Particulate Effects in Chocolate on Fat Bloom during Storage

By

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Abstract

Chocolate can be considered as a dispersion of solid particles in liquid fats. Solid particles such as crystallized cocoa butter, cocoa powder and sugar particles make up of 90-95% mass in chocolate while only 5-10% mass in chocolate at room temperature is in the liquid phase. Therefore, the nature of the solid particles is likely to have a significant effect on fat bloom in chocolate during storage. One theory about mechanisms of fat bloom during storage is that it is initiated by high-melting fats in cocoa butter dissolving in the low-melting fats, migrating to and recrystallizing on the chocolate surface. Thus, fat bloom is highly related to factors such as solid fat content (SFC), type of fats and storage conditions, which would change the fat dissolution, migration and recrystallization steps. Further, surface properties of nonfat particles also have a significant effect on particle interactions in chocolate potentially leading to different fat bloom results. Therefore, it can be hypothesized that particulate factors such as solid particle composition in the fat phase, particulate concentration, type of particles, shape and surface properties of particles influence bloom formation during storage.

Based on these rationales, four phases were designed to study the effect of fats and non-fat particles on bloom formation. In phase one, nonsugar chocolate model systems were designed with different SFCs while the effects of SFC and low-melting fat type on bloom whiteness were investigated. In phase two, effects of storage temperature fluctuation frequency of nonsugar model systems with canola oil were evaluated. In phase three, the chocolate model systems were modified by gradually replacing cocoa powder with four types of sugars at different levels to investigate the effects of sugar type and sugar concentration on fat bloom by three different bloom evaluations. Also, interactions of different sugar particles in cocoa butter

were quantified by Casson viscosity, sedimentation volume and surface tension in order to evaluate their correlation with bloom extent. In phase four, the sharp surface of sucrose was rounded and bloom in chocolate model systems with different sugar shape was investigated.

It was shown that increasing SFC and increasing temperature fluctuation frequency in nonsugar model systems, reducing sucrose concentration (more cocoa powder) and adding 0.5% lecithin in sucrose model systems significantly decreased the rate of bloom and the final bloom extent. Further, sucrose crystals had the highest bloom level over the other three types of sugar particles, whether crystalline or amorphous. Bloom results showed some strong and positive correlation with rheology sedimentation results in the sucrose system while the correlation was weak in the other three systems. Additionally, the surface modification process with regular washing and drying significantly reduced bloom in chocolate model systems. There was a negative correlation between surface circularity and bloom extents but the correlation was not strong in all sugar systems.

Overall, particulate factors such as SFC, sugar type, sugar concentration, presence of lecithin and particle surface influenced fat bloom during storage in chocolate model systems. These effects were strongest in sucrose systems compared to the other three sugars. There was some correlation between particle interaction and bloom as well as between surface circularity and bloom, but not all sugars followed the same trend.

Dedication

This dissertation is dedicated to my family and friends, my son, my mum and everyone who helped and contributed to this work.

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I would like to thank my advisor, Dr. Rich Hartel for the guidance throughout my graduate years. You taught me how to be a good scientist with passion, curiosity and rigorous work. Thank you for your understanding and support for me to balance research and family. This work cannot be finished without your endless conversations and numerous help with the edits. Thank you for providing me with opportunities for teaching. You taught me how to become an interesting and knowledgeable teacher.

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1 Introduction

Fat bloom in chocolate is a major issue in the confectionery industry. This whitish coating occurs on the chocolate surface with a loss of gloss, which greatly influences its appearance, quality and shelf life with reduced customer acceptance. There are several types of fat bloom including bloom caused by incompatible fats, bloom due to improper tempering, and storage bloom (Timms, 2003; Hartel and Lonchampt, 2004). Despite decades of research, the mechanisms of storage bloom are still unclear. There are several theories on the cause of storage bloom including the polymorphic transition theory and the fat dissolution, migration and recrystallization theory. The polymorphic transition theory suggests that the transition of cocoa butter in chocolate from the βV to βVI polymorph is the cause of bloom since βVI polymorph is always present in chocolate with bloom (Wille and Lutton, 1966; Berger at al., 1979; Cebula and Zeigleder, 1993; Sonwai and Rousseau, 2006). Bricknell and Hartel (1998) suggested that the transition from βV to βVI polymorph is not a sufficient cause of bloom as they found their chocolate with a polymorphic transition from βV to βVI had no visual bloom after storage. The fat dissolution, migration and recrystallization theory suggested that bloom is caused by temperature fluctuation with the following three steps: (1) dissolution of high-melting fats in cocoa butter in low-melting fats, (2) migration of liquid fats onto the chocolate surface by capillary flow or diffusion, and (3) recrystallization of cocoa butter on the chocolate surface (Hartel, 1999; Matsuda et al., 2001; Hartel and Lonchampt, 2004; Dahlenborg, 2014).

Based on the fat dissolution, migration and recrystallization theory, bloom may be caused by various factors that influence any or all of the three steps of bloom formation. Chocolate is a complex system made mainly with particles dispersed in the fat phase. There are about 90-

95% solid particles and only 5-10% mass is in the liquid fat (Hartel, 1999; Hartel et al., 2016). It is hypothesized that particles may have a great effect on bloom with its influence on the recrystallization and migration steps. Numerous factors may change the particulate effects on fat bloom. Solid fat content (SFC), the amount of crystalline fats in the fat phase, were reported to have negative correlation with bloom (Ramsom-Painter et al., 1997; Ali et al., 2001). Sugar particles also have different effects on bloom. Anhydrous glucose, maltitol and amorphous sugars were reported to reduce bloom (Cerbulis, 1969; Bricknell and Hartel, 1998; Son et al., 2018), whereas crystalline sucrose was reported to promote bloom (Bricknell and Hartel, 1998). Further, different types of emulsifiers were also found to be bloom inhibitors (Easton et al., 1952; DuRoss et al., 1965; Weyland, 1994; Hartel and Lonchampt, 2004). However, the mechanism of how these particles influence bloom is still unknown with little study to quantify the particulate effects in the chocolate system.

In this study, chocolate models systems were developed by dispersing cocoa powder in the fat phase (cocoa butter or cocoa butter stearin with liquid oils) while gradually replacing cocoa powder with sugar particles (sucrose, maltitol, corn syrup solids and polydextrose), with or without 0.5% lecithin. The bloom extents were evaluated by whiteness index, white area percentage and visual bloom level. Also, particulate interactions in the dispersion systems were quantified by Casson viscosity, sedimentation volume and contact angle. The aim of this study was to develop chocolate model systems and standard methods to evaluate bloom and particle interactions, to generate correlations between bloom extents and particulate effects, and to better understand mechanisms of fat bloom in chocolate thus providing useful information for the chocolate industry.

2. Literature review

2.1 Chocolate and chocolate ingredients

Chocolate is a solid confectionery product that is composed of cocoa, sources of fats (mainly milk fat and cocoa butter) and sugars. It is derived from cocoa beans, core of the cocoa tree *Theobroma cacao*, originating from the Amazon and Orinoco valleys in South America. As production of cocoa is strongly dependent on the climate conditions: 20-30°C (68-86°F), 1500-2500 mm of annual rainfall and 2000 hours of sunshine per year (Afoakwa, 2010), West Africa produces more than 70% of world cocoa (Côte d'Ivoire, 39%; Ghana, 20%, Nigeria, 6%) while Southeast Asia and South America share the rest of production (International Cocoa Organisation, ICCO, 2015).

The United States is one of the world's largest chocolate processing nations with more than 400,000 tons grindings per year (ICCO, 2008) and one of the world's major chocolate consumption countries (more than 5 kg per person per year; International Confectionery Association, 2007). In recent years, a rising popularity in the chocolate industry has led to trendy demands for more premium and innovative ingredients.

Chocolate is basically made from chocolate liquor (cocoa butter and cocoa solids), sugar, cocoa fat, milk fat, and milk solids. In general, chocolate shall contain, on a dry matter basis, not less than 15% total cocoa solids, of which not less than 18% shall be cocoa butter and not less than 14% fat-free cocoa solids (Codex Alimentarius Commission, 1981). In the United States, chocolate products are strictly regulated by the U.S. Food and Drug Administration (FDA) as shown in Table 2.1.

Table 2. 1 Regulations in naming and ingredients of chocolate products (FDA, 2018).

Product	Chocolate Liquor	Milk Solids	Sugar	Cocoa Fat	Milk Fat
Milk Chocolate	≥ 10%	≥ 12%			
Sweet Chocolate	≥ 15%	< 12%			
Semisweet or Bittersweet Dark Chocolate	≥ 35%	< 12%			
White Chocolate		≥ 14%	≤ 55%	≥ 20%	≥ 3.5%

2.1.1 Cocoa liquor

Cocoa liquor is a semi-solid product produced by grinding cocoa nibs (FDA, 2018). It generally contains about 50-60% cocoa butter, 17% carbohydrates, 11% protein, 6% tannins, and 1.5% theobromine (Wolke, 2005). After harvest, cocoa beans undergo several processes including fermentation, drying, cleaning, shell removal, breaking and winnowing, alkalization and roasting. Afterwards, the processed cocoa nibs are ground to produce cocoa liquor. Nib grinding is usually a two-stage process including a coarse grinding and a fine grinding stage, during which the particle size is significantly reduced. Final fineness is dependent on end use (Kamphuis, 2017). About 55% cocoa butter is locked in the solid form in the cellular structure of cocoa nibs. Nib grinding, which includes a thermal treatment, melts and releases cocoa butter from the nib cells, thus forming cocoa liquor, with a final particle size up to 30 μm (Afoakwa, 2010).

Microbiological quality control for cocoa liquor is very important because of the high microbial total plates counts (more than 10^6 colony-forming units per gram, cfu/g) and presence of salmonella in raw cocoa beans (Kamphuis, 2017). Thus, a microbial destruction process, by heating cocoa liquor in the storage tank at 90-100°C , is required to ensure the final product

with generally accepted specifications (Afoakwa, 2010; Kamphuis, 2017), as shown in Table 2.2.

Table 2. 2 Quality control parameters for cocoa liquor (Cargill Cocoa, 2007).

Parameter	Value	Method
Fat content	Minimum 53%[a]	IOCCC 37, 1990
Moisture content	Maximum 2.0%	IOCCC 26, 1990
Total Plate Count	Maximum 5000 cfu/g[b]	IOCCC 39, 1990
Molds	Maximum 50 cfu/g	IOCCC 39, 1990
Yeasts	Maximum 50 cfu/g	IOCCC 39, 1990
Enterobacteriaceae	Absent per gram	IOCCC 39, 1990
Escherichia coli	Absent per gram	IOCCC 39, 1990
Salmonella	Absent per 750 g	IOCCC 39, 1990

[a] May vary due to bean origin and harvest; [b] cfu/g: colony-forming units per gram.

Physical and chemical quality control is also important for cocoa liquor production. Flavor in cocoa liquor comes from the Maillard reaction during roasting by aroma precursors (free fatty acid, peptides and reducing sugars) in cocoa beans (Mohr et al., 1976; Barel et al., 1985; Misnawi et al., 2004a). Flavor compounds include alcohols, ethers, furans, thiazoles, pyrones, acids, esters, aldehydes, imines, amines, oxazoles, pyrazines and pyrroles (Hoskin and Dimick, 1994; Jinap et al., 1998; Puziah, et al., 1998; Misnawi et al., 2004a). Polyphenols are a class of compounds in cocoa liquor that not only act as antioxidants, but also contributes to stringency and bitterness (Misnawi et al., 2004a). Table 2.3 shows its composition in cocoa liquor with different origins. Cocoa flavor intensity is positively correlated with fermentation and roasting time while negatively related to the concentration of polyphenols in cocoa liquor (Counet et al., 2004; Misnawi et al., 2004a). The presence of polyphenols can lead to flavor deficiency due to a decrease in the availability of amino acids and reducing sugars for pyrazine formation, a flavor compound, and binding of existing pyrazine during roasting (Misnawi et al., 2004a;

Misnawi et al., 2004b). This problem of flavor deficiency cannot be overcome by high temperature during roasting as these polyphenols, such as procyanidins and tannin, are heat resistant (Misnawi, 2009). On the other hand, polyphenols can act as antioxidants, providing cocoa liquor with great shelf-life stability. In most cases, cocoa liquor can be stored for several weeks in liquid form and for more than 12 months in solid form (Kamphuis, 2017).

Table 2. 3 Polyphenol composition (%) in cocoa liquor prepared by Meiji Seika Kaisha Ltd. using cocoa beans imported from different countries (Natsume et al., 2000).

Cocoa liquor	Ecuador	Venezuela	Ghana	Colombia	Ivory Coast	Brazil
Total polyphenol	4.11	1.55	2.93	1.20	3.13	6.04
NP-LC						
Monomers	0.366	0.106	0.249	0.113	0.230	1.132
Dimers	0.295	0.087	0.185	0.076	0.178	0.788
Trimers	0.344	0.128	0.215	0.098	0.233	0.787
Tetramers	0.260	0.101	0.184	0.043	0.241	0.576
RP-LC						
Catechin	0.040	0.014	0.027	0.020	0.017	0.063
Epicatechin	0.227	0.974	0.137	0.059	0.125	0.577
NP-LC						
Procyanidin B2	0.124	0.034	0.058	0.024	0.061	0.197
Procyanidin C1	0.080	0.026	0.041	0.012	0.053	0.151
Cinnamtannin A2	0.144	0.064	0.089	0.034	0.138	0.315
Gal-EC-EC	0.018	0.006	0.013	0.004	0.016	0.038

Gal-EC-EC: galactopyranosyl-*ent*-(-)-epicatechin(2α→7, 4α→8)-(-)-epicatechi;
RP-LC: reserved-phase high-performance liquid chromatography;
NP-LC: normal-phase high-performance liquid chromatography.

Color is another important parameter in cocoa liquor processing, particularly for dark cocoa powder production. Traditionally, alkalization, by adding a solution of an alkali (e.g., potassium carbonate) to cocoa nibs, is used to darken the color of cocoa powder and reduce bitterness (Rodríguez et al., 2009; Kamphuis, 2017). The characteristic color in cocoa comes from roasting, together with the enzyme activity of polyphenol oxidase (PPO), with optimal

activity at pH 8.0 (Razzaque et al., 2000). The enzyme can oxidize polyphenols in cocoa liquor and generate melanoidins (pigments of brown color), thus darkening cocoa color and reducing bitterness (Biehl, 1986). This process needs to be cautiously monitored with skill and experience to ensure consistency in color and flavor control (Kamphuis, 2017).

As described before, cocoa liquor contains about 50% cocoa fat in mass. Cocoa liquor can be hydraulically pressed, by applying pressure up to 540 bar (540×10^5 N/m^2) with cycle times of around 15 min, to produce cocoa butter and cocoa powder (Kamphuis, 2017). In general, roughly 78-90% of cocoa butter can be obtained by pressing while the residual fats in cocoa cake can be separated by supercritical fluid extraction (Beckett, 2000). The remaining cocoa cake has a fat content of between 10 and 24% (Afoakwa, 2010). It is then kibbled and pulverized to reduce particle size to certain fineness. A cooling process is usually followed to produce the final cocoa powder to prevent discoloration (fat bloom) and lumping in the bags after packing caused by insufficient fat crystallization during filling (ADM Cocoa, 2006).

2.1.2 Cocoa butter

Cocoa butter is a special class of fat extracted from cocoa beans, with a characteristic chocolate odor. The aroma and flavor are generated during fermentation, drying and roasting after harvest. It is the key ingredient in chocolate, with its unique flavor, texture and melting behavior, and is crucial to the quality of chocolate during processing and storage. A detailed schematic of cocoa butter production is presented in Figure 2.1.

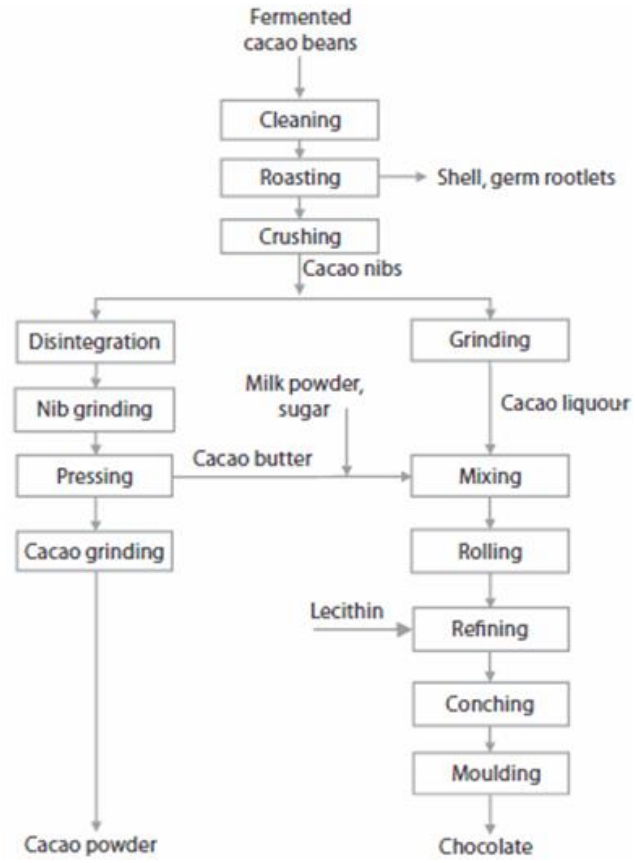


Figure 2. 1 A flow chart of cocoa butter and chocolate production. Adapted from Belitz et al. (2009).

2.1.2.1 Composition

The origin of cocoa butter has a substantial influence on the triacylglycerol (TAG) and fatty acid profile. Table 2.4 provides a comprehensive composition list for both TAGs and fatty acids in cocoa butter. In general, cocoa butters from South America are significantly higher in oleic acid than those from Africa, while South East Asia cocoa butters fall in between (Lipp and Anklam, 1998). Differences in the 2-oleic-disaturated TAG composition also impacts the melting behavior in chocolate (Jewell, 1981). In a TAG composition analysis among 28 different cocoa butters from different regions, cocoa butter from Bahia was identified to contain a relatively higher amount of di-unsaturated TAGs (like SOO, POO) and a significantly lower amount of monounsaturated TAGs (like POP, POS) (Podlaha et al., 1984).

Table 2. 4 Composition list of cocoa butter from different origins (Lipp and Anklam, 1998).

(a) TAG composition

Country of origin	Samoa	Ivory Coast	Ecuador	Malaysia	Ghana	Nigeria	Bahia
POL	0.8	0.6	0.7	0.6	0.6	0.8	1.1
MOO, MMP	0.3	0.2	0.3	0.5	0.2	0.2	0.2
PPL	1.6	1.9	1.9	1.5	1.9	1.9	1.7
OOO	0.2	0.8	0.8	0.8	0.5	0.4	0.9
SOL	0.5	0.9	0.8	0.7	0.4	0.8	1.0
POO	2.2	4.4	3.5	2.7	2.6	3.2	5.5
PSL	2.8	3.6	2.8	2.8	3.2	3.4	3.4
POP	16.4	15.9	15.3	13.8	15.2	14.8	14.0
SOO, PPP	3.7	6.0	4.8	3.8	4.5	5.1	8.4
SLS	2.1	1.8	1.5	2.0	2.1	1.9	2.1
POS	38.3	36.6	36.3	36.6	37.3	37.4	34.6
OOA	1.6	1.0	1.2	1.6	1.4	1.2	1.5
PPS	0.4	0.4	0.3	0.6		0.7	0.3
SOS	26.8	23.8	26.9	28.4	26.8	26.4	23.7
SPS	0.7	0.8	0.9	1.0	1.3	0.4	0.2
SOA	2.2	1.6	2.1	2.5	2.2	1.9	1.6

TAG: Triacylglycerol; P: Palmitic acid; S: Stearic acid; L: Linoleic acid; Ln: Linolenic acid; A: Arachidic acid; M: Myristic acid; O: Oleic acid.

(b) Fatty acid composition

Country of origin	Ecuador	Brazil	Ghana	Ivory Coast	Malaysia	Java
Palmitic acid	25.6	25.1	25.3	25.8	24.9	24.1
Stearic acid	36.0	33.3	37.6	36.9	37.4	37.3
Oleic aicd	34.6	36.5	32.7	32.9	33.5	34.3
Linoleic acid	2.6	3.5	2.8	2.8	2.6	2.7
Linolenic acid	0.1	0.2	0.2	0.2	0.2	0.2
Arachidic acid	1.0	1.2	1.2	1.2	1.2	1.2
Behenic acid	0.1	0.2	0.2	0.2	0.2	0.2

The melting point of cocoa butter is very sharp since the main fat components are of the same type, predominantly 1, 3-disaturated, 2-monounsaturated TAGs. Substantial amounts of 2-oleyl glycerides of palmitic and stearic acid (POP, POS, and SOS) are the main TAGs found in cocoa butter. Since cocoa butter is the major fat source in dark chocolate, physical parameters

like brittleness as well as quick and complete melting in mouth are unique in chocolate. As the continuous phase in chocolate, the fat phase is significant in building the melting and dispersion properties. Therefore, the major TAGs (POP, POS, and SOS) in cocoa butter are essential in providing the sharp melting around body temperature, resulting in a "cooling effect" in the mouth that is regarded as a criterion for high-quality chocolate.

Since stearic acid has a longer chain length than palmitic acid, SOS has the highest melting point among the three major TAGs while POP has the lowest. Thus, Asian cocoa butters, rich in SOS and POS, have higher solid fat content (SFC) than West African or South American cocoa butters, whereas South American cocoa butters have lower SFC, in general (Talbot, 2017).

2.1.2.2 Polymorphism

Polymorphism is the ability of a molecule to crystallize in different crystal packing configurations. Cocoa butter is highly polymorphic because it is rich in symmetrical monounsaturated triglycerides such as SOS (Talbot, 2017). There are 6 different polymorphic forms denoted by Roman numeral or Greek letter (Aronhime et al., 1998; Dimick, 1991). In general fats and oils, γ, α, β', β are primarily used to present the fat polymorphs with increasing stability and melting point. The γ polymorph is also called sub-α or β'_3 as its residence time is fairly short and instantly transforms to the α polymorph. However, with the primary α, β', β denotation, it is still not sufficient to present different polymorphs in cocoa butter as X-ray diffraction shows cocoa butter has 6 different polymorphs, as shown in Figure 2.2. As a result, Wille and Lutton (1966) reported the polymorphisms of cocoa butter using Form I to Form VI

and it is now popular in the confectionery industry to use the Wille and Lutton convention. Table 2.5 shows a summary of melting points of different polymorphs with different denotation (Lonchampt and Hartel, 2004).

