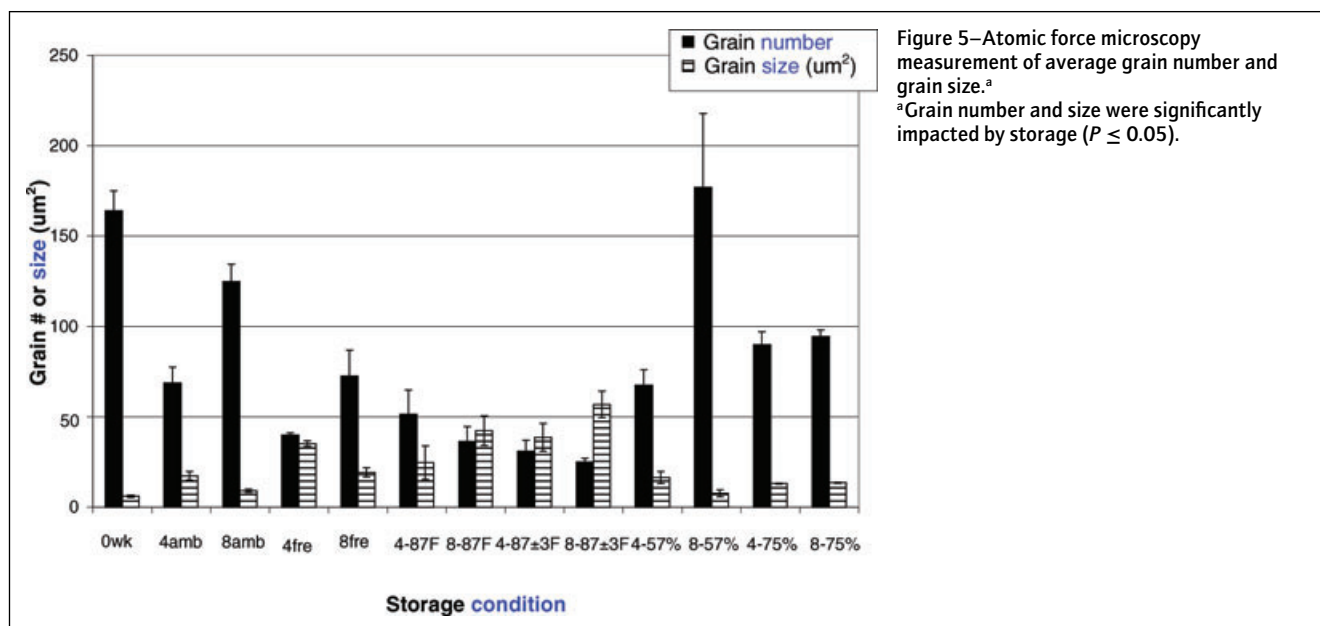


Figure 5—Atomic force microscopy measurement of average grain number and grain size.^a

^aGrain number and size were significantly impacted by storage ($P \leq 0.05$).

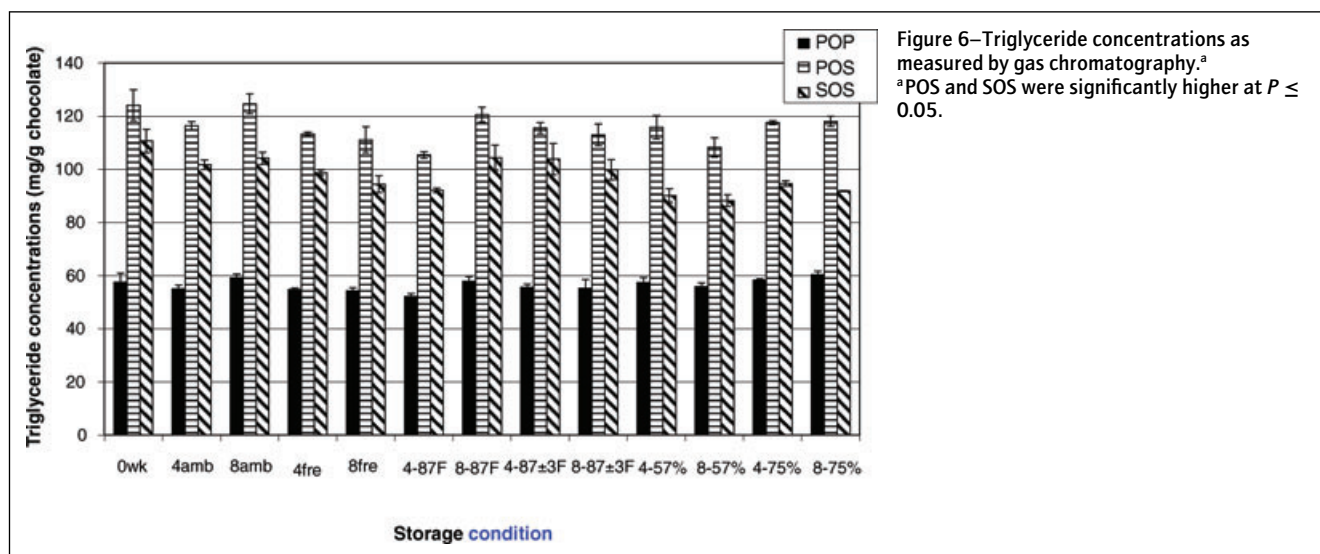


Figure 6—Triglyceride concentrations as measured by gas chromatography.^a

^aPOS and SOS were significantly higher at $P \leq 0.05$.

Table 3—ANOVA for sensory attributes rated for chocolate samples.^{a,b}

Modality/attribute	Judge	Treatment	Replication	J * T	J * R	T * R
Texture						
Hardness	4.27***	11.65***	13.19***	1.04	4.40***	2.90**
Cohesiveness	8.74***	13.14***	0.92	0.95	4.35***	1.53
Chewiness	2.85**	4.96***	2.65	1.63**	16.27***	3.87***
Fatty mouthcoating	5.24***	0.77	17.37***	0.91	8.70***	1.40
Dry mouthfeel	1.48	3.58***	1.21	1.50*	3.17**	1.96*
Toothpacking	7.59***	2.25*	5.86*	1.12	6.34***	1.92*
Melting	11.77***	47.08***	40.25***	0.95	38.89***	1.14
Flavor						
Bitter	2.93**	0.90	0.71	1.38*	7.96***	0.59
Sweet	1.80	2.30*	20.34***	0.94	3.15**	0.84
Cream	5.14***	3.02**	17.74***	1.07	14.44***	1.04
Chocolate	6.25***	1.54	9.61**	0.97	7.70***	0.59
Roasted	7.01***	0.83	3.00	1.15	14.35***	0.70

^aF-ratios used for source of variation.^b*, **, *** significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Discussion

On first glance, chocolate is a seemingly simple substance, but in reality it is a complex matrix containing a continuous phase of cocoa butter with sugar and cocoa particles uniformly dispersed throughout. The driving force behind the polymorphic transition from form V to VI is attributed to triglyceride packing, more specifically the “ethyl-ended holes” associated with SOS causing form V to be metastable and likely to drive the transition to form VI (Peschar and others 2004). Both form V and VI are triple-chain-packed β forms and are crystallized in a triclinic subcell (T_{\parallel}). Temperature fluctuations enhance the transition from form V to VI by increasing the liquid portion of chocolate as temperature increases and solidifying as temperature decreases. Higher temperatures also cause increased molecular mobility, which may be exacerbated with temperature cycling (Bricknell and Hartel 1998). Temperature fluctuations may increase the possibility of melt-mediated polymorphic transformation, where form V dissolves in the melt and form VI crystallizes upon cooling (Sato and Koyano 2001).

The link between lipid polymorphism and fat bloom is still unclear. Chocolate must first transition from form V to VI before fat bloom may occur; although chocolate can exist in form VI without fat bloom formation (Adenier and others 1993; Bricknell and Hartel 1998). Thus, lipid polymorphism and fat bloom are 2 related, yet separate, processes and must be treated as such during characterization, as apparent in dark chocolate samples stored 8 wk at high temperature without fluctuations. These samples had transitioned to form VI, but did not show a significant increase in color data, including WI. In contrast, temperature fluctuations dramatically impacted both polymorphic transition and fat bloom formation, due to increased molecular mobility of the liquid cocoa butter fraction.

The structural rearrangement associated with lipid polymorphism, and eventually fat bloom, causes a compaction within triglyceride planes, impacting textural perception and increasing melting point (Wille and Lutton 1966). Melting point determination indicated that dark chocolate that had transitioned to polymorphic form VI had a melting point of at least 35.5 °C, while