In general, fats display monotropic polymorphism, transforming from less stable form to more stable ones. Form I is formed from rapid cooling at lower temperature and quickly transforms to Form II and III. Forms I, II and III are all very unstable and exist only for seconds from chocolate melts and quickly transform to β'IV. The unstable polymorph β'IV appears mostly in freshly untempered or poorly tempered chocolate. Form βV, which could be transformed from the unstable β'IV in minutes to hours, is a metastable form. Form βVI, the most stable polymorph, cannot be obtained directly from chocolate melts and would take more than a year to be transformed from Form V (Langevelde et al., 2001). As a result, Form V is the most preferable polymorph in chocolates, partly for stability and partly for its melting temperature. A tempering process is required to control cocoa butter crystallization to promote the formation of βV.

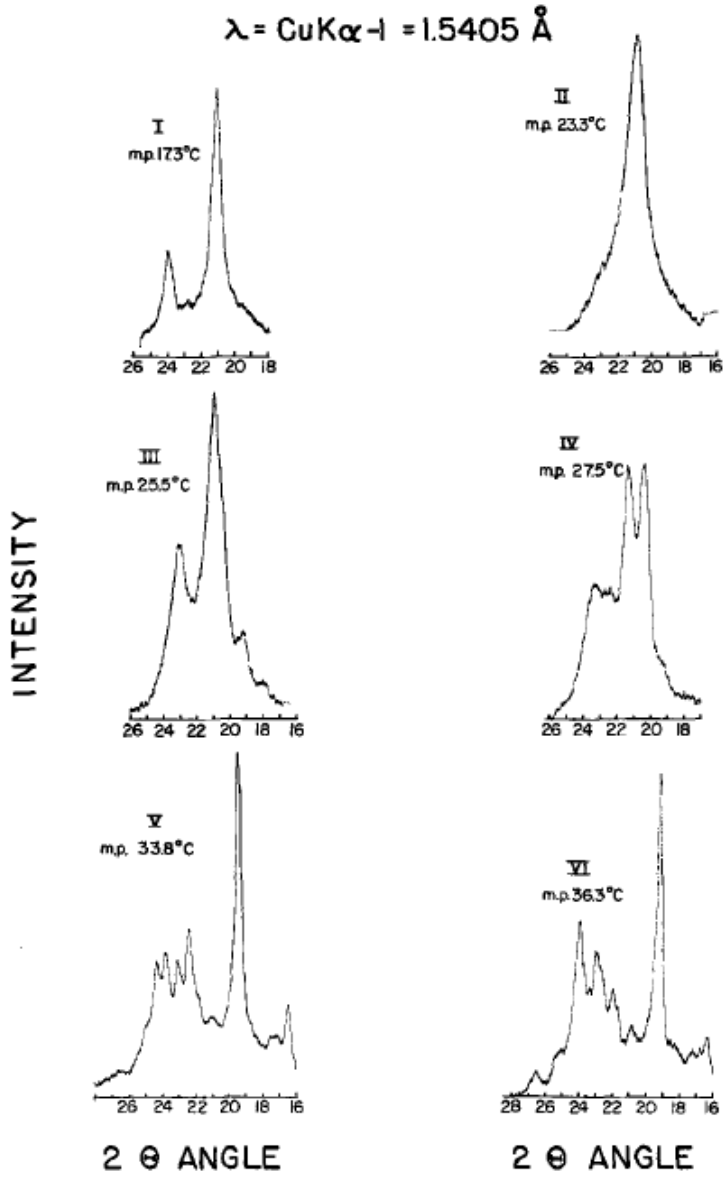


Figure 2. 2 Six polymorphs of cocoa butter from X-ray diffraction. Adapted from Wille and Lutton (1966).

Table 2. 5 Summary of melting points (°C) of different polymorphs with different denotation by different authors (Lonchampt and Hartel, 2004).

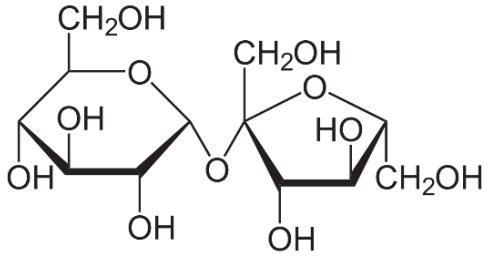
Vaeck, 1951	Duck, 1964	Wille and Lutton, 1966	Huygherbaert and Hendrickx, 1971	Lovegren et al., 1976	Dimick and Davis, 1986
18.0 (γ)	17.0 (γ)	17.3 (I)	14.9-16.1 (I)	13.0 (VI)	13.1 (I)
23.5 (α)	21-24 (α)	23.3 (II)	17.0-23.2 (II)	20.0 (V)	17.1 (II)
28.0 (β'')	28.0 (β'')	25.5 (III)	22.8-27.1 (III)	23.0 (IV)	22.4 (III)
-	33.0 (β')	27.3 (IV)	25.1-27.4 (IV)	25.0 (III)	26.4 (IV)
34.5 (β)	34.4 (β)	33.8 (V)	31.3-33.2 (V)	30.0 (II)	30.7 (V)
		36.3 (VI)	33.8-36.0 (VI)	33.5 (I)	33.8 (VI)

2.1.3 Sugar ingredients

Sugar contributes up to about 50% mass in chocolate, and is used as an inert ingredient to contribute to sweetness and affect flavor. As low as a 5% change in sugar can have a large flavor change in chocolate significant (Beckett, 1999). Also, acting as a particle in the semi-solid chocolate matrix, it is considered as an important ingredient that controls viscosity and texture. In a sensory study in milk chocolate, low-sugar samples were more bitter, gritty and roasted whereas high-sugar samples had higher milky/dairy, vanilla/caramel, hardness and sweetness intensities (Guinard and Mazzucchelli, 1999). Further, in a sugar-in-fat chocolate model system (Do et al., 2007), increasing sugar content while reducing fat content increased viscosity in the molten chocolate causing difficulties in the process. The final product also was reported to display poor eating qualities such as poor in-mouth melting properties, excessive hardness, and being difficult to swallow.

Crystalline sucrose is the predominant type of sugar in commercial chocolate. It is a disaccharide with chemically linked monosaccharides glucose and fructose. Figure 2.3 shows the structure of sucrose. In chocolate manufacturing, medium fine sugar with 0.6-1.0 mm grain size is predominantly used (Krüger, 2017). A two-stage refining process, using a two-roll pre-refiner to size the feed material followed by a five-roll refiner, is usually applied to accurately control the final particle size. In the final chocolate product, the maximum particle size should not exceed about 30 μm on an organoleptic aspect; and the minimum size is around 6 μm in order to achieve optimal flow properties in the chocolate mass (Niedieck, 1971; Niedieck, 1972; Do et al., 2007). Coarseness will be detected in chocolate when more than 20% of the particles are larger than 20 μm (Rostagno, 1969).

Figure 2. 3 Chemical structure of sucrose.



As a milk solid from dairy ingredients, lactose is a disaccharide made up of the monosaccharides glucose and galactose. Figure 2.4 shows the structure of lactose. Crystallized as a monohydrate with one water molecule, it is nonhygroscopic, low in sweetness intensity and forms harder crystals than sucrose (Krüger, 2017). Pure lactose can be successfully used to replace sucrose in chocolate (Hogenbirk, 1985; Müller, 2003; Bolenz et al., 2006). On the other hand, as a reducing sugar, lactose also participates in Mallard browning, affecting chocolate flavor and color (Krüger, 1999; Bolenz et al., 2006).

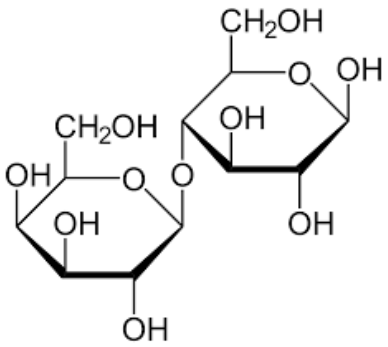


Figure 2. 4 Chemical structure of lactose.

Being difficult to dry, monosaccharides such as glucose and fructose are rarely used since extra moisture in chocolate will increase chocolate viscosity by favoring particulate interactions. On the other hand, a sensory study on milk chocolate showed that glucose leaves

a burning taste in the mouth and enhances the impression of sweetness whereas fructose gives a “pungent” sweetness (Krüger et al., 1987).

With low-calorie benefits, sugar-free chocolates are gaining in popularity in recent years and sugar alcohols are important sugar substitutes. Maltitol is a disaccharide alcohol with its structure shown in Figure 2.5. It is nonhygroscopic and has 75-90% of the sweetness of sucrose. As an anhydrous sweetener, it can be conched at temperatures as high as 80°C (Happel, 1995). It also contributes to similar rheological properties of chocolate to sucrose (Sokmen and Gunes, 2006), and thus is considered as a good sucrose alternative, sometimes in combination with polydextrose, in chocolate formulations.

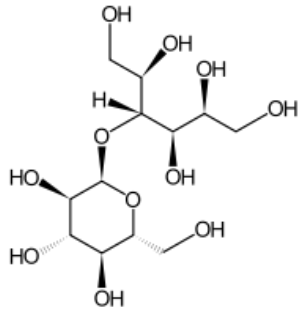


Figure 2. 5 Chemical structure of maltitol.

Amorphous sugar is a noncrystalline sugar solid lacking in the long-range molecular order found in crystals. It can be produced from a sugar solution by spraying drying at a high temperature or by thin film drying at an extremely low relative humidity. With its high hygroscopicity (Powers, 1980), it absorbs moisture rapidly, which is undesirable in viscosity control during manufacturing. On the other hand, it also causes mouthfeel issues as it absorbs moisture out of the saliva, resulting in an undesirable sensory feedback. Thus, it is not used in commercial chocolates.

2.1.4 Milk fat

The role of milk fat in chocolate is complex – it enriches chocolate flavor, changes chocolate texture and inhibits bloom. Acting as a flavor precursor, compounds in milk fat such as free fatty acids, lactones, ketones, esters, aldehydes and carbonyls provide a buttery and creamy flavor in chocolate (Skytte and Kaylegian, 2017). On the other hand, as a semi-liquid ingredient at room temperature, it is good at carrying other flavor compounds in chocolate. Milk fat also softens chocolate at room temperature, changes chocolate viscosity and retards setting of chocolate during manufacturing. Moreover, it is prone to lipid oxidation, generating off-flavors and affecting shelf life, especially when handled roughly and exposed to light, air and heat (Haylock and Dodds, 1999; Skytte and Kaylegian, 2017). This problem is even worse in white chocolate with the absence of the cocoa solids as a natural antioxidant.

2.1.4.1 Composition

The composition of milk fat is very complex; besides triglycerides, there are low levels of mono-, diglycerides, free fatty acid, phospholipids and sterols, as shown in Table 2.6. There are over 400 fatty acids identified in milk fat, with 65-70% saturated, 27-33% monounsaturated, 3.5-5.0% polyunsaturated (Skytte and Kaylegian, 2017) and 2-5% trans- fatty acids due to bacterial metabolism in the rumen (Parodi, 2004). Among them, 20 fatty acids make up the majority. The fatty acids are also variable, dependent on season of production. In general, summer milk fat has higher levels of unsaturated fats (Badings et al., 1983; Salamon et al., 2006). Table 2.7 shows the major fatty acids in both summer and winter milk fat.

Table 2. 6 Components in milk fat (Skytte and Kaylegian, 2017).

Component	Weight (%)
Triglycerides	98.3
Diglycerides	0.3
Monoglycerides	0.1
Free fatty acids	0.1
phospholipids	0.8
Sterols	0.35
Carotenoids	trace
Vitamins (mainly A, D and E)	trace
Flavor compounds	trace

Table 2. 7 Major fatty acids in milk fat (Skytte and Kaylegian, 2017).

Fatty acids	Typical Composition	Summer milk fat	Winter milk fat	Summer, maximum	Winter, minimum
C4:0	4.1	4.2	4.6	2.93	4.88
C6:0	2.4	2.3	2.7	1.54	2.65
C8:0	1.4	1.3	1.5	0.82	1.71
C10:0	2.9	2.6	3.4	1.77	4.08
C10:1	0.3	-	-	-	-
C12:0	3.5	3.0	4.2	1.99	4.69
C12:1	11.4	9.6	11.6	7.10	12.34
C14:1	-	-	-	0.51	1.04
C15:0	-	1.1	1.1	0.76	1.10
C16:0	23.2	23.3	29.1	24.95	32.08
C16:1	-	1.0	0.7	1.21	1.49
C17:0	-	0.8	0.9	0.41	0.57
C18:0	12.4	11.9	9.5	9.92	15.36
C18:1	25.2	28.1	21.3	21.22	31.59
C18:2	2.6	1.3	1.6	2.29	4.53
C18:3	0.9	1.1	1.4	0.20	0.64
C20:0	-	-	-	0.09	0.13
CLA	-	-	-	0.48	1.00
others	10.0	-	-		

CLA: conjugated linoleic acid.

There are also some regularities in the triglyceride structure: C4:0 and C6:0 are in the sn-3 position; C8:0 is in the sn-2 and sn-3 positions; C10:0, C12:0 and C14:0 are usually in the

sn-2 position; C16:0 is mainly equally distributed in the sn-1 and sn-3 positions; C18:0 is in the sn-1 position; and C18:1 is in the sn-1 or sn-3 position (Kaylegian and Lindsay, 1995; Parodi, 2004; MacGibbon and Taylor, 2006).

2.1.4.2 Polymorphism

The most stable polymorph of milk fat is the β' form. For the high-melting fractions (HMF), the stable form is β'-2 (Lonchampt and Hartel, 2004), whereas for the middle-melting fractions (MMF), the stable form is mainly β'-2 and some β'-3 (Ten Grotenhuis et al., 1999; Breitschuh and Windhab, 1998).

2.1.4.3 Interaction between milk fat and cocoa butter

The addition of milk fat in cocoa butter changes the hardness, melting point and temper degree in chocolate (Barna et al., 1992; Full et al., 1996). Milk fat and cocoa butter contain different stable crystal forms and are thus not completely compatible in the solid state (Skytte and Kaylegian, 2017). On the other hand, milk fat can be partially compatible in cocoa butter and become part of the continuous phase (liquid fat) but the addition extent is limited. It will not change the polymorphic type of cocoa butter unless the addition level is above 50% (Timms, 2003). In commercial chocolate, the milk fat addition level is mostly below 30%; thus, the change in polymorph of cocoa butter is not a concern in the industry. However, milk fat does lower the melting point of cocoa butter polymorphs, slow the rate of crystallization of cocoa butter and milk fat mixtures, and slow the rate of polymorphic transition in cocoa butter (Timms, 1980; Timms, 2003). Particularly, milk fat slows down the rate of βV to βVI transition, which

can partially explain the reason for the antibloom effect of milk fat in chocolate (Campell and Keeney, 1968; Dimick, 1991; Bricknell and Hartel, 1998).

Different milk fat fractions have different effects with cocoa butter. Figure 2.6 shows the isothermal diagrams of cocoa butter and milk fat blends. In general, milk fat softens the milk fat and cocoa butter mixture and presents a eutectic or diluting effect in the blend, and this softening effect is mainly contributed by low- and middle-melting triglycerides (Timms, 1980). Low-melting fractions (LMF) in milk fat have a diluting effect with cocoa butter due to their low solid fat contents (SFC) while middle-melting fractions (MMF), anhydrous milk fat (AMF) and high-melting fraction (HMF) in milk fat and cocoa butter present a eutectic phenomenon – the SFC of the blends is lower than that of either of the fats.

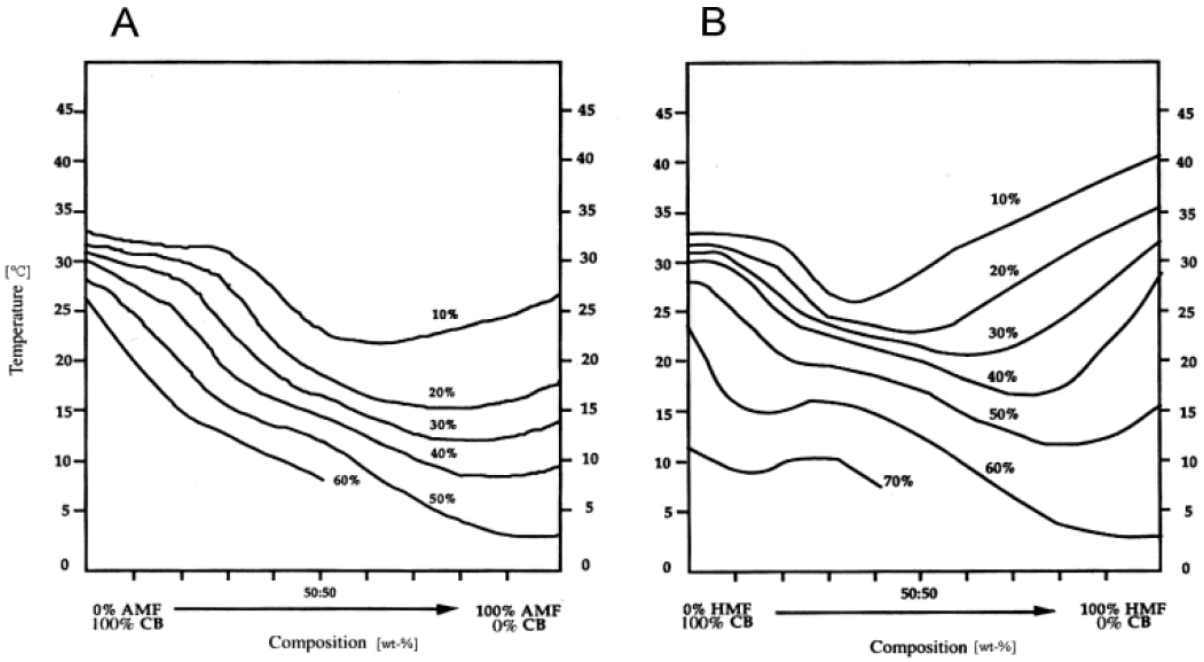


Figure 2. 6 Isothermal diagrams of blends of cocoa butter and (A) anhydrous milk fat (AMF); (B) high-melting fraction (HMF) of milk fat. Adapted from Hartel (1996).

Moreover, milk fat influences cocoa butter crystallization and changes the tempering requirements for chocolate. Low-melting fractions (LMF) in milk fat had a higher effect on retarding the nucleation step in cocoa butter crystallization than medium- and high-melting factions (Metin, 1997). Reddy et al. (1996) achieved a successful tempering in a previously untemperable chocolate by replacing 35-40% cocoa butter with HMF, MMF and LMF of milk fats, changing temperature and prolonging the crystallization time.

2.1.5 Emulsifier

Emulsifiers act as a surface active and viscosity control ingredient in chocolate to improve interactions between sugar and fat, thus reducing the amount of fat needed to achieve desirable flow properties. Emulsifiers are amphiphilic molecules that attach the polar head groups onto sugars while interacting with liquid fat with its alkyl chains, thus improving sugar-fat interactions and preventing sugar aggregation with entrapped fats unavailable for good flow properties (Wolf, 2017).

Lecithin is the most widely used emulsifier in chocolate, which mostly comes from soy. The main active components are phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI). Neutral oils also make up of over 30% mass in liquid lecithin, mainly oleic (C18:1) and palmitic acid (C16:0) in fatty acid composition (Vernier, 1998). PC is the most effective component in viscosity reduction effect (van Nieuwenhuyzen, 1997). Also, binary mixtures of phospholipids in lecithin are more effective in yield stress reduction than single ones (Arnold et al., 2014). By adding 0.1% to 0.3% lecithin, plastic viscosity and yield stress in chocolate are significantly reduced and the tolerance for moisture in chocolate is

increased. When lecithin mass reaches over 0.5%, yield stress begins to increase even though plastic viscosity still decreases slightly (Chevalley, 1999; Rector, 2000; Schantz & Rohm, 2005; Wolf, 2017). This may occur because (1) lecithin has reached the critical micelle concentration (CMC) and extra lecithin molecules attach to each other forming new micelles, or (2) the lipophilic heads attach to other lipophilic heads to form a second layer of lecithin thus decreasing the surfactant effectiveness (Wolf, 2017). This critical concentration of lecithin is based on the total surface area of sugar particles, usually influenced by the particle size and the sugar concentration. Figure 2.7 shows the effect of lecithin addition level on viscosity and yield stress in chocolate.

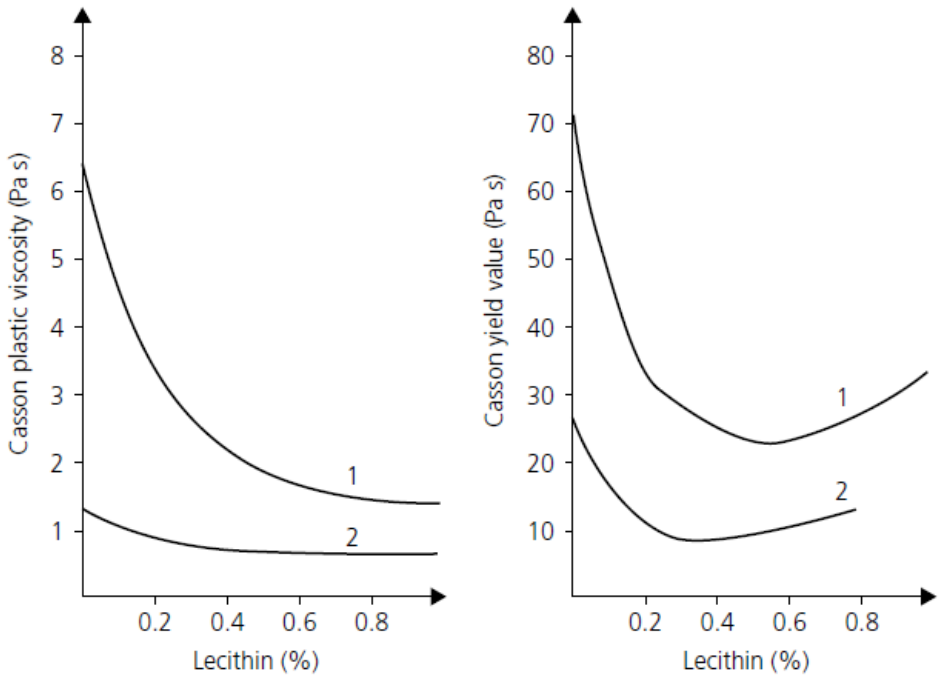


Figure 2. 7 Effect of lecithin addition level on viscosity and yield stress in two chocolates: (1) 33.5% fat, 1.1% moisture; (2) 39.5% fat, 0.8% moisture. Adapted from Wolf (2017).

Another widely used emulsifier in chocolate is YN, a synthetic lecithin produced from partially hardened rapeseed oil by glycerolysis, phosphorylation and neutralization (Wolf,

2017). Compared to lecithin, it is more consistent, more efficient, and has neutral flavor. It is also used to control viscosity in chocolate.

Polyglycerol polyricinoleate (PGPR), a polyglycerol mixture produced from polycondensation of castor oil and glycerol, is another widely approved emulsifier in the chocolate industry (Afoakwa, 2010). There is no significant effect from PGPR on viscosity control, but it is good at yield stress reduction. Adding 0.5-0.1% PGPR could reduce yield stress in chocolate to 0 (Rector, 2000; Schantz & Rohm, 2005), with a ready flow and rapid settling behavior (Afoakwa, 2010). It could replace cocoa butter to coat solid particles in the dispersed phase to achieve optimal flow thus reducing costs from cocoa butter. It stays at the particle interface, with its hydrophobic portion coating particles and its hydrophilic portion extending into the continuous phase. Combining lecithin and PGPR is a good strategy in flow property control (Schantz & Rohm, 2005). Adding PGPR can balance out the viscosity reduction effect from lecithin resulting in a product with a decreased yield stress and a slightly increased plastic viscosity (Vernier, 1998; Rector, 2000; Schantz & Rohm, 2005).

Besides flow property control, emulsifiers can also influence the tempering process, which as a result influences the viscosity in well-tempered chocolate (Dhonsi and Stapley, 2006; Radujko et al., 2011; Hartel, 2013).

2.2 Microstructure of chocolate

Chocolate is considered a dispersion of solid particles in a fat matrix. Figure 2.8 shows the structure of melted milk chocolate under confocal microscopy. On average, chocolate has about 32% fats and 68% nonfat particles. Since the typical SFC of cocoa butter at room

temperature is around 75%, that means there are 90-95% solid particles in the solidified chocolate matrix and only 5-10% liquid mass (Hartel, 1999; Hartel et al., 2016). Microstructure of chocolate greatly influences its texture, melting behavior and flow properties when melted. Further, since the liquid oil plays an important role in the dissolution and migration step during bloom formation and particles influence the migration and recrystallization of cocoa butter, microstructure also may affect fat bloom in chocolate during storage.

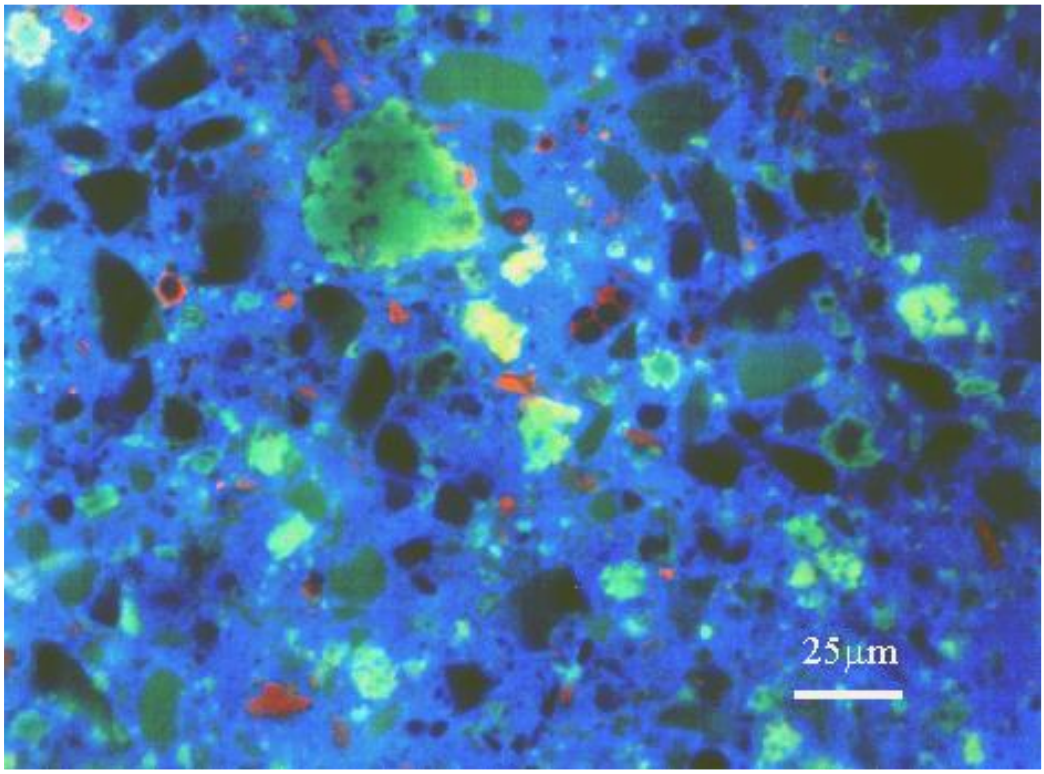


Figure 2. 8 Composition of melted milk chocolate by confocal microscopy (Dark green: sugar; bright green: milk protein; red: cocoa solids; blue: liquid fat; black: cocoa solids and sugars). Source: Mark Audy, DPC, Moorepark.

2.2.1 Fat phase in chocolate

When melted, fats in chocolate coat the particles and fill the gap between them to reduce the resistance to flow. However, some of the fats may be trapped between the solid aggregates or in cocoa particles, and not contribute to the flow (Wolf, 2017). In general, fat content

influences rheology in melted chocolate, with apparent viscosity decreasing as fat content increases. This is because the extra fats can dilute particles, lubricating the flow (Beckett, 2000; Afoakwa, 2010). In chocolates with low fat content (~25%), the distance between particles is short with high solid particle packing intensity and strong particle-particle interactions. The effect of additional 1% fat on viscosity in low fat content chocolate is quite significant, whereas this effect is very small in chocolate with fat content above 35% (Chevalley, 1999; Beckett, 2000; Wolf, 2017). Yield stress, on the other hand, is more dependent on the particulate interactions in the system and is less affected by the additional 1% fat content (Chevalley, 1999; Wolf, 2017), as shown in Figure 2.9. Flocculation and aggregation may also occur in low fat dispersions, increasing the restrictions of mobility and compartmentalization in the matrix (Afoakwa, 2010).

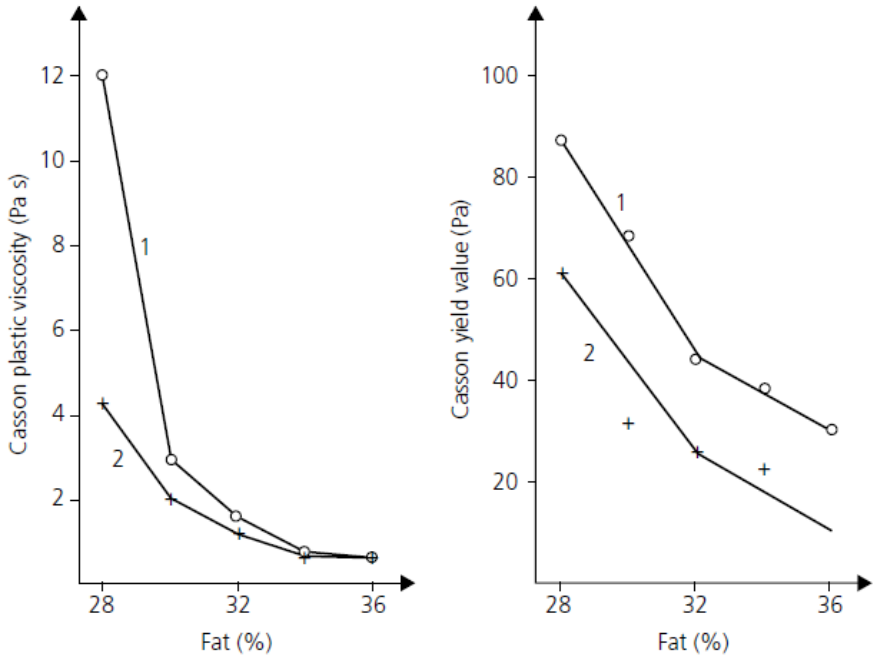


Figure 2. 9 The effect of fat content on Casson viscosity and Casson yield stress in chocolate with 0.25% lecithin and different particle distributions: (1) 5.7% particles > 20 μm; (2) 16% particles > 20 μm. Adapted from Chevalley (1999).

Solid fat content (SFC), representing the amount of fat in the solid phase at certain temperature, is also an important parameter for microstructure of chocolate. In principle, SFC decreases when temperature increases, dependent on the fat composition in chocolate. Lower SFC indicates more liquid fat in the continuous phase, resulting in a softer chocolate. Thus, SFC is strongly related to the hardness and melting behaviors in chocolate (Full et al., 2006).

2.2.2 Particles in chocolate

The nature of particles, such as the size, shape, and particle size distribution, greatly influence rheological properties in melted chocolate (Afoakwa et al., 2008a; Afoakwa et al., 2009a; Ziegler and Hogg, 2017; Wolf, 2017). In general, apparent viscosity decreases with an increasing particle size, broader particle size distribution and more spherical shape (Ziegler and Hogg, 2017). Viscosity can be reduced when the total surface area of particles that fats need to cover is decreased as the average particle size is reduced or the jagged edges of particles become smooth and round (St. John et al., 1995; Wolf, 2017). Particles with similar size are loosely packed together and the space between them needs to be filled by either liquid fats or smaller particles, as shown in Figure 2.10. As a result, in a bimodal system, where coarse and fine particles are combined, the fat needed to fill the empty space is less resulting in a lower viscosity (Fischer, 1994; Ziegler and Hogg, 2017; Wolf, 2017). Finer particles and a combination of coarse and fine particles may also lead to more particle interaction thus resulting in an increased yield value (Ziegler and Hogg, 2017; Wolf, 2017).

Solid particles in chocolate systems promote particle interactions in terms of distance between nonfat particles as well as fat crystals. Crystalline fat particles interact with each other

by van der Waals forces and water bridges (Johansson and Bergenstahl, 1992a). This interaction is strongly related to fat composition and morphological state of the fat crystals such as crystal shape, polymorphism, size and crystal rigidity (deMan and Beers, 1988). In a sugar-in-fat dispersion system, van der Waals forces and water bridges are also the two predominant forces in sugar particle interactions (Johansson and Bergenstahl, 1992a; Barbin et al., 2005). One potential mechanism would be that the original contacts between sugar particles, at an extremely short distance, are primarily formed under van der Waals forces, contributing to the high storage modulus in the dispersion system. Then, network rearrangement between particles is formed by water bridges, with a changed equilibrium distance between particles, greatly contributing to the strong interaction in the dispersion system (Johansson and Bergenstahl, 1992a).

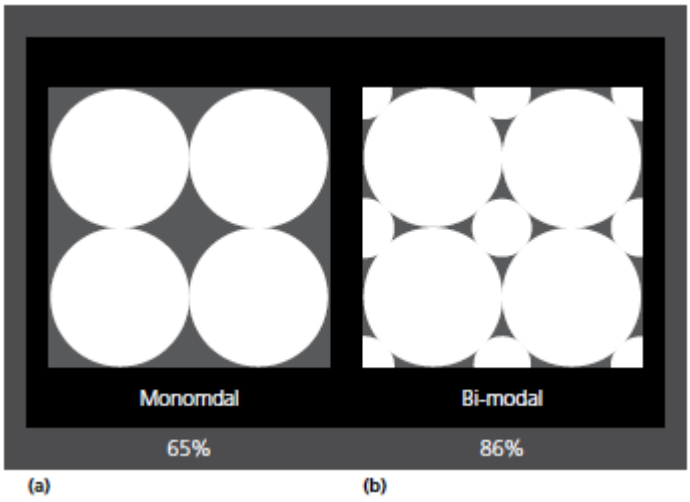


Figure 2. 10 Demonstration of packing structures in two system with different particle size distribution: (a) Monomodal, same particle size, (b) Bi-modal, combination of coarse and fine particles. Adapted from Wolf (2017).

Surfactants, which usually adsorb on the sugar crystals and fats in the dispersion, generally reduce apparent viscosity. Elastic properties of both fat and sugar dispersions can also be

decreased by surfactants, resulting in a decreased yield value and storage modulus. The effect can be the opposite when the concentration of surfactant is extremely high (Johansson and Bergenstahl, 1992a). More importantly, surfactant may change the particle surface energetics and the particle interaction energies (Bricknell and Hartel, 1998). Moreover, the nature of the interaction, whether steric interaction or specific polar interactions, and network structure may also be changed (Johansson and Bergenstahl, 1992a).

Particulate interactions can be quantified by rheology and sedimentation volume (Johansson and Bergenstahl, 1992a; Barbin et al., 2005). With weak particle interactions in the dispersion, particles may settle individually in a compact sediment with little resistance to flow, resulting in a lower viscosity and lower sedimentation volume (Barbin et al., 2005). Decreased adhesion is also related to a reduction in storage modulus and yield values (Johansson and Bergenstahl, 1992a).

As particulate interaction may modify the rheological and microstructure characteristics of chocolate matrix, it may also have an influence on bloom (Glicerina et al., 2016). An AFM study showed that the heterogeneity in the dispersed particulate network had a substantial influence on the morphology and crystallization pathway of the fat phase (Rousseau and Sonwai, 2008). Replacing sugar particles with cocoa powder or milk solids may change the surface area of particles, thus decreasing the migration rate in chocolate, as the shape of the nonsugar particles are irregular (Smith, 1998). Reducing particle size in chocolate also reduces migration due to the same mechanism (Timms, 2003). Moreover, a recent study, by comparing the surface wetting properties of chocolates with water and diiodomethane, showed that the hydrophilic particle phase (mainly sucrose) predominantly contributes to chocolate surface

roughness alterations, one indicator in the bloom process (Reinke et al., 2015), thus supporting that sugar particles may affect bloom by changing chocolate surface properties.

2.3 Tempering

Tempering is an essential process during chocolate making. The objective of tempering is to develop sufficient amount of seed crystals to prevent heterogeneous crystallization in the subsequent cooling process (Giddey and Clerc, 1961). By the end of successful tempering, the chocolate should have the largest amount of βV fat crystals with the smallest size (to prevent crystallization during storage) (Seguine, 1991). Basically during a tempering process, the chocolate is initially heated to around 50°C to destroy any crystal structures and then cooled to a crystallization temperature (~26°C) to crystallize out β'IV crystals together with small amounts of βV crystals. Finally the chocolate is heated again to around 32°C to melt β'IV crystals, leaving βV crystals solely in the system. A seeding process may be incorporated in the cooling stage during tempering to speed the cooling process and improve βV crystallization. A typical tempering chart is shown in Figure 2.11.

The degree of temper can be evaluated by a cooling rate curve. This is done by placing freshly tempered chocolate in a cool water bath and recording the temperature of the chocolate every several seconds to construct a temperature versus time curve. In principle, if there is a plateau with slope=0, the chocolate is well-tempered; if the slope is positive, the chocolate is under-tempered; if the slope is negative, the chocolate is over-tempered (Windhab, 2017). Figure 2.12 shows the cooling curves in chocolates with different tempering extent.

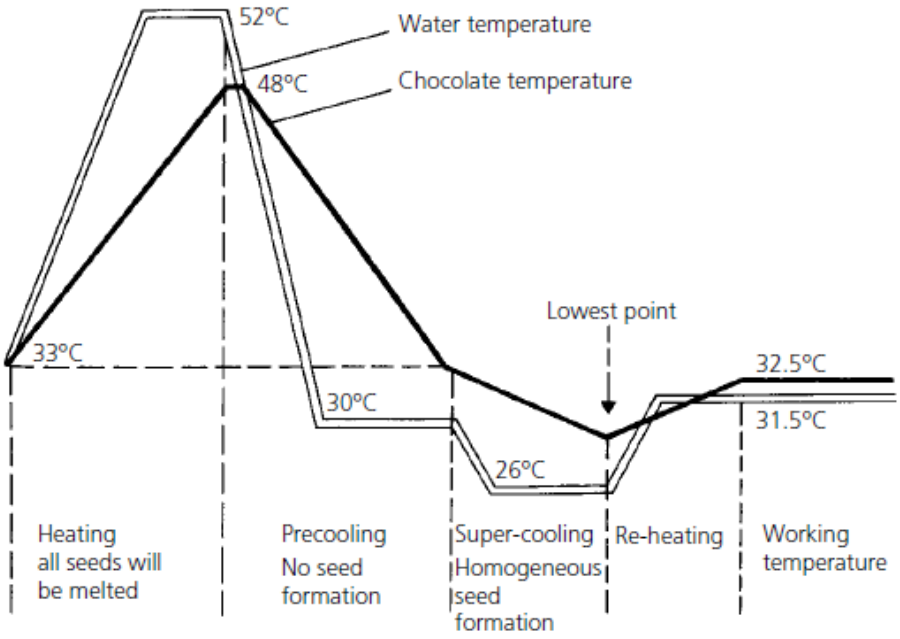


Figure 2. 11 A typical tempering chart in chocolate. Adapted from Windhab (2017).

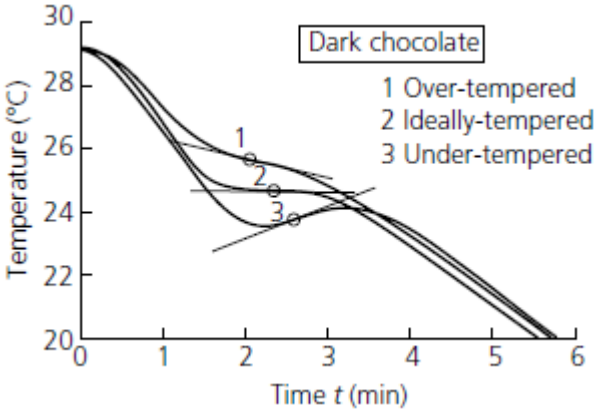


Figure 2. 12 Cooling curves in chocolates with different tempering extent. Adapted from Windhab (2017).

Tempering is an essential process during chocolate manufacturing to prevent bloom and prolong chocolate shelf life. Surface properties, melting properties, internal crystal network structure and inter-particle interactions in chocolates can be altered during different tempering processes, when the chocolate is under-tempered, well-tempered or over-tempered (Afoakwa et al., 2008b; Afoakwa et al., 2009b). Especially, adding seeds during tempering can accelerate

cocoa butter crystallization and improve cocoa butter polymorphism (Hachiya et al., 1989a; Hachiya et al., 1989b; Hachiya et al., 1989c).

One good strategy for preventing storage bloom is to add "permanent" seeds during tempering. Seeding with βVI cocoa butter and β1 SOS (1,3-distearoyl-2-oleoylglycerol) can improve bloom stability during storage with fluctuating temperature while seeding with β2 BOB (1,3-dibehenoyl-2-oleoylglycerol) has an even better improvement and 5% seeding can prevent bloom completely (Hachiya et al., 1989d). This is because part of BOB remains solid when added to 5% in chocolate at up to 40°C and can act as a seeding agent for any liquid fat to recrystallize during storage. As a result, cocoa butter is always in a βV polymorph with BOB addition (Timms, 2003). Unfortunately, this is not legal in the chocolate industry. Chocolate can also be produced with special tempering process, by seeding with βVI SOS to crystallize cocoa butter to βVI polymorph during tempering (Giddey and Clerc, 1961), but the sensory properties are not desirable due to the high-melting point (Timms, 2003). Zeng et al. (2002) also documented a tempering process, by adding a special βVI cocoa butter seed in precooled chocolate and processing at 34°C, that could crystallize chocolate in βV instead of βVI to achieve significantly accelerated solidification and increased bloom stability with a good mouthfeel. Moreover, a post-tempering process by warming the freshly enrobed chocolate to 28-31°C for 0.5-2 hours followed by a fast cooling to normal storage temperature can also improve bloom stability in filled chocolates, by stimulating fat migration between center and shell and accomplishing fat concentration equilibrium (Ziegleder and Mikle, 1995).

2.4 Types of bloom

Chocolate bloom is a whitish coating that occurs on the surface of chocolate, with loss of its initial gloss. It is a quality issue rather than a safety issue as the “moldy” surface appears undesirable to consumers reducing customer acceptance and sales volume; thus, chocolate bloom is a severe problem in the industry despite decades of studies. The “moldy” appearance comes mainly from two sources: fats and sugars in the chocolate; therefore, basically there are two major types of bloom: sugar bloom and fat bloom. Sugar bloom is related to the interaction of sugar in the chocolate and moisture in the atmosphere or in the chocolate. When chocolate has hygroscopic ingredients or ingredients with high moisture content, or chocolate is placed in a high-humidity environment, sugar crystals on the chocolate surface tend to absorb moisture. When later dried, the dissolved sugar recrystallizes on the chocolate surface leaving a white layer with larger sugar crystals that can be clearly visible. Fat bloom, on the other hand, is fairly complicated, with different theories and mechanisms. As it is the focus in this study, bloom referred in this thesis is always fat bloom.

2.4.1 Bloom from incompatible fats

Bloom can be caused by the incompatibility between cocoa butter and foreign fats. When an incompatible foreign fat is added to cocoa butter, the fat crystal system is unstable and will tend to phase separate in several weeks or months. This phase separation phenomenon in cocoa butter has been studied by various investigators (Paulicka, 1970; Paulicka, 1973; Timms, 1984; Bigalli, 1988; Given et al., 1989) and its effects on bloom are complex. It usually takes several weeks for the phase separation in incompatible fat blends to become evident (Timms, 2003).

The phase separation of these incompatible fats can lead to polymorphic transition of cocoa butter to a more stable state causing bloom formation (Paulicka, 1970; Timms, 2002). On the other hand, the softening effect reduces SFC and provides a large amount of liquid fats with the high mobility, which promotes fat migration (Timms, 2003). In general, when compound chocolate contains more than 10% lauric-type fats or high-trans-type fats, bloom would eventually occur (Timms, 2003).

Incompatibility problems usually occur in compound coatings. Cocoa butter equivalents (CBE), such as shea butter and sal fat are completely compatible with cocoa butter due to its similar TAG composition to cocoa butter and the same polymorphism. Cocoa butter substitute (CBS) or lauric hard butter, where trilaurin is the main TAG, has the least compatibility with cocoa butter, as low as 5%, due to its significant difference in TAG composition. Bloom occurs rapidly when the concentration of cocoa butter (CB) in the CB-CBS blend is very high (Laustsen, 1991; Seguine, 2001). Cocoa butter replacers (CBR), also called cocoa butter extenders or hydrogenated domestic butter, cannot replace cocoa butter fully and its compatibility with cocoa butter is between CBE and CBS. A eutectic effect will be observed when above 15-20% cocoa butter is replaced (Lonchampt and Hartel, 2004). It is less bloom resistant than its fractionated portion (Laustsen, 1991).