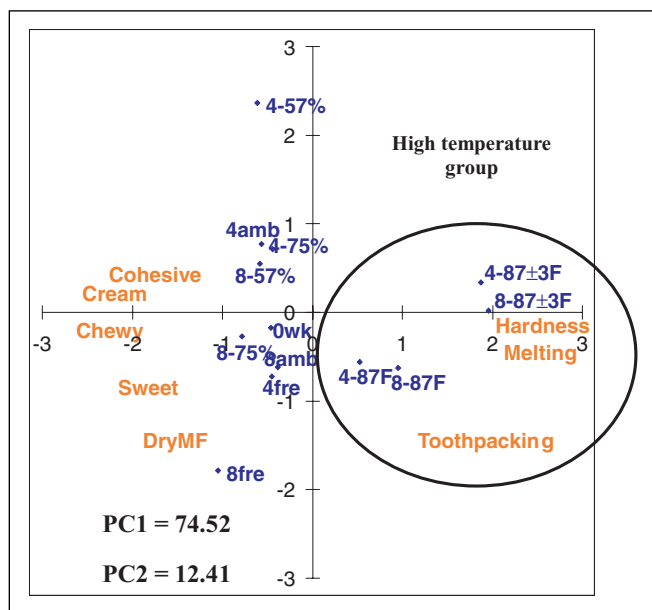


Figure 7—Principal component analysis biplot of significant sensory attributes.

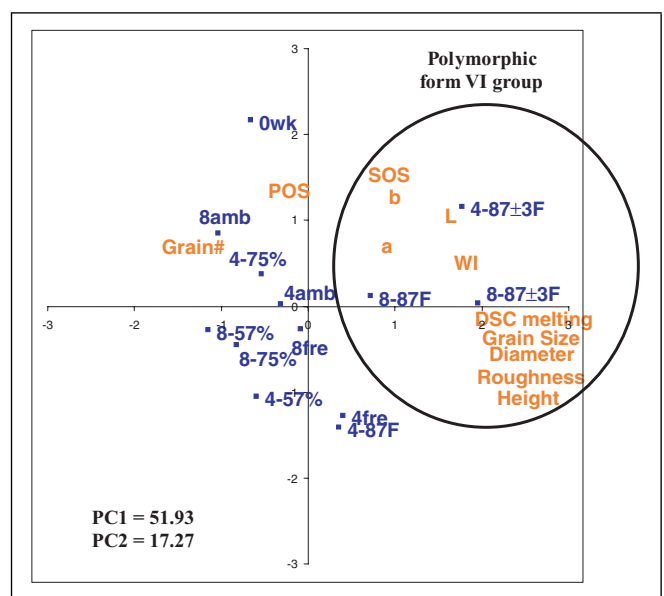


Figure 8—Principal component analysis biplot of instrumental color, AFM, DSC, and TAG results.

samples that contained fat bloom had a melting point slightly higher ($>36^{\circ}\text{C}$). Chocolate stored 4 wk at high temperature without fluctuations was on the verge of transitioning to form VI according to XRD pattern analysis. DSC results also showed a melting point of 35.4°C , very close to that of the chocolate in form VI. A definite phase contrast was notable in all samples that had transitioned to form VI, suggesting a potential phase shift or separation in chocolate stored at high temperatures.

It is likely that the driving force behind the polymorphic transition to form VI is specific triglyceride rearrangements. Concentrations of POP, POS, and SOS triglycerides in chocolate did not vary with blooming. The triglyceride found in the highest concentration in cocoa butter is POS, which dominates the structure of the triclinic subcell (Peschar and others 2004). Cocoa butter in form V and SOS had similar triglyceride packing, while POS and cocoa butter in form VI also had similar structures (Peschar and others 2004). Therefore, slight melting of the chocolate during temperature fluctuations may cause POS to melt, thus recrystallizing in form VI. Although triglyceride concentrations did not change in a storage-dependent manner, their structural rearrangements are the driving force behind lipid polymorphism.

The polymorphic transition to form VI was consistent with a significant increase in surface roughness ($>600\text{ nm}$), decrease in grain number (<37 grains in an area of $50 \times 50\ \mu\text{m}$), and increase in grain size ($>38\ \mu\text{m}^2$). Chocolate in polymorphic form VI that also contained fat bloom (such as after storage at high temperature with fluctuations) had a slight decrease in surface roughness, fewer larger grains, and a definitive compositional difference in the chocolate microstructure. Crystal shape also varied depending on storage condition. Chocolate stored at high temperature without fluctuations had rough, jagged crystals. Samples stored with fluctuating temperature had smoother, but larger, crystals, which could increase light diffraction off the surface, thus creating a lighter appearance. Grain size significantly impacts physical perception, as crystal sizes $>20\ \mu\text{m}$ may be perceived as grainy by the human palate (Rostagno 1969; Hoskin 1994). Since cocoa butter is the continuous phase in chocolate and structural rearrangement occurs with storage in various conditions, it is believed that the microstructural and organoleptic properties may also be altered. Dulling of the chocolate surface is due to clusters of fat crystals measuring $>5\ \mu\text{m}$ that protrude from the surface and scatter light (Lohman and Hartel 1994). Milk chocolate measured by AFM has a surface roughness of 278 nm, which increased to 736 nm when severely bloomed, similar to the current study (Hodge and Rousseau 2002).