2.4.2 Bloom from improper tempering

Bloom in under-tempered chocolate is related to the polymorphic transition. Crystallization time is longer in under-tempered chocolate as the nuclei formed in the tempering process are not sufficient for good crystallization and nucleation occurs during

cooling instead of crystal growth alone (Hartel and Lonchampt, 2004). As a result, unstable polymorph β'IV formed during this uncontrolled crystallization process leads to the β'IV-βV or β'IV-βVI transition (Timms, 2002; Hartel and Lonchampt, 2004).The polymorphic transition also causes contraction in the crystalline matrix forming gaps between crystals and pushing particles such as cocoa solids and sugars into the gaps (Kinta and Hartel, 2010). This phase separation between fat and dry matter as a result of re-crystallization and mass transfer limitation becomes visible on the surface as bloom (Hartel and Lonchampt, 2004).

Similarly, like under-tempered bloom, a polymorphic transition of β'IV-βV or β'IV-βVI transition is related to untempered bloom, as well as a separation of sugar and cocoa particles from the fat phase (Hartel and Lonchampt, 2004). Kinta and Hatta (2005) proposed a mechanism of untempered bloom in detail: (1) All seed crystals are destroyed when heated above the melting temperature; (2) During the cooling process, few nuclei can be formed due to lack of tempering; (3) Fats in chocolate, especially for SOS, crystallize on the nuclei, keep growing and form a cross-link network, incorporating sugar and cocoa solids inside the network in a spherical xenomorphic shape; (4) Due to the inhomogeneity in the matrix, a part of chocolate surface turn light brown due to the low fat content and high sugar content while the rest remains dark, resulting in a visual bloom.

In contrast, over-tempering produces excess amount of nuclei, which limit the extent of crystallization, thus resulting in an undesirable mass contraction in chocolate and a dull appearance on the surface. Bloom in over-tempered chocolate is related to the light diffraction on the rough surface as a result of the cracks and large crystals appearing during the tempering (Musser, 1973).

Cooling rate after tempering is another factor that affects fat bloom. Fast cooling can form pores and cracks on the chocolate surface, which would favor liquid fat migration and promote bloom. Further, fast cooling can also promote unstable β'IV fat crystals to form, followed by the β'IV-βV or β'IV-βVI transition causing bloom formation (Kleinert, 1962).

2.4.3 Storage bloom

Even though chocolate is well-tempered with metastable βV fat crystals, bloom could still occur during storage. As βV is still not the most stable form, a polymorphic transition of fat crystals from βV to βVI is favored during storage, especially under abusive storage conditions, which can lead to bloom formation (Timms, 1984). This usually occurs during storage in a long term, in months or years, with whitish spots and loss of gloss, where the whitish areas contain fat crystals that change the diffraction/scattering of the light on the chocolate surface (Timms, 2003). Usually the surface is rougher in bloomed chocolate, but the correlation between roughness and bloom is not strong especially at the early stage (Hodge and Rousseau, 2002). Therefore, visual bloom must be related to morphology and size of the fat crystals on the chocolate surface (Bricknell and Hartel, 1998; Timms, 2003). The recrystallized fat must extrude out of the surface to give diffuse reflectance, thus resulting in visual bloom (Hartel et al., 2016). The mechanism of storage bloom is very complicated and will be discussed in detail in the next section.

2.5 Mechanisms of storage bloom

2.5.1 Theories

Numerous theories have been generated, for decades, to explain the causes of fat bloom in chocolate during storage. However, till now the true intrinsic mechanism is still unclear. It is very likely that bloom is not caused simply by one factor. Despite the complexity in bloom mechanisms, most bloom theories are mainly based on two aspects: the polymorphic change in cocoa butter, and fat migration and recrystallization in chocolate.

2.5.1.1 Fat dissolution, migration and recrystallization

During storage, it is almost impossible to maintain constant storage temperature. When temperature increases, the SFC of chocolate decreases, leaving more fats in the liquid phase, with high mobility. On the other hand, the solubility of high-melting fats in chocolate in the liquid fats also increases, providing more solid fats dissolved in the liquid fats, with potential to migrate. The volume of these fats in the liquid phase is larger than when in the solid phase, resulting in over-pressure pushing liquid fats to the surface through pores and crevices (Timms, 2003). As temperature fluctuates, temperature gradients also form within chocolate as the surface experiences greater fluctuations than the center, providing a concentration difference in triglycerides between the surface and the core, which is also the driving force for liquid fats to migrate to the surface. As a result, liquid fats migrate to the chocolate surface, probably by both capillary force and diffusion (Aguilera et al, 2004; Lonchampt and Hartel, 2004). When temperature decreases, SFC decreases and the solubility of high-melting fats decreases, leaving more fats to be recrystallized. This temperature fluctuation favors recrystallization, which also

encourages polymorphic transition in cocoa butter (Timms, 2003; Lonchampt and Hartel, 2004).

Bloom is also accelerated in filled chocolate and chocolate with added nuts where the center of chocolate is high in fat content. This is basically caused by migration of unsaturated fat from center to surface, while the migration level of liquid fat from surface to center is less (Lonchampt and Hartel, 2004). In filled chocolate, bloom is also greater when the fat phase does not enrobe the nonfat particles completely due to improper conching process or undesirable flow behavior in chocolate (Kleinert, 1962).

In sum, bloom is caused by liquid oil migration due to diffusion from the fat concentration difference between the surface and center, or/and capillary flow between interparticle passages and connected pores, providing more opportunity for fat recrystallization on the chocolate surface (Hartel, 1999; Hartel and Lonchampt, 2004; Dahlenborg, 2014). This process, which is usually enhanced by temperature fluctuation, may occur as the following steps: (1) Dissolution of the higher melting fatty acids in the lower melting ones; (2) Migration of liquid fat, together with dissolved fats, to the surface; (3) Recrystallization of higher melting fats on the surface (Hartel, 1999; Matsuda et al., 2001).

2.5.1.2 Polymorphic transition

A polymorphic transition of cocoa butter from βV to βVI is thought to occur in all bloomed chocolate; in storage bloom, the whitish crystal layers on a bloomed chocolate surface have been found to be βVI crystals (Berger at al., 1979, Sonwai and Rousseau, 2006). This polymorphic transition can be initiated either by heterogeneous nucleation on the surfaces of

the βV crystals and sugar particles, or secondary nucleation of triglycerides in the liquid phase after separation from βV crystals (Sato and Koyano, 2001).

In filled chocolate, this polymorphic transition mechanism and oil migration mechanism are synergistic. Enhancing oil migration rate increases polymorphic transition rate (Smith, 1998; Timms, 2003; Smith et al., 2007) and polymorphic transition causes an increase in the liquid phase and thus encourages oil migration (Ziegleder et al, 2001; Timms, 2003).

However, Bricknell and Hartel (1998) pointed out that the polymorphic transition from βV to βVI alone is not sufficient to cause visual bloom, as they found chocolates made with amorphous sugars did not present visual bloom, even though X-ray diffraction showed that these chocolates did undergo a polymorphic transition to βVI. Thus, even though polymorphic transition is an essential factor in bloom formation, it is still unclear whether it is the cause or the result of fat bloom.

2.5.2 Factors influencing storage bloom

2.5.2.1 SFC

Solid fat content (SFC) is the amount of crystallized fat in the total fat mass at certain temperature. It represents the ratio of the solid and liquid part in the fat system. In literature, no one has studied the effect of SFC directly since there are always other factors involved as well.

Adding low-melting fats to chocolate reduces SFC and affects bloom. Ali et al. (2001) and Smith et al. (2007) reported that decreasing SFC by adding nut oil or a blend of cotton oil and palm mid-fraction (PMF) would accelerate polymorphic transition of fats, as well as fat

migration, which could potentially promote bloom. Sahari et al. (2013) reported that increasing modified tea seed oil concentration from 10% to 20% in cocoa butter (reducing SFC) increased bloom extent. On the other hand, high-melting fats with high SFC can have other effects. Lohman and Hartel (1994) reported high melting milk fat fractions reduced bloom in chocolate and they hypothesized one explanation would be due to the high SFC and complex crystalline structure from the high melting fats. Other theories may also explain this anti-bloom effect. As discussed before, high SFC reduces the amount of liquid fat (carrying dissolved solid fat) in the system. This liquid fat has high mobility and could potentially participate in the migration and recrystallization of cocoa butter, leading to bloom. Also, high-melting fats with high SFC usually slow down the polymorphic transition in cocoa butter (Schlichter-Aronhime and Garti, 1988).

Interestingly, in a study on palm kernel oil-based compound coatings, the induction time for bloom and time to achieve bloom completely were found to be significantly and negatively correlated with SFC (Ransom-Painter et al., 1997). This research suggested that the mechanisms of bloom in compound coatings might be different from that in chocolates, and the changes in crystalline structure within each coating may contribute to different bloom results.

2.5.2.2 Sugar particles

Several studies have shown that some types of sugar particles can act as bloom inhibitors. Cerbulis (1969) investigated the effect of several sweeteners in chocolate and reported that anhydrous glucose had significant reduction in bloom level, whereas fructose as well as

cerelose, a commercial preparation of glucose containing about 9% water, were found to have slight bloom inhibition compared to chocolate control samples made with sucrose. Son et al. (2018) reported the anti-bloom effect of maltitol and D-tagatose in chocolate compared to control samples with sucrose, but the potential mechanism was not clarified. Bricknell and Hartel (1998) reported the inhibition effect on visual bloom from amorphous sugar (spray dried corn syrup) even though a polymorphic transition of cocoa butter from βV to βVI did occur and they credited this phenomena to the difference in the shape of recrystallized fat on the chocolate surface influenced by amorphous sugar. Several mechanisms have been postulated about the anti-bloom effect from amorphous sugars; namely, the change in migration rate with microstructure change, fat recrystallization inhibition or reactant mobility reduction (Lonchampt and Hartel, 2004).

2.5.2.3 Milk fat

It has been well accepted that milk fat can act as a bloom inhibitor in chocolate (Hartel, 1996; Kaylegian, 1998; Timms, 2003; Lonchampt and Hartel, 2004). Adding 1-2% milk fat to dark chocolate is sufficient to retard bloom formation (Skytte and Kaylegian, 2017). Several studies have shown that hydrogenated milk fat was able to delay fat bloom in chocolate, and the effect of fully-hydrogenated milk fat was more significant than partially- and unhydrogenated milk fat (Campbell and Keeney, 1968; Campbell at al., 1969, Hendrickx et al., 1971). Further, the melting point of milk fat fraction matters in bloom inhibiting effect. HMF is better at bloom prevention than intact milk fat (Jebson, 1974; Bricknell and Hartel, 1998). The higher the melting point of milk fat, the longer the delay of fat bloom (Lohman and Hartel,

1994, Dimick et al., 1996). On the other hand, LMF actually promotes bloom instead of inhibiting it (Lonchampt and Hartel, 2004). This might be due to the extra liquid fat in LMF to enhance the mobility of the triglycerides (Lohman and Hartel, 1994).

The mechanisms of the anti-bloom effect from milk fat are still uncertain. Adding milk fat into chocolate softens the texture and lowers SFC (Timms and Parekh, 1980; Jewell and Bradford, 1981; Lohman and Hartel, 1994), but even this softened chocolate is more resistant to bloom than regular chocolate. The effect of milk fat on cocoa butter crystallization might be one explanation. The addition of milk fat reduced crystal size, crystallization rate and polymorphic transition rate of cocoa butter (Vaeck, 1960; Chapman et al., 1971; Hartel, 1996; Bricknell and Hartel, 1998; Lonchampt and Hartel, 2004). Cebula and Ziegleder (1993) reported a type of chocolate made with 2-5% milk fat that did not bloom for a year when stored at 23°C and stayed at βV polymorph as the milk fat retarded the cocoa butter polymorphic transition. Also, the reduction of polymorphic transition in cocoa butter prevents microscopic cracks from occurring (Kleinert, 1961). Further, minor components (polar lipids) in milk fat change cocoa butter crystallization by changing crystal morphology (Tietz and Hartel, 2000; Wright et al., 2000). The dispersed phase in chocolate is also very important to bloom formation. Milk fat may interact with solid particles to affect bloom rate (Hartel, 1999; Liang and Hartel, 2004).

2.5.2.4 Emulsifiers

Several emulsifiers have been found to be anti-bloom agents in chocolate. Span 60, Tween 60, sorbitan monostearate, polysorbate 60, sorbitan tristearate and polyglycerol ester were all

reported to have greatly effective inhibition effects on fat bloom, although they cannot be used in chocolates (Easton et al., 1952, DuRoss et al., 1965; Weyland, 1994). Lecithins and monoacylglycerols also reduce bloom by retarding polymorphic transition of cocoa butter (Garti et al., 1982). Potential mechanisms of the anti-bloom effects from emulsifiers could be related to the interaction between sugar and fats, intermediated by emulsifier (Lonchampt and Hartel, 2004). One theory is that a monolayer of emulsifiers adsorbed on fat crystals makes them more polar, whereas a layer of emulsifiers on sugar crystals makes them more apolar (Johansson and Bergenstahl, 1992a; Johansson and Bergenstahl, 1992b; Dedinaite et al., 1998). The resulting interactions between emulsifier, sugar and fats may influence bloom formation through a change in the microstructure of chocolate (Johansson and Bergenstahl, 1992a). For example, particle interactions may influence fat migration or the recrystallization of cocoa butter. Further, emulsifiers may change crystallization in cocoa butter by acting as co-crystallizer, increasing seed numbers, changing growth rate, reducing crystal size, increasing the melting point of cocoa butter and retarding the polymorphic interaction (Nakae, 2000; Lonchampt and Hartel, 2004; Miyasaki, 2016).

2.5.2.5 Storage conditions

Temperature is an important factor in storage bloom. In general, the lower the temperature, the slower bloom would occur (Lonchampt and Hartel, 2004). Chocolate may have optimal shelf properties when stored below 18°C, without temperature fluctuations (Ali et al., 2001; Lonchampt and Hartel, 2004; Quevedo et al., 2005). When storage temperature increases, the SFC of chocolate decreases providing more liquid fat with high mobility. Oil migration rate in

chocolate is increased with an increasing storage temperature (Ziegleder, 1997; Guiheneuf et al., 1997; Jinap et al., 2000; Ali et al., 2001; Miquel et al., 2001; Choi et al., 2005). As fat migration is thought to be an important step in storage bloom in chocolate, temperature may play an important role in the process. When the storage temperature is below the melting point of βV (between 18-30°C), bloom rate increases dramatically as temperature increases. Bloom also occurs even rapidly once the temperature goes just above the melting point of βV, to 32-34°C. Since the chocolate is partially melted, when it cools again, crystallization is uncontrolled, forming unstable crystal forms (Lonchampt and Hartel, 2004). Also, Walter and Cornillon (2001) reported that a thermal history of " annealing" chocolate at 28 or 32°C for 3 days before finally storing it at 21°C significantly induced fat bloom; thus, preventing extreme thermal changes before storage is also the key.

Temperature fluctuation is another factor that affects bloom. When temperature increases, the SFC goes down, providing more liquid fat and more dissolved fat with high mobility to migrate to the chocolate surface. When temperature goes down again, recrystallization of cocoa butter can occur on the surface. As a result, temperature fluctuations reduce the bloom induction time and enhance bloom rate. Thus, several studies used temperature fluctuation to enhance bloom (Lohman and Hartel, 1994; Tietz and Hartel, 2000; Ali et al., 2001). Quevedo et al. (2005) found that more rapid temperature fluctuation between 16°C and 26°C from every 12 hours to every 3 hours increased surface roughness of chocolate, which might be an indication of bloom extent. However, Rothkopf et al. (2017) found that cycling between 18°C and 25.5°C every 7 days resulted in greater bloom than cycling every 2.5 days. Thus, the effect of temperature fluctuation frequency is complex, and can be different with different chocolate

microstructure and storage conditions. Also, the storage temperature before the cycling treatment has an effect on the bloom extent. Ali et al. (2001) reported that chocolate prestored at 30°C for 8 weeks showed faster and greater bloom compared to chocolate prestored at 18°C for 8 weeks when a temperature cycle between 30°C for 8 hours and 20°C for 16 hours was applied in both samples. Rothkopf et al. (2017) pointed out that the starting temperature during temperature cycling was important to bloom as chocolate first stored at the higher temperature presented earlier WI increase when samples were both stored at temperatures cycled between 25.5°C and 18°C every 7 or 2.5 hours. Furthermore, Hodge and Rousseau (2002) found that increasing the maximum temperature in the cycling from 32°C to 34°C increased surface roughness of chocolate. Thus, the interval between the maximum and minimum temperatures during cycling is also important.

2.5.3 Storage bloom summary

The mechanisms of chocolate bloom are very complex and need to be explained by multiple factors together. Any factor that changes polymorphism of cocoa butter, fat crystallization and fat migration may have great influence on bloom formation. Figure 2.13 summarizes important factors promoting or inhibiting bloom formation.

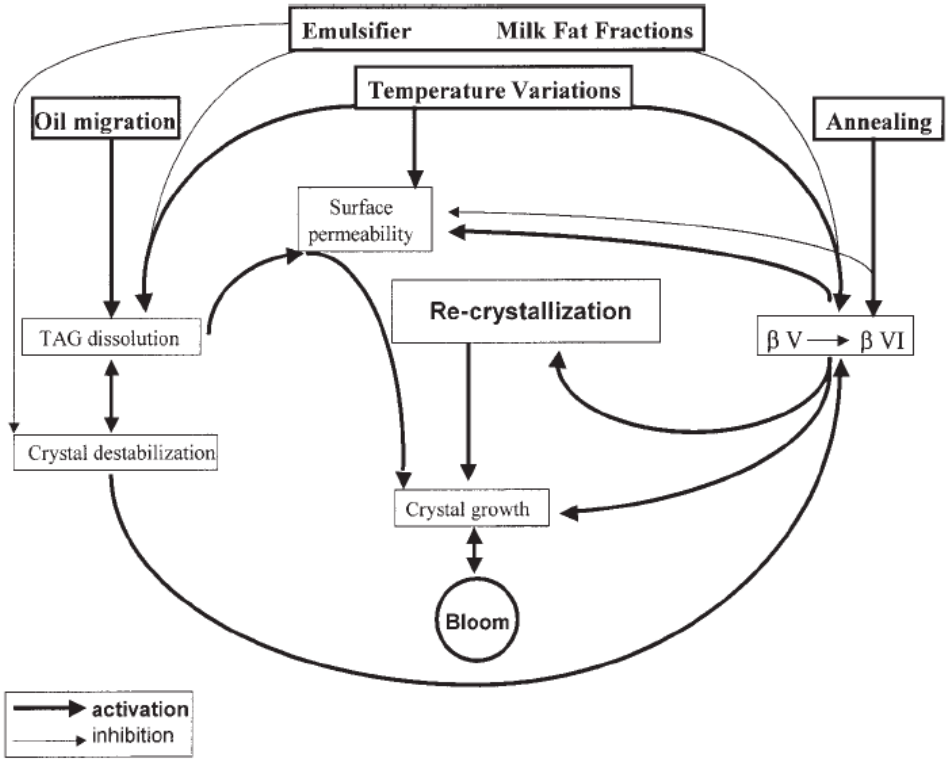


Figure 2. 13 A summary of mechanisms of chocolate bloom. Adapted from Hartel and Lonchampt (2004).

2.6 Chocolate evaluation technologies

2.6.1 DSC

Differential scanning calorimetry (DSC) is a thermoanalytical technique where a sample and a reference sealed in aluminum pans are maintained at nearly the same temperature increasing linearly as a function of time. The principal idea behind this technology is that the heat flow would change (either exothermically or endothermically) compared to the reference when a sample undergoes phase transition, to maintain both pans at the same temperature. The difference in the amount of heat flow required, which is also known as the enthalpy, is measured as a function of temperature to determine the amount of heat absorbed or released during such transitions. In food materials, DSC is able to measure the melting point, glass transition temperature, and heat of fusion (Tan and Che Man, 2000). The heat of fusion is calculated by

integrating the peak corresponding to a given transition. The heat of fusion could be expressed according to the following equation:

$$\Delta H = KA \quad (2.1)$$

ΔH is the heat of fusion, K is the calorimetric constant, and A is the area under the curve.

The DSC method is fast and sensitive, which enables the parameters to be determined easily. In chocolate studies, it is used for tracking crystallization kinetics and determining crystal polymorphs in cocoa butter (Spigno et al., 2001; Timms, 2003).

2.6.2 TD-NMR

Nuclear magnetic resonance (NMR) method is based on the phenomena that nuclei in a magnetic field absorb and reemit electromagnetic radiation. The intramolecular magnetic field around an atom in a molecule changes the resonance frequency, which provides detailed information about the electronic structure of a molecule. Time-Domain NMR (TD-NMR) or Low Resolution NMR is a well-known type of NMR. Based on analyzing the absolute or relative amplitude of the free induction decay and/or spin-echo, this technique is widely applied in quality control as well as research and development in the food industry.

Solid fat content (SFC), which is the ratio of fat in crystalline (solid) phase to total fat at a certain temperature, is analyzed by TD-NMR. As a result of slower nuclear dynamics, the interaction between nuclei in solids and other solid neighboring nuclei means solid nuclei relax quickly (<10 μsec), which can be easily distinguished from other slower relaxing nuclei in the liquid state. After application of one 90° radio frequency (RF) pulse, SFC is calculated from a diagram of the time evolution of the NMR response as the sample nuclei relax after the pulse

is removed. A representative free induction decay (FID) signal diagram is showed in Figure 2.14.

SFC can be calculated directly by measuring the NMR signals from nuclei in the solid and liquid state (11μsec after the RF pulse was terminated) and the liquid state alone (70μsec after the RF pulse was terminated) (Method Cd 16b-93; AOCS, 2009). Using the following equation, commercial instruments with certain software could calculate SFC directly.

$$SFC = \frac{F*(S_{SL}-S_L)}{F*(S_{SL}-S_L)+S_L+D} * 100 \quad (2.2)$$

Here, S_{SL} is the NMR signal from nuclei in the solid and liquid state (obtained 11 μsec after the RF pulse was terminated); S_L is the NMR signal from liquid nuclei (obtained 70 μsec after the pulse was terminated); F is an empirical correction factor gained from daily calibration depending on the detector dead time and crystal type; and D is the digital offset factor established during calibration.

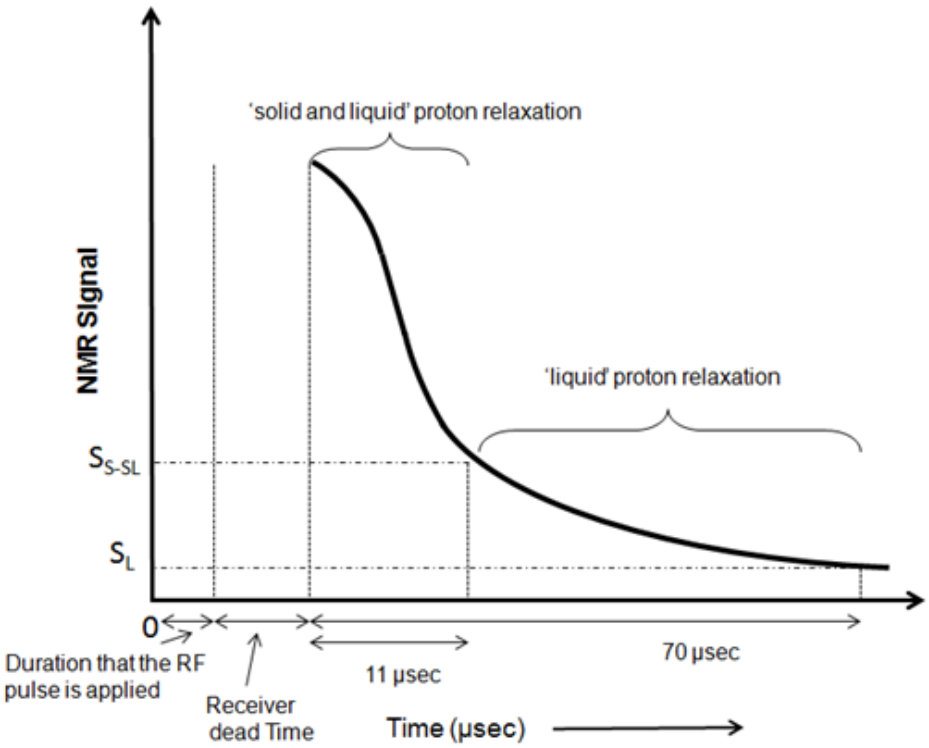


Figure 2. 14 Free induction decay (FID) signal diagram as a function of time. Adapted from Peyronel and Marangoni (2014).

2.6.3 Rheology

As discussed before, liquid chocolate is a dispersion of solid particles in liquid fats. The matrix system is complex due to high amounts of particles. At rest, the particles are randomly dispersed in the system. As a result, the apparent viscosity is relatively high. When a shear force is applied, chocolate starts to move in the flow direction and the particles are also aligned in this direction. As a result, there is a decrease in resistance to flow when shear rate is increased until a pseudo-equilibrium is reached. In other words, liquid chocolate is a shear-thinning structure (Wolf, 2017). Figure 2.15 shows the change between these two states.

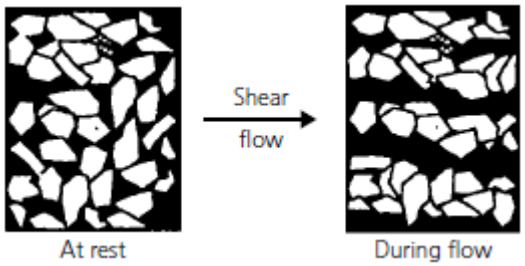


Figure 2. 15 A schematic diagram of chocolate network at rest and during flow. Adapted from Wolf (2017).

In order to obtain a flow curve, a rheometer is used to perform rotational tests. Three geometries are most commonly used: cup and bob, cone and plate and parallel plate (Mezger, 2006; Goodwin and Hughes, 2008), as shown in Figure 2.16. When using the cup and bob geometry, the chocolate is placed in the cup and the bob is immersed in the chocolate. The shear rate is fixed and controlled by the driven motor of the rheometer while the shear stress can be measured by the torsion spring connected to the rotating bob (Wolf, 2017).

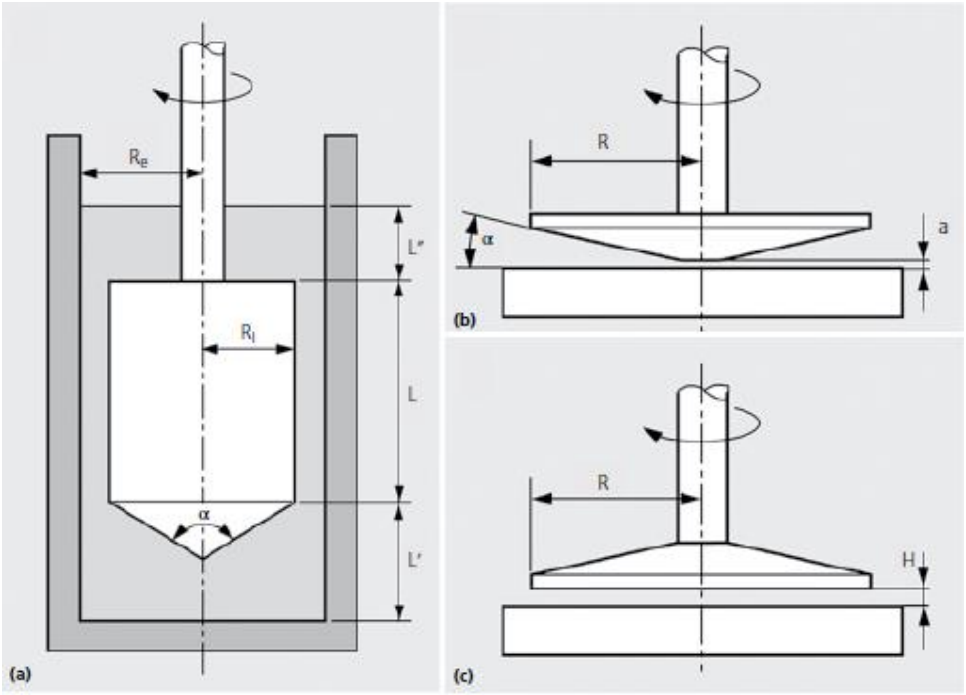


Figure 2. 16 Typical geometries of a rheometer: (a) cup and bob, (b) cone and plate, (c) parallel plate. Adapted from Wolf (2017).

One way to evaluate the flow behavior of chocolate is to fit a mathematical model to the flow curve. Casson model (Eqn. 2.3) is the most widely used model in the chocolate industry.

$$\sqrt{\sigma} = \sqrt{\sigma_c} + \sqrt{\eta_c}\sqrt{\dot{\gamma}} \tag{2.3}$$

σ is the shear stress, $\dot{\gamma}$ is the shear rate, σ_c is the Casson yield stress, and η_c is the Casson viscosity, which is viscosity at viscosity at infinite shear rate (Metz et al., 1979; Seguine, 1988). Casson yield stress and Casson viscosity can be used as important parameters to present chocolate flow behavior and particle interactions.

2.6.4 Tempermeter

In the chocolate industry, the degree of temper is very important for quality control and bloom prevention. Commercial tempermeters are basically thermometers that track cooling curves of tempered chocolates. The principle of the tempermeter is based on the phenomenon

that heat is released when the cocoa butter is crystallized during cooling, which will offset the heat that is lost due to cooling from the surrounding. As a result, there will be a plateau in the cooling curve.

To operate the tempermeter, the chocolate samples are placed in a metal cup, precooled in cold water bath, and the temperature is recorded by a sensor probe placed in the chocolate as shown in Figure 2.17. The data are usually sent to a recording software where a temperature vs. time cooling curve is plotted. Typical cooling curves are shown in Figure 2.12. The slope of the plateau is then calculated by the software, and usually chocolate with slope between -1 and 1 can be considered as well-tempered. A tempermeter is advantageous for its convenience and being able to quantify the temper degree, but the information it provides about chocolate crystallization is still limited (Loeser, 2017).

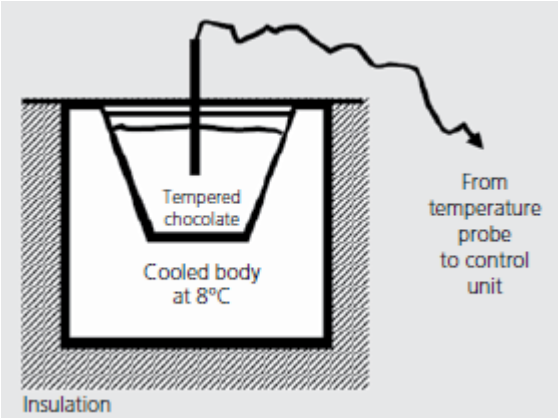


Figure 2. 17 Setup of a commercial tempermeter. Adapted from Loeser (2017).

2.6.5 Tristimulus colorimetry

One of the most convenient ways to evaluate the appearance of a chocolate surface is using a tristimulus colorimeter to measure surface color. Dark chocolate usually presents a brown color, which gets whiter when bloom develops on the surface and can be tracked by a

tristimulus colorimeter. Essential parts of a typical colorimeter are a white light source, three to four color filters, an array of photometers and a computer to process color data. In general, three photoelectric measurements are usually taken by three color filters corresponding to the three primary colors in the spectrum (red, green and blue), which can be combined to match most colors. Some more advanced colorimeters also use a fourth filter to simulate the blue part of the CIE function (Joshi and Brimelow, 2002). The detector's spectral response is in the color-matching functions: $\bar{x}_\lambda$, $\bar{y}_\lambda$ and $\bar{z}_\lambda$, which are later transformed to tristimulus values *X*, *Y*, and *Z*. By using the formulae from Hunter and Harold (1987), the final data are then transformed and presented in CIELAB (*L**, *a**, *b**), HunterLab or *Y*, *x*, *y*. A summary of this procedure is shown in Figure 2.18.

CIELAB is usually used to present chocolate color data. CIELAB system is established by International Commission on Illumination (French: Commission internationale de l'éclairage, CIE). There are three coordinates: *L**, luminance ranging from 0 (black) to 100 (white); *a**, negative values represents green while positive values represent red; and *b**, negative values represent blue and positive values represent yellow (Kong and Singh, 2011). A star after the *L*, *a*, *b* letters is generally used to differentiate from HunterLab.

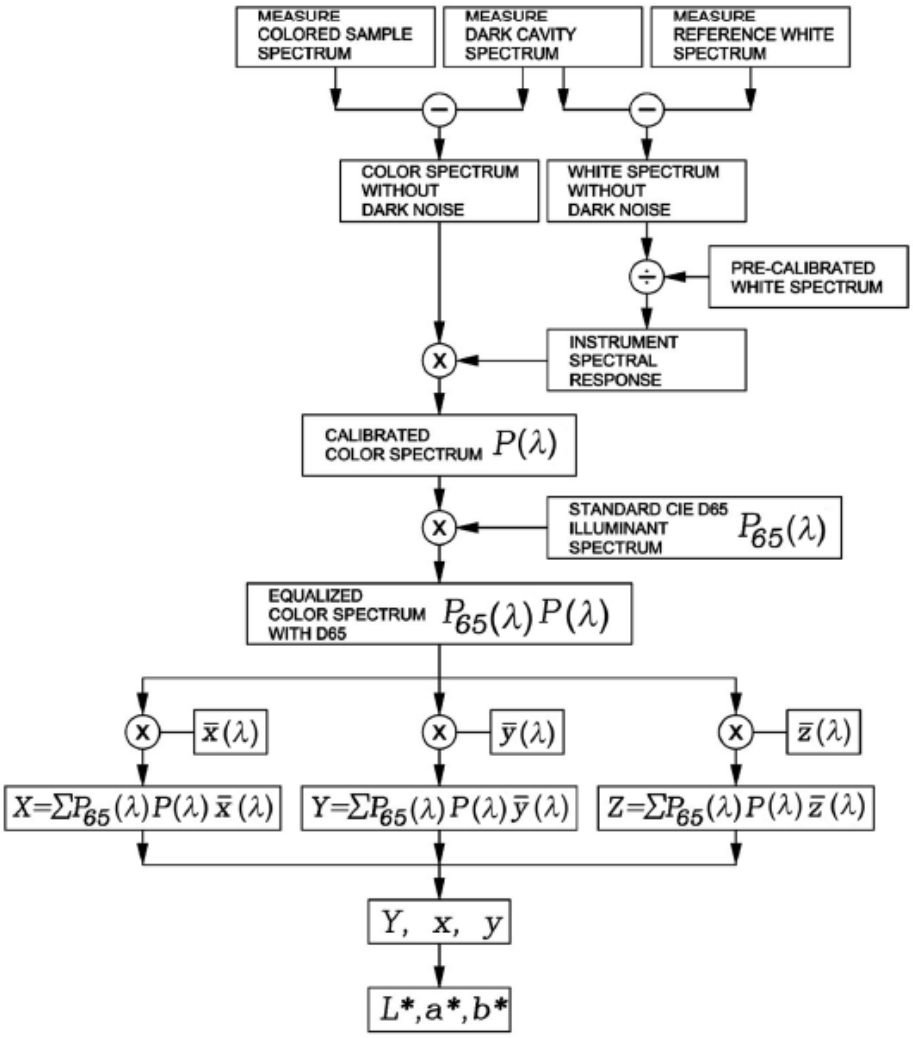


Figure 2. 18 Procedures of tristimulus colorimetry measurements. Adapted from Malacara (2011).

2.7 Summary and research directions

Chocolate is a complex system. Bloom is also complicated and is influenced by multiple factors. In this research, the effects of the nature of particles in chocolate on fat bloom formation during storage are studied. It is hypothesized that particulate factors such as SFC, particulate concentration, type of particles, presence of emulsifiers, shape and surface properties of particles all contribute to bloom formation during storage. It is our objective to develop a standard method and model systems to evaluate these effects on storage bloom.

Comparison of three different methods of bloom evaluation and construction of correlations between particle interactions and bloom formation are also involved. Establishing a connection between bloom and formulation factors could provide useful data for formula development and storage control in the chocolate industry and perhaps even other industries.

3. Materials and methods

In order to investigate particulate effects on fat bloom in chocolate during storage, this study was carried out in several stages. First, nonsugar chocolate model systems were designed with different solid fat contents (SFC) while the effects of SFC and low-melting fat type on bloom whiteness were investigated. Next, the effects of SFC and storage temperature fluctuation frequency on bloom whiteness were investigated. Further, the chocolate model systems were modified by gradually replacing cocoa powder with four types of sugars at different levels and the effects of sugar type and sugar concentration on fat bloom were investigated by three different bloom evaluations. Lastly, the sharp surface of sucrose was rounded and bloom in chocolate model systems with different sugar surfaces was investigated. A correlation between particle interaction and fat bloom in chocolate was also constructed.

3.1 Effects of fat phase on fat bloom in chocolate model systems

The aim of this phase is to evaluate the effect of SFC and composition of fats on fat bloom in chocolate model system. The fat phase in the chocolate model systems were formulated by mixing fractionated cocoa butter with different types oils with ratios obtained from the SFC isothermal diagrams to ensure the designated SFC. And bloom were evaluated by whiteness index (WI) values.

3.1.1 Materials

Four liquid oils were used as low-melting fats in chocolate model systems including refined peanut oil (PNO; Golden Peanut Company, Alpharetta, GA), refined cottonseed oil

(CSO; AAK, Louisville, KY), canola oil (CNO; Adam vegetable oils, Arbuckle, CA), and oleic sunflower oil (SFO; Adam vegetable oils, Arbuckle, CA). Cocoa butter stearin (CB-S), a high-melting fraction of cocoa butter (CB; ADM cocoa, Milwaukee, WI), was used as the high-melting fat in the chocolate model system. The fractionation process was conducted by the following steps: (1) melt the CB at 60°C for 1 h to destroy any thermal history and crystal structures; (2) crystallize the melt statically by cooling at 26°C without physical disturbance for 24 h; and (3) filter off the solid phase by vacuum filtration. The resulting solid component was designated as CB-S. Defatted cocoa powder (CP; ADM cocoa, Milwaukee, WI), containing about 0.5% fat, was used as nonfat particles in the dispersed phase.

3.1.2 SFC profile and isothermal diagrams of binary fat mixtures

SFC profiles of binary fat mixtures were measured, to calculate fat formulations for chocolate model systems with 45, 55, and 65% SFC, by a Bruker Minispec mq20 nuclear magnetic resonance (NMR) (Bruker, Woodlands, TX), together with Minispec software. Standards with 0, 32, and 73.1% SFC were used for calibration. The binary fat mixtures were prepared by mixing high-melting and low-melting fats at oil concentrations ranging from 0% to 100% (0, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, and 100%), with either 5% or 10% increment, depending on whether the final SFC was close to the targeted 45%-65% zone. Roughly 2 mL of the fat mixtures were melted at 60°C for 30 min, filled and capped in the NMR tubes for measurement. A tempering process was followed according to the IUPAC method (IUPAC, 1987) by melting the samples at 80°C for 20 min, quenching to 0°C for 90min, holding at 26°C for 40 h, cooling to 0°C for 90 min and finally equilibrating the samples at the

experimental temperatures, from 0°C to 40°C with 5°C increment, for 1 h before taking SFC measurements on the NMR. Samples with different oil compositions were prepared in quadruplicate and each replicate was poured into three NMR tubes as a sub-sample for NMR measurements. The SFC values were taken as the average of the 12 readings. The isothermal diagrams for each binary system were generated by plotting the average SFCs versus low-melting fat concentrations at the designated temperatures. In order to evaluate the efficiency of CB fractionation, SFC of CB samples as a control was also tested using the same method described before and the measurements were taken in triplicate.

3.1.3 Chocolate model systems

Twelve chocolate model systems were formulated by dispersing 75 g defatted cocoa powder in 75 g fat mixtures containing CB-S and one type of the 4 liquid fats, with 3 levels of SFC (45, 55, and 65%). To determine the fat ratio in the fat mixture, a simple linear regression, between the liquid oil composition and SFC, was conducted on the 20°C isothermal curve for each liquid oil type; the oil composition in each model system, as shown in Table 3.1, was calculated by applying the designated SFC (45, 55, and 65%) into the regression model.

Table 3. 1 Oil composition in the fat mixtures with cocoa butter stearin required to attain different solid fat content (SFC).

SFC (%)	PNO (%)	CSO (%)	SFO (%)	CNO (%)
45	29.46 ± 0.80^{a}	26.18 ± 0.93^{b}	29.85 ± 0.74^{a}	29.65 ± 0.57^{a}
55	16.53 ± 0.64^{c}	14.82 ± 0.59^{d}	17.23 ± 0.58^{c}	17.30 ± 0.40^{c}
65	3.68 ± 0.63^{e}	3.45 ± 0.51^{e}	4.61 ± 0.52^{e}	4.95 ± 0.38^{e}

$^{a,b,c,d,e.}$ Means not connected with the same letter are significantly different (α=0.05).

PNO: Peanut oil; CSO: Cottonseed oil; SFO: Sunflower oil; CNO: Canola oil.

3.1.4 Tempering

In order to eliminate the possibility of bloom due to improper tempering, all chocolate model systems were tempered according to Kleinert's cyclo-thermic tempering method (Kleinert, 1970). Temperatures in the tempering profiles were adjusted by every 0.5°C based on a trial and error principle (Barna et al., 1992) on each replicate until the tempered chocolate passed all of the temper measurements described afterwards to ensure good tempering. To temper the model systems, the samples were melted at 50°C for 30 min to completely remove any crystal structure, cooled to T_1 until the viscosity reached a maximum, reheated to T_2 until the viscosity became constant, recooled to T_3 until viscosity reached a maximum again, and finally held at T_4. The optimal tempering profiles found in this way for each model system are shown in Table 3.2. Each model system was tempered in quadruplicate. There is a slight trend in Table 3.2 showing that higher temperatures at each stage were sufficient to reach good tempering for samples with higher SFC. This is probably because more solid fats in the system provide more driving force for fat crystallization. Moreover, higher SFC results in an increase in viscosity in the system; thus, higher temperature is needed to keep the viscosity consistent.

Table 3. 2 Tempering profiles (ºC) for chocolate model systems with different solid fat content (SFC).

SFC	T_1	T_2	T_3	T_4
45%	24.0	28.5	27.0	30.5
55%	25.0	29.5	28.0	31.5
65%	25.5	30.0	28.5	32.0

T_1: Initial cooling temperature; T_2: Temperature which chocolate was warmed to; T_3: Temperature which chocolate was recooled to; T_4: Final holding temperature.

In order to ensure good tempering, freshly tempered chocolate was examined by two methods. First, a strip of wax paper was dipped into the chocolate sample, and held at room temperature for several minutes. If the chocolate on the wax paper was solidified in one minute, with a glossy surface and a good snap, the chocolate was considered well-tempered. To confirm, the freshly tempered chocolate was transferred into a temper meter cup surrounded by ice water bath and a cooling rate curve was generated by recording the temperature of the chocolate sample every 10 s. Typical cooling rate curves for different degree of temper are shown in Figure 2.12. A well-tempered chocolate should have a flat plateau in the second temperature phase. Representative cooling rate curves for each SFC are shown in Figure 3.1, confirming that all model systems were well-tempered.

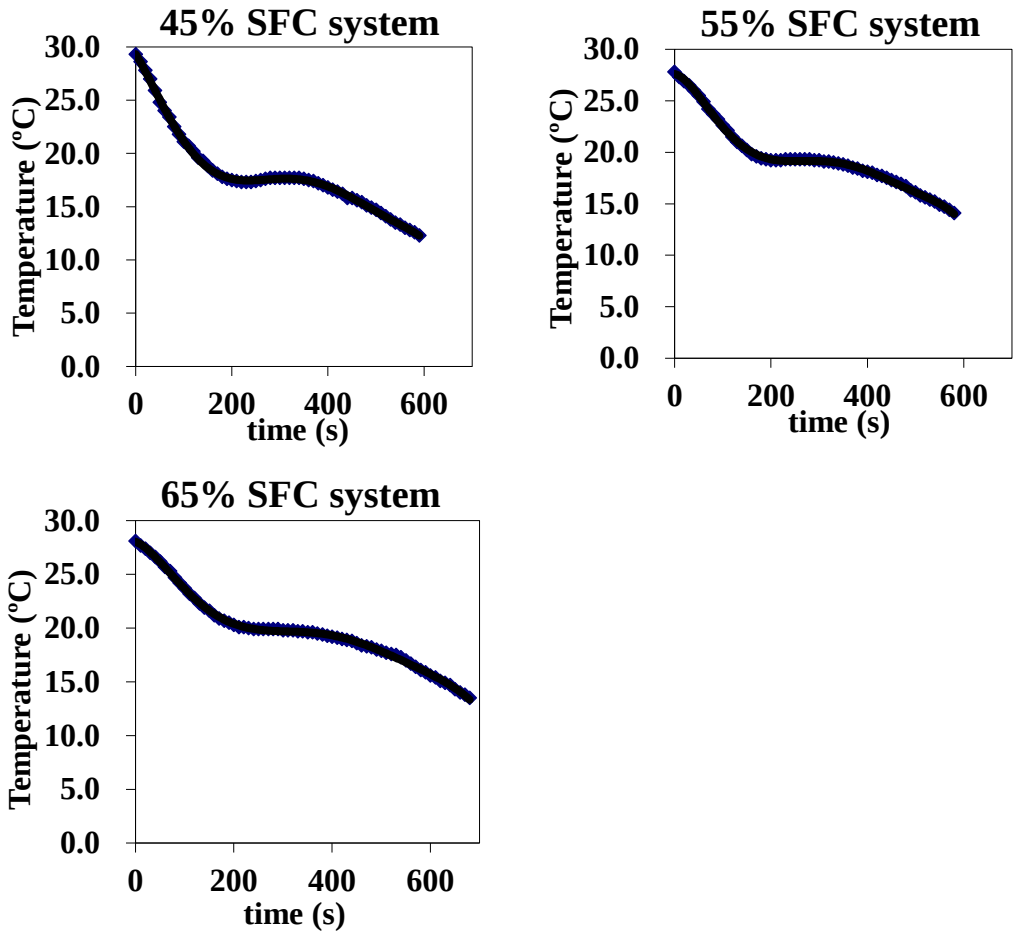


Figure 3. 1 Representative cooling rate curves for chocolate model systems with different solid fat contents (SFC).