Chocolate stored at high temperature without fluctuations developed several sharp, needle-like crystals and were not as hard as samples stored with temperature fluctuations containing a crystal network of several crystal agglomerations or overlapping crystals. Although SFC strongly influences mechanical properties of chocolate, it is not a good predictor of hardness (Narine and Marangoni 1999). Hardness of lipid blends with the same SFC may vary (Braipson-Danthine and Deroanne 2004). Smaller particle size in chocolate has been shown to be highly correlated with increased hardness (Afoakwa and others 2009). Therefore, hardness is not attributed to SFC, but to the crystalline microstructure and particle size distribution.

Chocolate texture was determined by both instrumental and sensory measurements. Texture analyzer results did not discriminate samples in the same manner as descriptive analysis, possibly due to product deformation during analysis. As chocolate tran-

sitioned to form VI and formed fat bloom, the samples became harder and more fracturable. Chocolate stored at high temperature with fluctuations would crumble when pressure was applied. It is possible that the instrument could not separate these 2 attributes or that an optimal deformation level (percent compression) could not be found. Previous research demonstrated that instrumental texture analysis is not reliable and only instrumental and sensory hardness are correlated (Full and others 1996; Guinard and Mazzucchelli 1999; Lee and others 1999; Gambaro and others 2002; Peschar and others 2004; Andrae-Nightingale and others 2009). Unfortunately, sensory panels are difficult to maintain and may be less reproducible than instrumental measures.

Overall, sensory results indicated that the polymorphic transition to form VI caused chocolate samples to be harder, less cohesive (more fracturable), less chewy, more toothpacking, and have a longer melting time, less sweet taste, and less cream flavor. As fat bloom formed on chocolate stored at high temperature with fluctuations, these undesirable organoleptic qualities were intensified. The decrease in sweet taste and cream flavor could be due to SFC and volatile release of the chocolate. In previous studies, solid fat index (SFI) of milk chocolate had a significant impact on sweetness and flavor perception through sensory testing (Daget and Vallis 1994). A lower SFI caused an increase in sweetness and flavor perception, because most volatiles are already in the liquid phase of the lipid matrix. Unfortunately, SFC or SFI determination using nuclear magnetic resonance (NMR) is a destructive process because chocolate must be melted to fit in glass tubes. Tempering and storage in glass tubes may not have the same effect as storage open to atmospheric conditions. Solid state NMR (SS-NMR) is a possible nondestructive method, but equipment and time limitations did not allow use of this method in the current study.

Chocolate storage studies are not new, but few have intertwined studies of quality from both a sensory and instrumental approach. This is the first time a descriptive sensory panel and multiple instrumental analyses have been used to determine the impact of lipid polymorphism and fat bloom formation on stored chocolate samples. The current study provides a very good starting point for future studies to be conducted relating sensory and instrumental data to newly manufactured chocolate, which is currently underway in our laboratory. The results of this study are very relevant to the chocolate industry, as large companies may do the research “in-house” but the results are not widely distributed and many small companies do not have the means to carry on research of their own.

Conclusions

Storage of dark chocolate in various conditions significantly impacted several texture attributes. Chocolate stored 8 wk at high temperature and 4 wk with fluctuations caused polymorphic transition from form V to VI as determined by XRD and DSC. Temperature fluctuations exacerbated the polymorphic transition. Samples that had transitioned to polymorphic form VI (high temperature with and without fluctuations) were harder, more fracturable, more toothpacking, had a longer melt time, less sweet taste, and less cream flavor. AFM phase imaging showed that these samples had a compositional difference noticeable at the nanoscale level, possibly due to phase separation during the polymorphic transition. These samples also had significantly rougher surfaces, and fewer, larger grains than chocolate stored at ambient, frozen, and high relative humidity conditions. More specifically, storage of chocolate at high temperature without fluctuations resulted in

larger, needle-like grains, while chocolate stored with temperature fluctuations resulted in large, smooth grains due to melting and recrystallization of lipid crystals. Chocolate stored at high temperature (form VI) were the most visually and texturally compromised. Storage in all conditions had a detrimental effect; while storage at high temperature with fluctuations caused the most negative changes. Overall, storing chocolate at constant temperature and relative humidity is recommended.

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