3.1.5 Chocolate storage

After tempering, the chocolate samples were molded into plastic discs, cooled at room temperature overnight, and then transferred into a Sanyo MIR-253 (Sanyo Scientific, IL) incubator with programmable temperature cycler. The temperature was cycled between 20°C and 30°C every 7 h, with 99 cycles.

3.1.6 Whiteness index

A Minolta CR-3000 (Minolta, NJ) colorimeter was used to quantify the surface changes of chocolate. Calibration was done with a white standard plate (Y=91.96, x=0.3170, y=0.3329). Afterwards, L*, a*, b* values were measured in triplicate on each chocolate disc surface, and whiteness index (WI) was calculated according to the following equation (Lohman and Hartel 1994).

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}} \quad (3.1)$$

The WI values were tracked at day 0, 1, 7, 14, 21, and 28 as an indicator of bloom extent. Higher WI values indicate higher bloom extents. Bloom progression curves were constructed by plotting WI values with time at different SFCs in different model systems to compare bloom behavior.

3.1.7 DSC

In order to track the polymorphic transition of chocolate samples at different storage time, a differential scanning calorimetry (DSC) (Perkin Elmer DSC 8500, Shelton, CT), with liquid nitrogen (Airgas, Rador, PA) as the cooling medium, was used to measure the melting points.

To prepare the samples, about 6 mg of well-tempered chocolate (CSO system with 65% SFC) at day 0 and 28, as well as a freshly under-tempered chocolate with visible whitish spots on the surface, were sampled from chocolate discs and sealed in an aluminum pan (TA Instrument, New Castle, DE) using a crimper, and put into the DSC chamber, together with an empty pan as a reference. The samples were measured by holding at 15°C for 1 min and then screening from 15 to 60°C at 5°C/min. The DSC curves were analyzed with DSC data analysis software (Pyris, Perkin Elmer, Norwalk, CT) and the melting point (peak value) was calculated automatically from the software. The measurements were conducted in duplicate.

3.2 Effects of temperature fluctuation frequency on fat bloom during storage

The focus of this phase was to evaluate the effect of temperature fluctuation frequency on bloom in model systems with different SFCs. Only CNO systems were studied in this phase, and three temperature fluctuation frequencies (every 3, 7, or 11 hours) were selected by storing chocolate model systems in the oven with different temperature cycling programs. Bloom evaluation methods were similar to the previous phase.

3.2.1 Materials

CNO (Adam vegetable oils, Arbuckle, CA) was used as the low-melting fat in the chocolate model system. CB-S, fractionated according to the method described in Section 3.1.1 was used as the high-melting fat. Defatted cocoa powder (ADM cocoa, Milwaukee, WI) was used as nonfat particles in the dispersed phase. Yucatan BR SEMI-SWEET chocolate (ADM

cocoa, Milwaukee, WI), containing 46.4% total sugar and 33.0% fat content, was used as a commercial chocolate.

3.2.2 Experimental Design

A 3×3 full factorial with two factors, temperature fluctuation frequency and SFC was conducted. Also, Yucatan BR SEMI-SWEET chocolate was the control to compare bloom progression in commercial chocolate and model systems. Table 3.3 shows the factorial design.

Table 3. 3 Experimental design for chocolate model systems with three different solid fat contents (SFC) (45, 55, and 65%) when temperature was cycled between 20°C and 30°C at different fluctuation frequencies (every 3 hours, 7 hours, 11 hours).

SFC (%)	Temperature fluctuation frequency
45	Every 3 hours
45	Every 7 hours
45	Every 11 hours
55	Every 3 hours
55	Every 7 hours
55	Every 11 hours
65	Every 3 hours
65	Every 7 hours
65	Every 11 hours

3.2.3 Tempering

Model systems with 45, 55, and 65% SFC were prepared according to the formula described in Section 3.1.3. All samples were tempered in triplicate according to the Kleinert method (Kleinert, 1980) using the tempering profile in Table 3.4. The tempering measurements were examined as previously described in Section 3.1.4.

Table 3. 4 Tempering profiles (°C) for chocolate systems.

chocolate system	T_1	T_2	T_3	T_4
45% SFC model system	24.0	28.5	27.0	30.5
55% SFC model system	25.0	29.5	28.0	31.5
65% SFC model system	25.5	30.0	28.5	32.0
commercial chocolate	25.0	29.5	28.0	31.5

T_1: Initial cooling temperature; T_2: Temperature that chocolate was warmed to; T_3: Temperature that chocolate was recooled to; T_4: Final holding temperature. SFC: solid fat content.

3.2.4 Chocolate storage

After tempering, the chocolate samples were deposited into plastic disc molds. Each tempered chocolate was molded into two disks, cooled at room temperature overnight and stored in a Sanyo MIR-253 (Sanyo Scientific, IL) incubator with programmable temperature cycler. In this case, the temperature was cycled between 20 and 30°C with three different temperature fluctuation frequencies (every 3 hours, 7 hours and 11 hours) with 99 cycles.

3.2.5 Whiteness index

The WI values were tracked at day 0, 1, 3, 7, 14, 21, and 28 by a Minolta CR-3000 (Minolta, NJ) colorimeter, as described in Section 3.1.6.

3.2.6 SFC during storage

In order to track the SFC of chocolate samples during storage using different temperature fluctuation frequencies, CNO and CB-S were mixed using the formula described in Section 3.1.3 to make 45, 55, and 65% SFC model systems. About 2 mL samples were melted, poured into the NMR tubes and tempered using the IUPAC method described in Section 3.1.2. Samples

were prepared in triplicate. The sample tubes were then equilibrated at room temperature overnight and stored in the incubator using the same temperature cycling program as chocolate samples (cycling between 20 and 30°C every 3, 7 and 11 hours). By the end of each cycle (either at 20 or 30°C), the SFC values were taken by NMR. SFC measurements were repeated over three temperature cycles.

3.3 Effects of different nonfat particles on fat bloom during storage

The aim of this phase was to evaluate the effects of sugar type, sugar concentration, and the presence of lecithin on fat bloom during storage in chocolate. To formulate chocolate model systems, four types of sugar (sucrose, maltitol, corn syrup solids and polydextrose), either crystalline or amorphous, were used at different levels. Bloom was evaluated by three methods including WI measurement, stereomicroscopy and visual analysis.

3.3.1 Materials

Chocolate model systems in this phase were made by dispersing particles (containing sugar and cocoa solids) in the fat phase. Cocoa butter (ADM Cocoa, Milwaukee, WI) was used as the fat phase. Defatted cocoa powder (ADM Cocoa, Koog aan de Zaan, The Netherlands), containing about 0.5% fat, was used as nonsugar particles in the dispersed phase. Four different types of sugar including two crystalline sugars, sucrose (C&H Sugar, Yonkers, NY) and maltitol (Sweet Pearl P200, Roquette, Keokuk, IA), and two amorphous sugars, polydextrose (PD, STAR-LITE III F, Tate and Lyle, Decatur, IL) and spray dried corn syrup solids (CSS, 42 DE, STAR-DRI 200, Tate and Lyle, Decatur, IL), were selected to compare the effects of sugar

type and sugar concentration on bloom. Lecithin (W. A. Cleary Products Inc., Somerset, NJ) was used as the emulsifier.

3.3.2 Chocolate model systems and experimental design

In order to control the particle size in the model system, sugar particles were ground using a coffee grinder and sieved by a RO-TAP sieving machine (The W.S. Tyler Company, Cleveland, Ohio) to a final particle size between 45-90 microns (maltitol, CSS, PD samples were only sieved but not ground due to their fine particle sizes initially). To make a chocolate model system as a control, 75 g defatted cocoa powder was mixed with 75 g cocoa butter (67% SFC). Other model systems were formulated by replacing 25, 50, and 75% of the cocoa powder with sugar particles on a volume basis. This is important because the density difference between sugar and cocoa particles is not negligible. For example, in 25 ml particles, the weight of sucrose is 18.2 g while the weight of cocoa powder is 13.1 g. In order to keep the total surface of particles and total volume in the dispersed phase constant, volume basis is preferred to give the chocolate model systems a consistent microstructure. To compare the effects of lecithin on bloom formation, 0.5% lecithin (referenced to the mass in the control sample) was also added to some model systems. Table 3.5 shows the basic formulation of model systems with four different sugar replacement levels and four different sugar types.

Table 3. 5 Basic formulation of chocolate model systems adjusted from the model system made with 75 g cocoa butter and 75 g cocoa powder, where cocoa powder was gradually replaced by sugar particles at three different levels (25, 50, and 75%) on a volume basis and four different sugar types (sucrose, maltitol, CSS, PD).

Sugar type	Cocoa powder replacement level (%)	Cocoa butter (g)	Cocoa powder (g)	Sugar (g)
Sucrose	25	75	56.25	26.05
	50	75	37.50	52.10
	75	75	18.75	78.15
Maltitol	25	75	56.25	26.26
	50	75	37.50	52.52
	75	75	18.75	78.77
CSS	25	75	56.25	26.07
	50	75	37.50	52.14
	75	75	18.75	78.20
PD	25	75	56.25	26.21
	50	75	37.50	52.42
	75	75	18.75	78.64

CSS: corn syrup solids; PD: polydextrose.

In this phase, three bloom experiments were conducted: (1) effect of sugar particle types in replacement of cocoa powder, (2) effect of lecithin with different sugar fraction, and (3) effect of lecithin with different types of sugar particles.

In the first experiment, model systems with 4 levels of sugar concentration and 4 types of sugar were compared. To simplify the systems, no lecithin was added. This is a 4×4 full factorial design as shown in Table 3.6.

In the second experiment, sucrose was the only sugar used in all model systems. Chocolate model systems with 4 levels of sucrose concentration and two levels of lecithin concentration were compared. This is a 4×2 full factorial design as shown in Table 3.7.

Table 3. 6 Experimental design for chocolate model systems adjusted from the model system made with 75 g cocoa butter and 75 g cocoa powder, where cocoa powder was gradually replaced by sugar particles at different levels on a volume basis and four different sugar types.

Sugar type	Cocoa powder replacement level (%)
Sucrose	0
Sucrose	25
Sucrose	50
Sucrose	75
Maltitol	0
Maltitol	25
Maltitol	50
Maltitol	75
CSS	0
CSS	25
CSS	50
CSS	75
PD	0
PD	25
PD	50
PD	75

CSS: corn syrup solids; PD: polydextrose.

Table 3. 7 Experimental design for chocolate model systems adjusted from the model system made with 75 g cocoa butter and 75 g cocoa powder, where cocoa powder was gradually replaced by sucrose at different levels on a volume basis with or without 0.5% (g/g) lecithin.

Cocoa powder replacement level (%)	Lecithin concentration (%)
0	0
25	0
50	0
75	0
0	0.5
25	0.5
50	0.5
75	0.5

In the third experiment, all model systems used 50% as the sugar replacement level. Chocolate model systems with 4 sugar types and two levels of lecithin concentration were compared. This is a 4×2 full factorial design as shown in Table 3.8.

Table 3. 8 Experimental design for chocolate model systems adjusted from the model system made with 75 g cocoa butter and 75 g cocoa powder, where cocoa powder was replaced by 4 different sugar particles by 50% on a volume basis with or without 0.5% (g/g) lecithin.

Sugar type	Lecithin concentration (%)
Sucrose	0
Maltitol	0
CSS	0
PD	0
Sucrose	0.5
Maltitol	0.5
CSS	0.5
PD	0.5

CSS: corn syrup solids; PD: polydextrose.

3.3.3 Tempering

All samples were tempered in triplicate according to Kleinert method (Kleinert, 1980) using the tempering profile in Table 3.9. The tempering measurements were examined as previously described in Section 3.1.4. A commercial temper meter (TRICOR System Inc., Elgin, IL) that automatically calculates the slope of the plateau in the second temperature stage was also used to check degree of temper. A well-tempered chocolate should have a plateau with a slope between -1 and 1. Representative cooling rate curves for each sugar levels are shown Figure 3.2, confirming that all model systems were well-tempered.

Table 3. 9 Temperatures (°C) for tempering profiles for chocolate model systems adjusted from the model system made with 75 g cocoa butter and 75 g cocoa powder, where cocoa powder was gradually replaced by sugar particles at different levels on a volume basis.

Cocoa powder replacement level (v/v)	T_1	T_2	T_3	T_4
0%	25.5	30	27.5	32
25%	25.5	30	27.5	32
50%	25.5	30	27.5	32
75%	25.5	30	27.5	32

T_1: Initial cooling temperature; T_2: Temperature which chocolate was warmed to; T_3: Temperature which chocolate was recooled to; T_4: Final holding temperature.

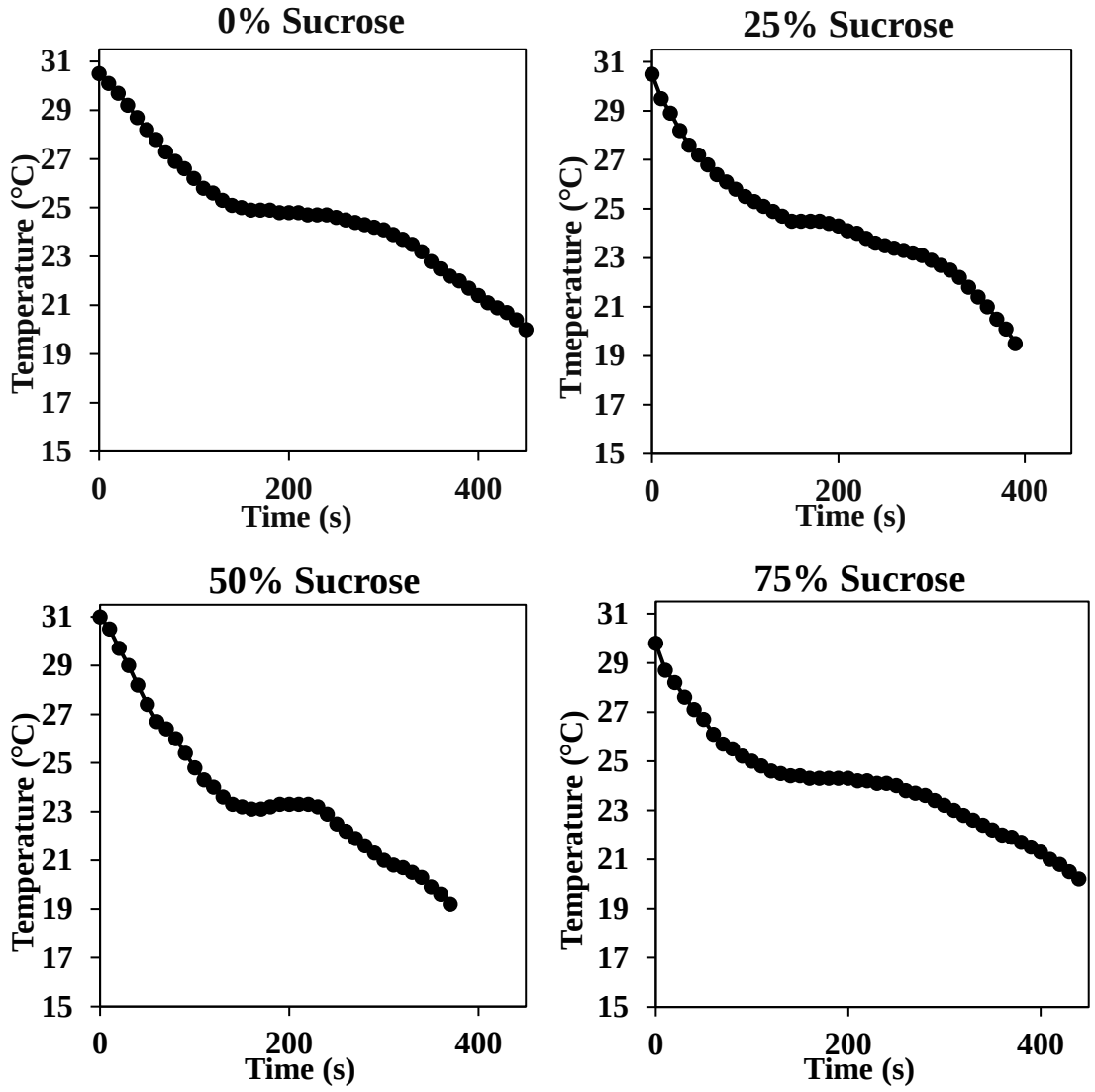


Figure 3. 2 Representative cooling rate curves for chocolate model systems adjusted from the model system made with 75 g cocoa butter and 75 g cocoa powder, where cocoa powder was gradually replaced by sugar particles at different levels (0, 25, 50, and 75%) on a volume basis.

3.3.4 Chocolate storage

After tempering, the chocolate samples were poured into plastic disc molds. Each tempered chocolate was molded into two disks, cooled at room temperature overnight and stored in a Sanyo MIR-253 (Sanyo Scientific, IL) incubator with programmable temperature cycler. The temperature was cycled between 20 and 30°C every 6 hours with 99 cycles.

3.3.5 Bloom Evaluation

Bloom progression in chocolates was tracked at day 0, 1, 7, 14, 21, and 28. In this phase, three different methods of bloom evaluation were conducted and compared.

3.3.5.1 Whiteness Index

Whiteness index (WI) was evaluated by a Minolta CR-3000 (Minolta, NJ) colorimeter, as described in Section 3.1.6.

3.3.5.2 White area percentage

A stereomicroscope (Nikon SMZ-10 microscope; Nikon Inc., Japan) with a magnification of 22.5X was used to scan the surface of chocolate disks. Images of the chocolate surface were taken by a digital camera system (Nikon DS-5m camera head, Nikon DS-L1 control unit; Nikon Inc., Japan). Eight pictures were taken on each chocolate disk. The percentage of whitish area was analyzed by image analysis software Image J (Wayne Rasband, National Institute of Health, USA), based on the percentage of white pixels detected from the picture.

3.3.5.3 Visual Analysis

Chocolate surface appearance was also rated by visual analysis where the scoring scale ranged from 0 to 5 with 0.5 increments. The scale was generated and modified in reference to previous sensory studies (Cerbulis, 1969; Guinard and Mazzucchelli, 1999; Andrae, 2006) with 6 levels of bloom to quantify the surface change in the chocolate model systems:

0: there is no difference from the original appearance;

1: the surface loses glossiness and looks dull, but there is no visual bloom yet;

2: whitish spots appear but they are almost not perceptible;

3: the chocolate surface presents obvious whitish spots and areas;

4: major part of chocolate disks turn gray or white; and

5: all parts of chocolate disks turn extremely white.

3.3.6 Particle interactions

In order to quantify the difference in the physical properties of sugar particles, three experiments were conducted to evaluate the interaction between sugar particles in melted cocoa butter. Results from three different interaction quantification methods were compared.

3.3.6.1 Rheology

Viscosity is a good indication of the interaction between particles. In a sugar-in-fat dispersion system, viscosity has a strong and positive correlation with the forces of particulate interaction (Johansson and Bergenstahl, 1992a; Barbin et al., 2005). To measure viscosity, sugar-in-fat dispersions were made by mixing sugar particles with melted cocoa butter at

different sugar concentrations, according to the formulation shown in Table 3.10. The concentrations of sugar particles in the rheology test were identical to the sugar concentrations in the chocolate model systems in Section 3.3.2. Afterwards, the samples were presheared at 45°C at 900 rpm overnight and transferred to a Discovery HR-2 rheometer (TA Instruments, New Castle, DE) where a flow sweep with a shear rate scan from 1 to 1000 1/s at 40°C was conducted. After the test, the shear stress was plotted versus shear rate and a Casson model, a standard viscosity model in chocolate rheology, was used to calculate Casson viscosity and Casson yield stress. Casson viscosity and Casson yield stress was calculated automatically by TRIOS software connected to the rheometer (TA Instruments, New Castle, Delaware). Higher viscosity indicates higher particle interactions (Barbin et al., 2005).

Table 3. 10 Compositions of sugar-in-fat dispersions with cocoa butter and sugar particles for the rheology test, with different sugar concentrations and four different sugar types (sucrose, maltitol, corn syrup solids, and polydextrose).

Sugar type	Cocoa butter (g)	Sugar (g)
Sucrose	75	26.05
	75	52.10
	75	78.15
Maltitol	75	26.26
	75	52.52
	75	78.77
CSS	75	26.07
	75	52.14
	75	78.20
PD	75	26.21
	75	52.42
	75	78.64

CSS: corn syrup solids; PD: polydextrose.

3.3.6.2 Sedimentation Volume

Sedimentation volume has a strong and positive correlation with viscosity of a sugar-in-fat dispersion system (Barbin et al., 2005), thus being another important indicator of particulate interaction. A sedimentation test was conducted by dispersing 10wt % sugar particles in melted cocoa butter at 40°C, and stirring at 900 rpm overnight. The samples were then transferred to a tube with constant total volume (14 mL). Samples were left in the oven at 40°C for 24 h in order to allow complete settling of the particles. Sedimentation volume was measured, with higher sedimentation volume indicating greater particle interactions (Barbin et al., 2005).

3.3.6.3 Contact angle

Contact angle is the angle formed between a solid surface and the tangent of the droplet surface curvature at the three-phase boundary where a liquid, gas and solid intersect. It can represent the wetting properties of a surface with certain material. When the contact angle is larger than 90°, the surface is considered as a nonwetting material, whereas a surface with a contact angle smaller than 90° is considered a wetting material (Yuan and Lee, 2013), as shown in Figure 3.3. The shape of a liquid sessile droplet on a flat and homogenous surface in equilibrium is only dependent on the interaction between the droplet and surface, intrinsic material properties, temperature and pressure (Palzer et al., 2001; Yuan and Lee, 2013). The Young equation (Eqn. 3.2) gives a correlation between intrinsic contact angle θ, and the surface energies of a liquid (σ_{lg}) and a solid (σ_{sg}) and interfacial tension between the solid and liquid (σ_{sl}) (Young, 1805; Whyman et al., 2008; Keijbets, et al., 2009).

$$\cos\theta = \frac{\sigma_{sg} - \sigma_{sl}}{\sigma_{lg}} \qquad (3.2)$$

In practical experiments, the measured contact angle from a sessile droplet test may deviate from Equation 3.2 as a result of imperfection of surface preparation, heterogeneity of samples, and the interaction between solid surface and liquid droplet (Reinke et al., 2015).

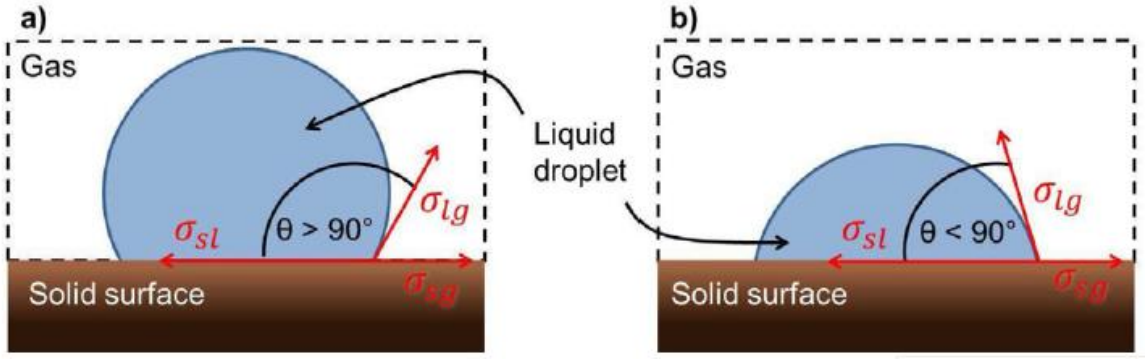


Figure 3. 3 Contact angles (θ) of a liquid sessile droplet on a solid surface: (a) nonwetting material, θ>90°, (b) wetting material, θ<90°. Adapted from Reinke et al. (2015).

Static contact angle can be measured using an optical tensiometer by a sessile drop method. A metal ball with 4.0000 mm diameter was used to calibrate the length and angles of the images from the camera, performed automatically from the software. To measure contact angle, sugar particles (sucrose, maltitol, CSS and PD) were packed firmly together as a compressed tablet with a flat surface layer by a powder press with one droplet of melted cocoa butter at 50°C placed on the top of the sugar layer by a tiny syringe. A ThetaLite optical tensiometer (Biolin Scientific, Espoo, Finland), together with OneAttension software (Biolin Scientific, Espoo, Finland), was able to record the real-time contact angles using the sessile drop method and the Young-Laplace equation. The contact angle after equilibrium (when the contact angle values were stable) was measured 10 times for each surface.

In order to eliminate the effect of surface porosity on the contact angle results, commercial large sucrose crystals (Rock candy crystals, Richardson Brands Co., Canajoharie, NY) were used as a control. Flat surfaces from the crystals were selected and the contact angles between

the surface and one droplet of melted cocoa butter at 50ºC were measured using the same method described previously. The contact angle after equilibrium (when the contact angle values were stable) was measured 10 times. To further eliminate the effect of surface impurity on contact angle results, the large sucrose crystals were dipped into deionized water for 1 s. Visible water residues were removed by Kimwipes and the sugar crystals were further dried in an oven at 60ºC for 1 h and cooled at room temperature for 0.5 h. Flat surfaces from the crystals were selected and the contact angles were measured using the same method described previously. The contact angle after equilibrium was measured 7 times.

3.4 Effects of circularity of sugar particle surface on fat bloom during storage

The key to this phase was to find a reliable method to modify the sharp surface of crystalline sugar, making it round and smooth, and change the particle circularity. According to a patent from the Hershey Company (St. John et al., 1995), this can be done by moisture addition and forcing sugar recrystallization during the conching process. When water is added into the chocolate mix, ultrafine sugar particles below 10 μm as well as jagged and angular edges on the larger sugars will be dissolved (St. John et al., 1995). During the conching process, temperature is so high that the mixture is dried and sugar in the solution recrystallizes on the larger sugar crystals to decrease surface energy, further rounding and smoothing the surfaces. In this phase, bloom in chocolate model systems with different surface circularities were compared by three different methods (WI measurement, stereomicroscopy and visual analysis).

3.4.1 Materials

Chocolate model systems were constructed by dispersing particles, including sugars and cocoa solids, into the fat phase, similar to the method described in Section 3.3.2. Cocoa butter (ADM Cocoa, Milwaukee, WI) was used as the fat phase. Defatted cocoa powder (ADM Cocoa, Koog aan de Zaan, The Netherlands), containing about 0.5% fat, was used as nonsugar particles in the dispersed phase. Lecithin (W. A. Cleary Products Inc., Somerset, NJ) was used as the emulsifier. Sucrose (C&H Sugar, Yonkers, NY) was used as the sugar particle to replace cocoa solids. It was refined to a particle size between 45 to 90 μm using the method in Section 3.3.2.

3.4.2 Sugar surface modification

A white chocolate mix was formulated by adding 40 g melted cocoa butter, 60 g sucrose (45-90 μm), and 0.22 g lecithin. The mixture was placed in a mixer with an external bath temperature of 60°C at 138 rpm and 2.4 g distilled deionized water was slowly added into the mixture while mixing. Within 10 min, 0.28 g lecithin was added to prevent crystal agglomeration, with continued mixing at 60°C for 24 h to ensure moisture was totally removed.

In order to confirm that the surface rounding was successful, two measurements were conducted. First, one droplet of the melted white chocolate mix was diluted by 1 mL mineral oil and a diluted droplet was analyzed by a Nikon QImaging Fast 1294 optical microscope (Nikon Inc., Japan), together with an Image-Pro Plus software (Media Cybernectics Inc., Rockville, MD) under 200X magnification. The circularity of the particles was checked using Image J software (Wayne Rasband, National Institute of Health, USA). A white chocolate mix containing 40 g melted cocoa butter, 60 g sucrose (45-90 μm), and 0.22 g lecithin, without any

modification process, was used as a control and the particle shape of the unmodified sucrose was checked using the same method described previously to compare. Between 60 and 100 particles were analyzed in both white chocolate mix systems. If the surface rounding was successful, the circularity of the particles after the modification should be increased. Secondly, a flow sweep at 40°C on the white chocolate mix before and after the surface modification was conducted by scanning the shear rate from 1 to 1000 1/s by the rheometer. Shear stress was plotted at different shear rate and a Casson model was fitted to obtain the Casson viscosity. The Hershey's patent shows that this modification method should result in a reduction in the Casson viscosity (St. John et al., 1995).

3.4.3 Chocolate model system and experimental design

In this phase, model systems with 4 levels of sucrose concentration and 2 types of particle surface were compared. To make a chocolate model system as a control, 75 g defatted cocoa powder was mixed with 75 g cocoa butter (67% SFC). Other model systems were formulated by replacing 25, 50, and 75% cocoa powder on a volume basis with either unmodified sucrose particles (45-90 μm), or surface-modified sucrose particles. As the surface-modified sucrose particles had already been incorporated in a white chocolate mix described in Section 3.4.2, to make model systems with surface-modified sucrose, the white chocolate was mixed with extra cocoa butter, lecithin and cocoa powder with formulations shown in Table 3.11. Lecithin was also added at 0.5% (referenced to the mass in the control sample) to all model systems. This is a 4×2 full factorial design as shown in Table 3.12.

Table 3. 11 Formulations of surface-modified model systems where cocoa powder in a chocolate model system made with 75 g cocoa butter and 75 g cocoa powder was gradually replaced by sugar particles at different levels (25, 50 and 75%) on a volume basis.

Cocoa powder replacement level (v/v)	25%	50%	75%
White chocolate mix (g)	43.633	87.266	130.899
Cocoa butter (g)	57.634	40.267	22.901
Lecithin (g)	0.533	0.316	0.099
Cocoa powder (g)	56.250	37.500	18.750

Table 3. 12 Experimental design for chocolate model systems where cocoa powder in a chocolate model system made with 75 g cocoa butter and 75 g cocoa powder was gradually replaced by sugar particles with or without surface modification at different replacement levels (0, 25, 50 and 75%) on a volume basis.

Sucrose surface	Cocoa powder replacement level (%)
Unmodified	0
Unmodified	25
Unmodified	50
Unmodified	75
Modified	0
Modified	25
Modified	50
Modified	75

3.4.4 Tempering

All samples were tempered in triplicate according to the Kleinert method (Kleinert, 1980) using the tempering profile in Table 3.9. The degree of temper was checked as previously described in Section 3.3.3.

3.4.5 Chocolate storage

After tempering, the chocolate samples were poured into plastic disc molds. Each tempered chocolate was molded into two disks, cooled at room temperature overnight and

stored in a Sanyo MIR-253 (Sanyo Scientific, IL) incubator with programmable temperature cycler. The temperature was cycled between 20°C and 30°C every 6 hours with 99 cycles.

3.4.6 Bloom Evaluation

Bloom progression in chocolates was tracked at day 0, 1, 7, 14, 21, and 28. In this phase, three different methods of bloom evaluation, including whiteness index measurement, stereomicroscopy and visual analysis were conducted and compared using the methods as described in Section 3.3.5.

3.4.7 "Water-free Wash"

In order to evaluate the effect of surface modification process on bloom formation, a "Water-free Wash" was conducted in parallel to the regular sucrose surface modification by repeating each of the modification steps in Section 3.4.2 expect for not adding 2.4 g distilled deionized water. Chocolate model systems were made by the same formulation in Table 3.11, tempered and stored using exactly the same method as described in Section 3.4.4 and Section 3.4.5. Bloom was evaluated with the methods described in Section 3.3.5 and was compared to the bloom results all of the model systems described in this phase.

3.4.8 Particle surface circularity

In order to compare the shape of different particles (maltitol, polydextrose, corn syrup solids), a white chocolate mix was made by mixing 60% (v/v) in 40% (v/v) melted cocoa butter. Then, one droplet of the white chocolate mix was diluted by 1 mL mineral oil and a diluted

droplet was analyzed by a Nikon QImaging Fast 1294 optical microscope (Nikon Inc., Japan), together with an Image-Pro Plus software (Media Cybernectics Inc., Rockville, MD) under 200X magnification. The circularity of the particles was checked using Image J software (Wayne Rasband, National Institute of Health, USA). About 100 particles were analyzed in these 3 white chocolate mix systems. The circularity of "control-washed" sucrose described in Section 3.4.7 was also evaluated with the same method.

3.5 Statistics

Data were analyzed by JMP statistical software (JMP Pro 13.1.0, SAS Institute Inc., Cary, NC). A full factorial was used as the experimental design type for data analysis in all phases. Two-way ANOVA were used to evaluate the significance of the effect of each factor and a Tukey's Honest Significance Difference (HSD) test or student's t-test was conducted to evaluate the difference between each factorial group. A test with $p<0.05$ was considered significant.

4. Results and Discussion

This study investigated the effect of particulate factors such as solids particle composition in the fat phase, particulate concentration, type of particles, shape and surface properties of particles, as well as storage conditions such as temperature fluctuation frequency on fat bloom formation in chocolate model systems during storage. Bloom results were evaluated by three different methods and explained by particulate interactions as well as particle surface properties. This information can provide useful knowledge for the optimization of formulation and storage control in chocolate products.

4.1 Effects of fat phase on fat bloom in chocolate model systems

Since there is no systematic study in literature on the bloom behavior in chocolate model systems with designated solid fat content (SFC), the objective of this phase was to develop a standard method and model systems studying bloom in nonsugar chocolate model systems by using different composition in the fat phase. The effects of SFC and liquid oil type on fat bloom during storage were evaluated by means of whiteness index measurements. Changing fat crystal content and liquid oil type may change the microstructure of the chocolate matrix, thus having an effect on the migration and recrystallization steps in chocolate bloom. Therefore, any difference caused by these two factors may be detected by the whiteness index results.

4.1.1 SFC profiles and isothermal diagrams of binary fat mixtures

4.1.1.1 SFC profiles

SFC profiles of binary fat mixtures were constructed by plotting the SFCs versus temperatures at different oil compositions to show the trends in the melting properties. In order to investigate the efficiency of cocoa butter fractionation, SFC profiles of CB and CB-S were compared, as shown in Figure 4.1. Figure 4.2 to 4.5 show the SFC profiles of binary blends of CB-S with PNO, CSO, SFO and CNO at oil compositions from 0% to 100%.

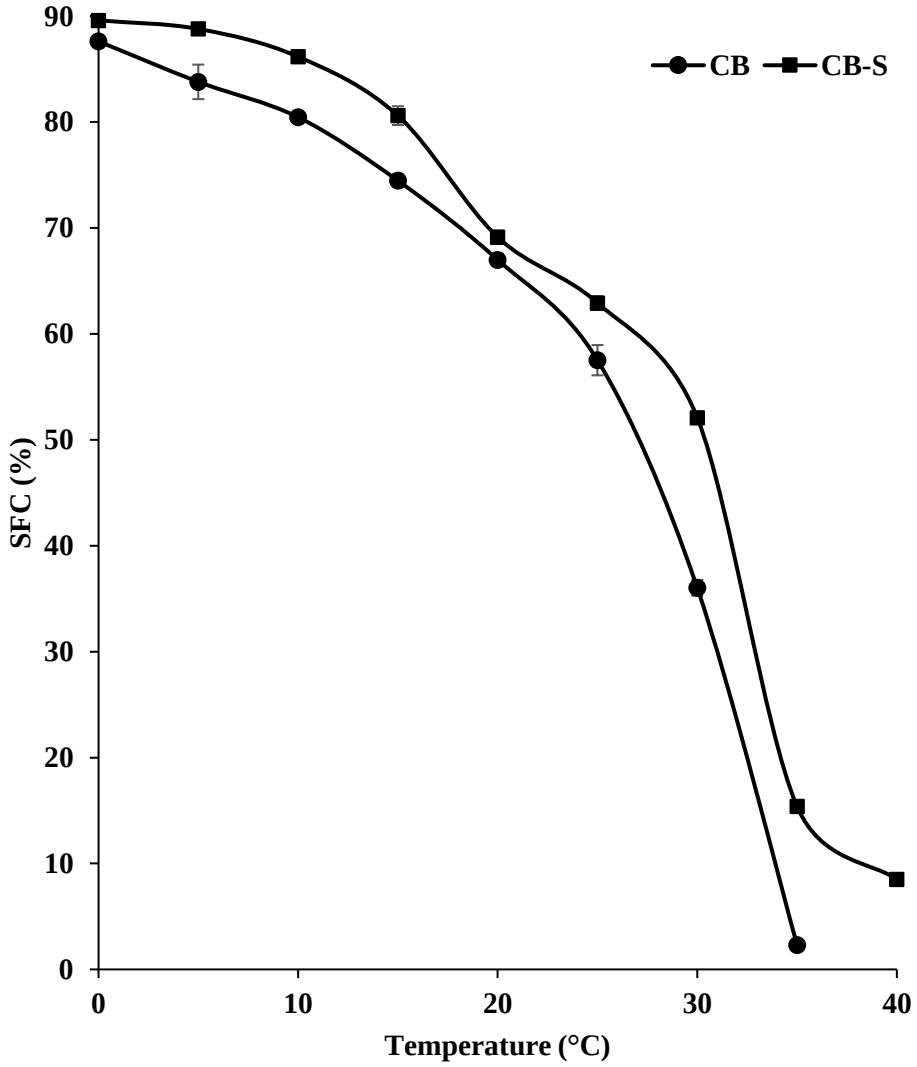


Figure 4. 1 A comparison of SFC profiles of cocoa butter (CB) versus cocoa butter stearin (CB-S) as a single fat system.

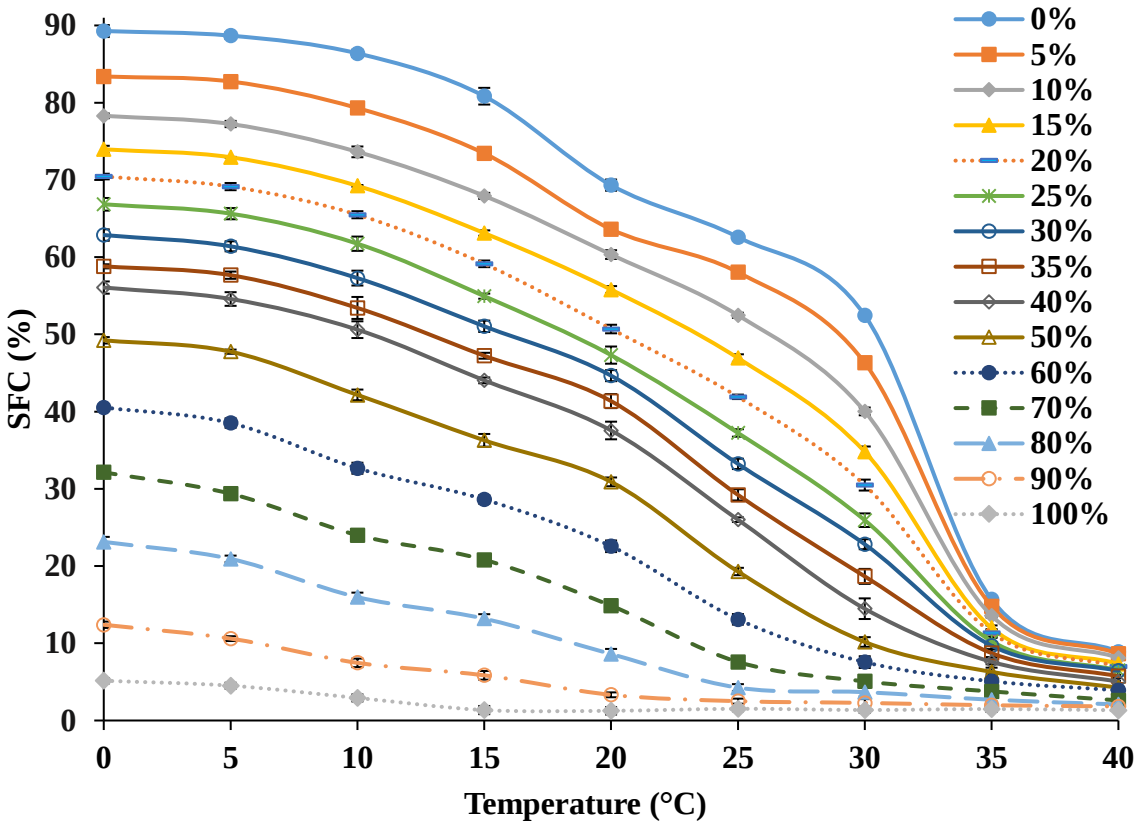


Figure 4. 2 Solid fat content (SFC) profiles of binary blends of CB-S with peanut oil (PNO) at oil compositions from 0% to 100%.

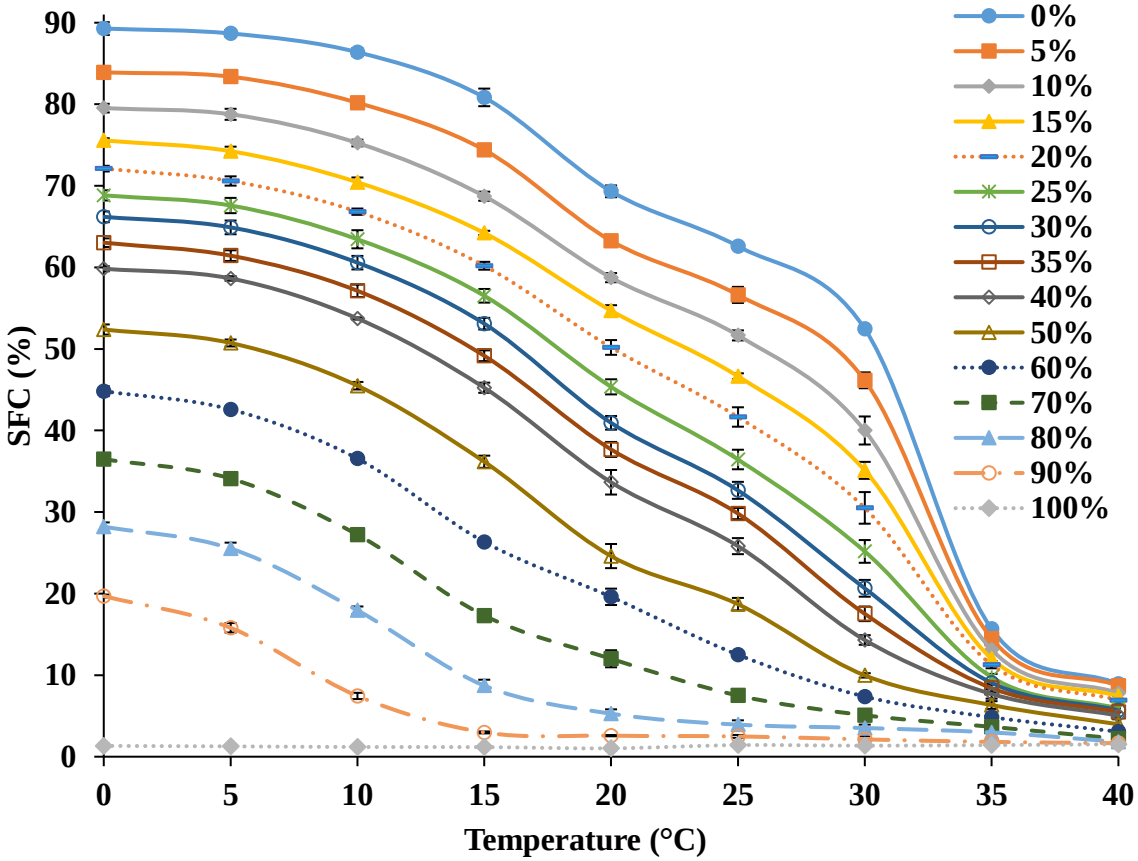


Figure 4. 3 Solid fat content (SFC) profiles of binary blends of CB-S with cottonseed oil (CSO) at oil compositions from 0% to 100%.

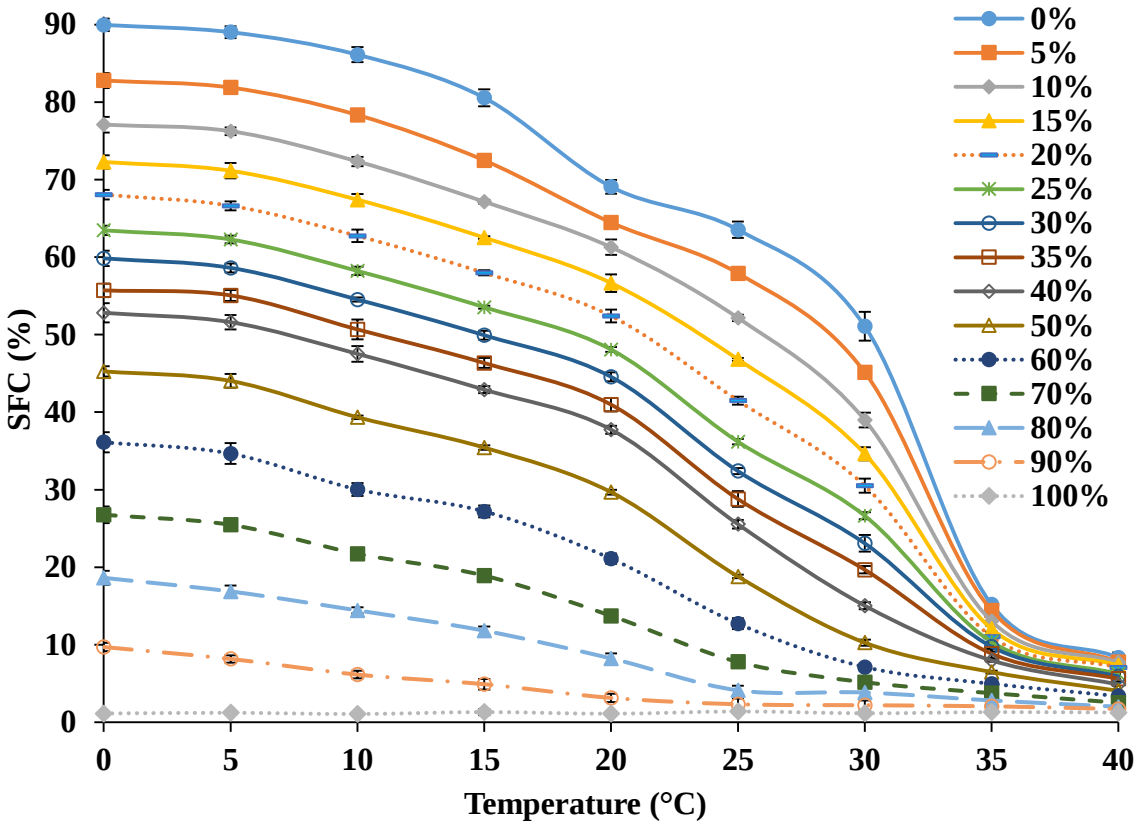


Figure 4. 4 Solid fat content (SFC) profiles of binary blends of CB-S with sunflower oil (SFO) at oil compositions from 0% to 100%.

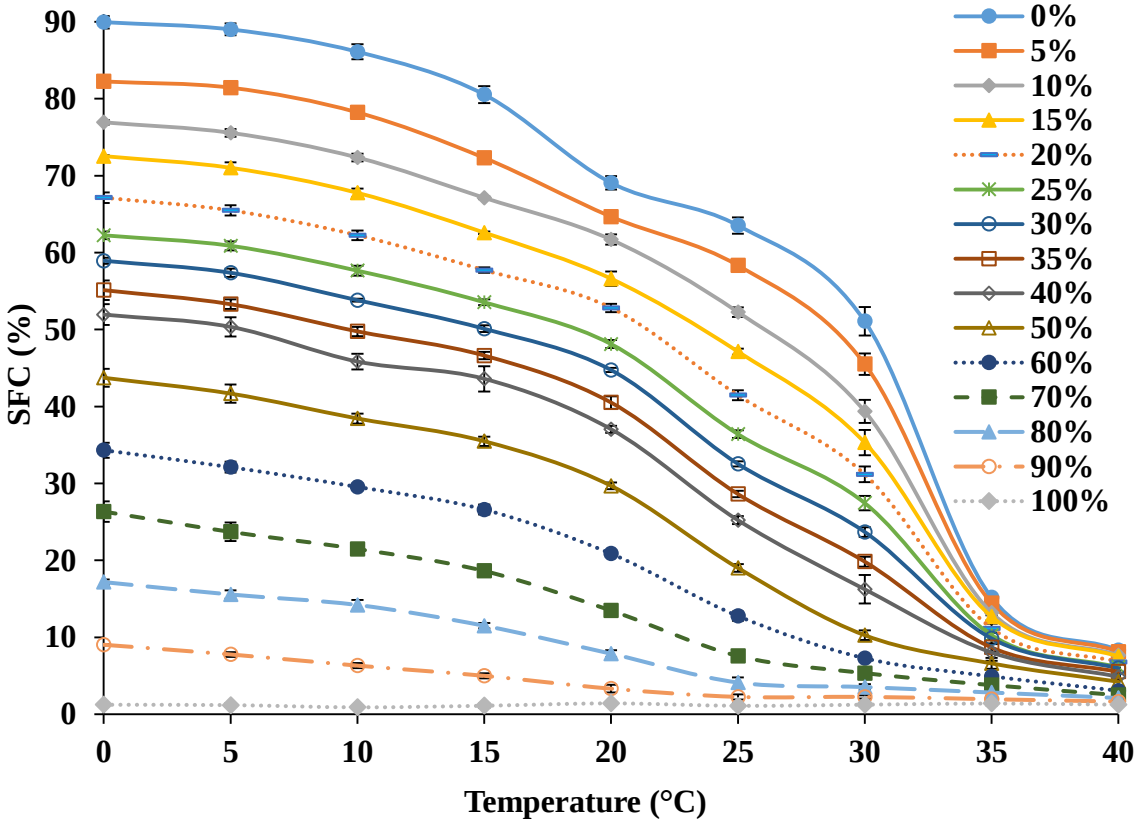


Figure 4. 5 Solid fat content (SFC) profiles of binary blends of CB-S with canola oil (CNO) at oil compositions from 0% to 100%.

As shown in Figure 4.1, the SFC of CB started at around 88% at 0°C, and dropped to about 67% at room temperature (20°C). This high value of SFC at room temperature can contribute to the typical textural properties of chocolate such as good snap and proper hardness (Ribeiro et al., 2012). The SFC of CB then decreased dramatically after 25°C and finally to around 2% by 35ºC. This is caused by the main TAGs in cocoa butter (POP, POS and SOS), which can explain the sharp melting of chocolate around body temperature during oral processing (Bigalli, 1988). The trend in SFC of CB is consistent with the SFC of medium melting CB from Ivory Coast as reported by Bricknell (1997). The shape of the melting curve of CB-S was similar to CB and the trend is consistent with the SFC of high-melting CB as reported by Bricknell (1997). SFC at every temperature point was always higher than CB, which is expected as the fractionation process removed some low-melting fats of CB, increasing the total SFC. Interestingly, the SFC of CB-S did not drop to near 0% at 40°C. And this may be caused by the extremely high-melting TAGs in CB such as PPP, PPS and SPS, since fractionation concentrated these TAGs, enhancing their effects on SFC.

Comparing Figures 4.2 to 4.4, the melting profiles of binary blends between CB and four different oils were very similar, in general. The SFC decreased with increased temperatures as the low-melting fats gradually melted into liquids. The slopes of the curves were even steeper after 30°C, which indicates a sharp melting when temperature is near body temperature. Again, the SFC in blends with higher CB-S concentration did not reach 0% at 40°C and this is due to the high melting fats in CB-S, as explained previously. To note, the trend in SFC profiles in CNO and SFO systems were almost identical, which matches the results on the melting profiles of binary fat blends of CB-S with CNO or SFO, reported by Zhou (2004). On the other hand,

the difference in SFC in different oil systems only became significant when the temperature was extremely low. Notably, blends with PNO and CSO at 0°C or 5°C had a higher SFC than SFO and CNO. This is due to slightly higher amounts of saturated fatty acids such as palmitic acid and stearic acid in PNO and CSO, compared to SFO and CNO, resulting in relatively higher melting points in PNO (-2 to 3°C) and CSO (0 to 4°C) than SFO (-16 to -18°C) and CNO (around -10°C) (Weiss; 1983; Bockisch,1998).

4.1.1.2 Isothermal diagrams of binary fat mixtures

Isothermal diagrams of binary fat mixtures were constructed from the SFC profiles (Figures 4.2 to 4.5) by plotting SFCs of binary fat mixtures on the y-axis and concentrations of different liquid fats on the x-axis with different temperatures as different series on the graph. Compared to the SFC profiles, isothermal diagram has advantages to better present the interaction between two fats by analyzing the linearity of the curves, allowing prediction of SFC at any oil concentration at certain temperature by directly applying regression models to the curves. Ideally, binary fat mixtures with no incompatibility will exhibit linearity on the isothermal SFC curve as adding extra liquid oil would simply dilute the SFC due to the mass replacement. Figures 4.6 to 4.9 show the isothermal SFC diagrams of binary fat blends of CB-S with PNO, CSO, SFO and CNO at different temperatures ranging from 0 to 40°C.

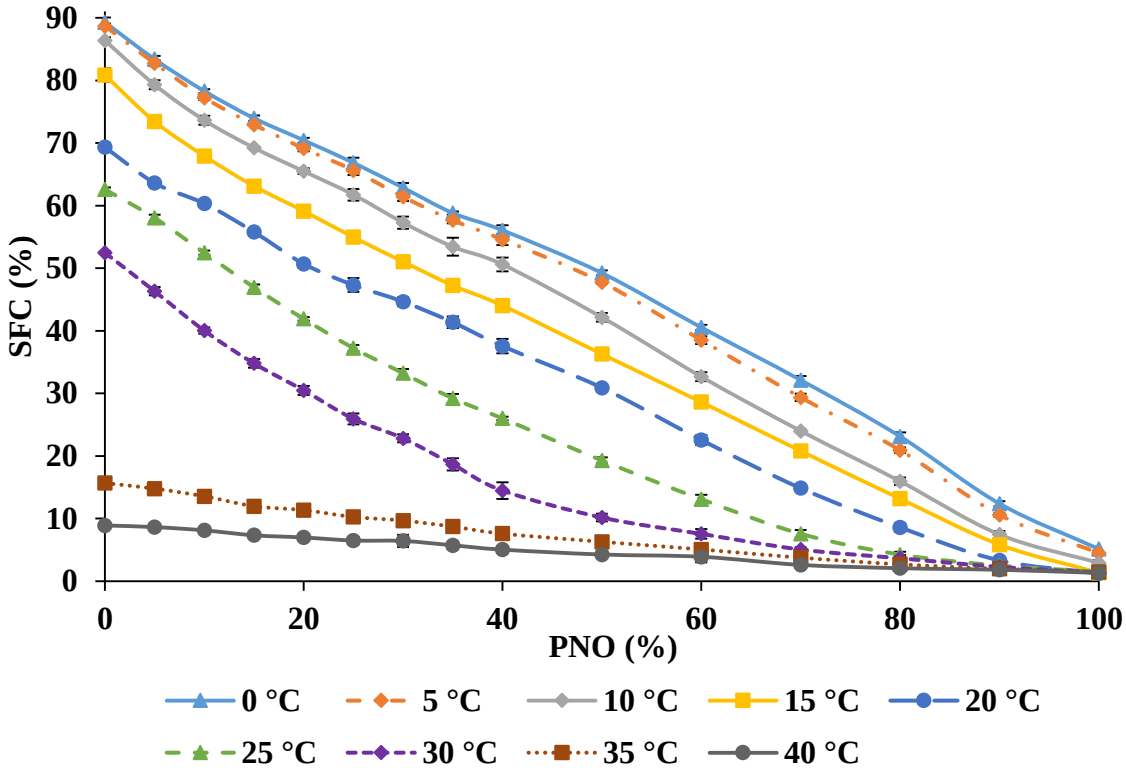


Figure 4. 6 Isothermal solid fat content (SFC) diagrams of binary fat blends of cocoa butter stearin (CB-S) with peanut oil (PNO) at temperatures ranging from 0 to 40°C.

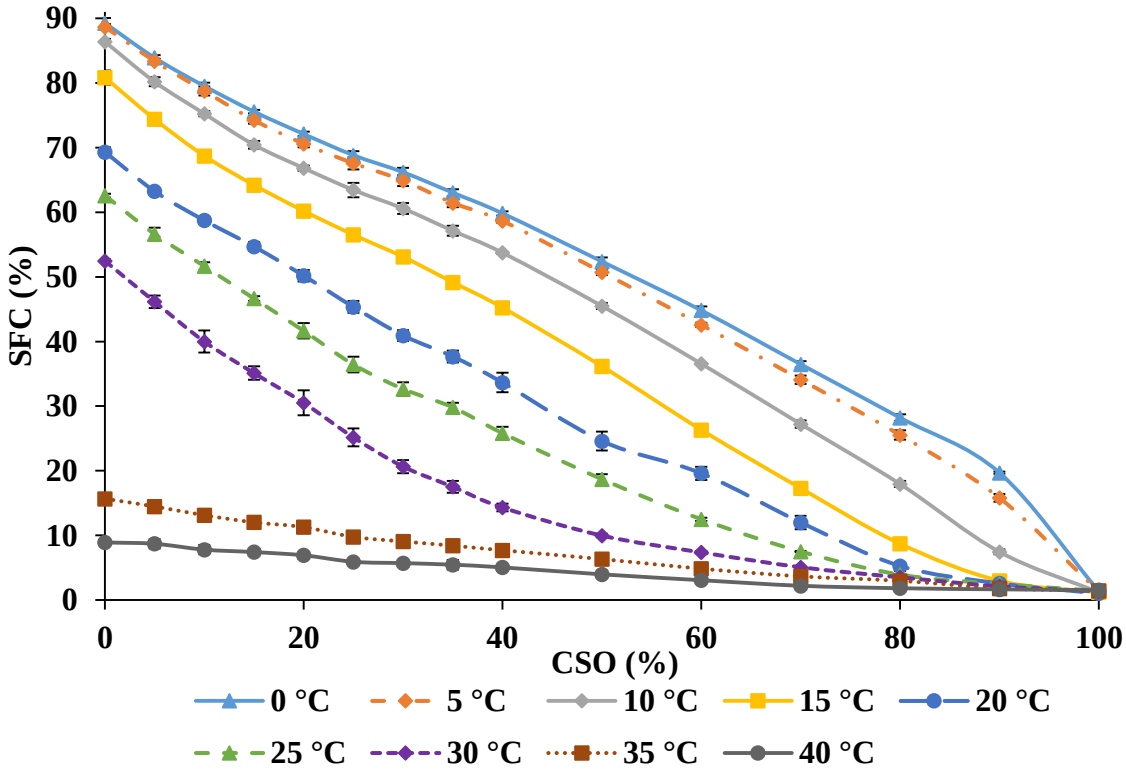


Figure 4. 7 Isothermal solid fat content (SFC) diagrams of binary fat blends of cocoa butter stearin (CB-S) with cottonseed oil (CSO) at temperatures ranging from 0 to 40°C.

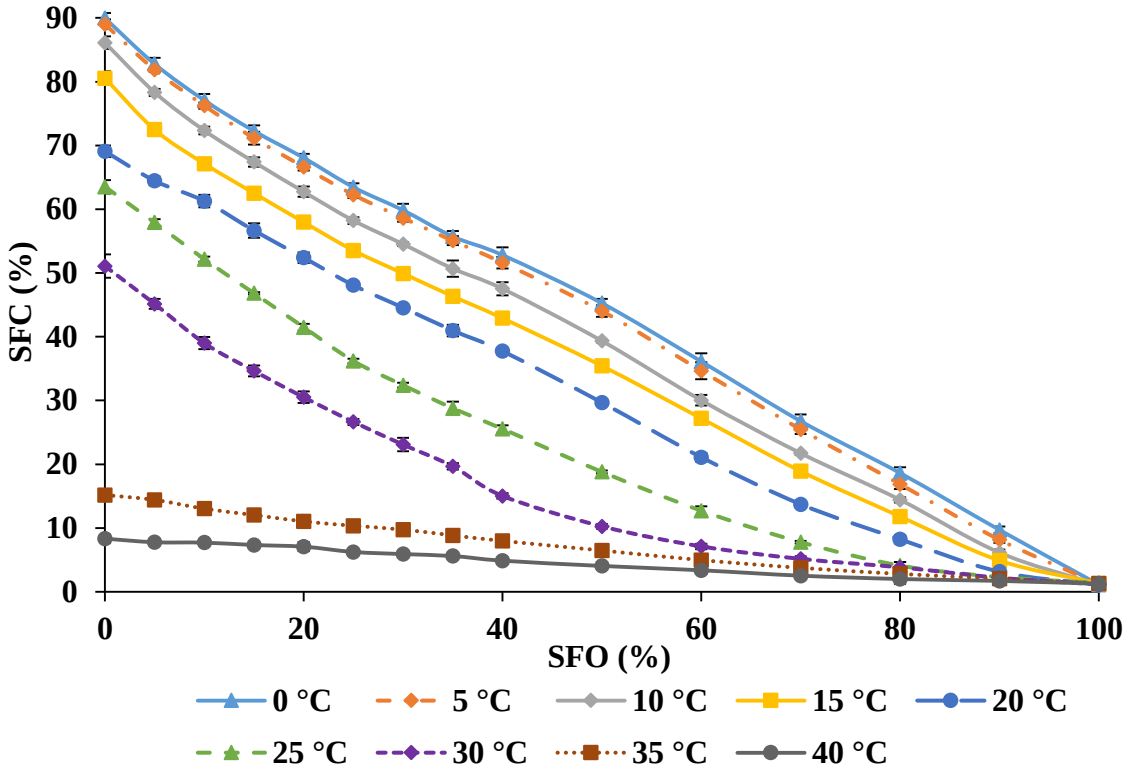


Figure 4. 8 Isothermal solid fat content (SFC) diagrams of binary fat blends of cocoa butter stearin (CB-S) with sunflower oil (SFO) at temperatures ranging from 0 to 40°C.

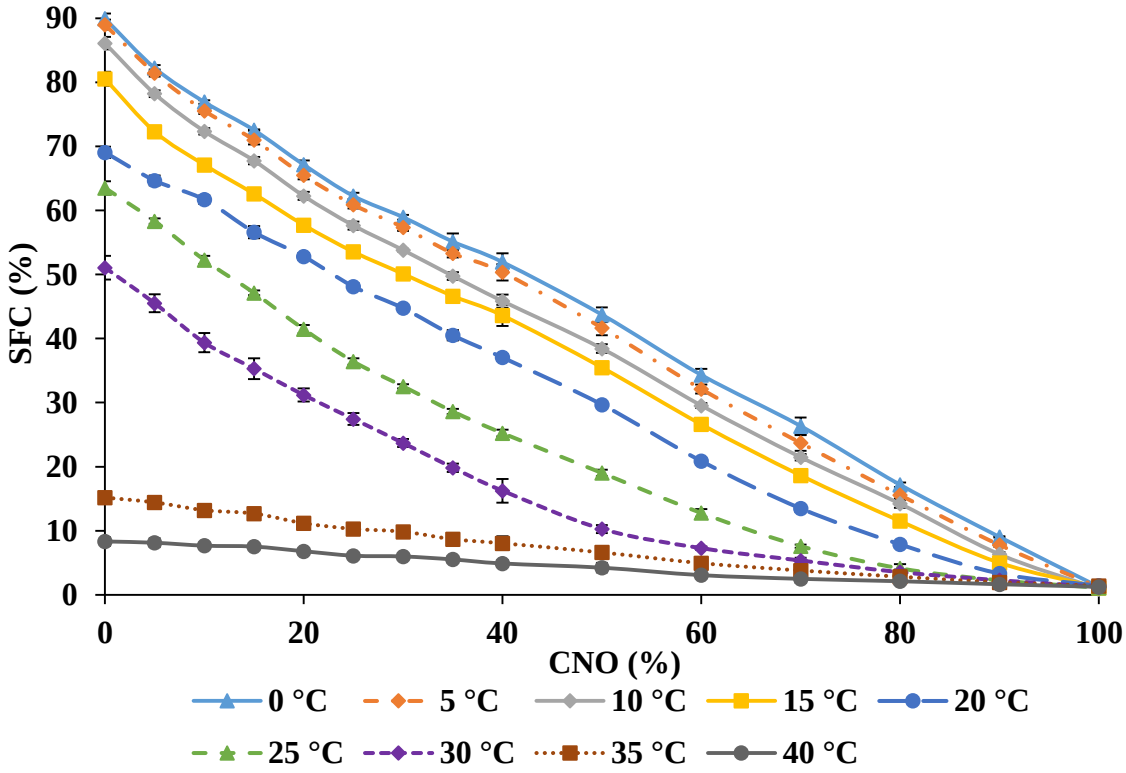


Figure 4. 9 Isothermal solid fat content (SFC) diagrams of binary fat blends of cocoa butter stearin (CB-S) with canola oil (CNO) at temperatures ranging from 0 to 40°C.

In general, the isothermal SFC diagrams in the four oil systems were very similar; the SFCs of binary fat systems all decreased when the oil composition was increased as the liquid fats diluted SFC in replacement of the solid mass. CNO and SFO binary systems were nearly identical, which matches previous CNO and SFO isothermal diagrams in literature (Zhou, 2004). The 0°C and 5°C isothermal SFC curves for PNO and CSO were slightly higher than those for SFO and CNO due to their higher melting points, which agrees with the results of the melting profiles discussed previously.

When temperatures were below 20°C, the linearity of the isothermal diagrams was fairly good (R^2>0.98, p<0.0001), whereas higher temperatures reduced linearity a bit especially at 25°C and 30°C, when CB-S partially melted. Generally speaking, the isothermal curves in all four systems decreased continuously, presenting a simple dilution effect, which matches the previous literature on the function of vegetable oils in chocolate (Zhou, 2004; Lonchampt and Hartel, 2004).

In order to calculate the formulation of model systems with the designated 45%, 55%, and 65% SFC, a simple linear regression was conducted on the 20°C isothermal curves in the four oil systems. The oil concentration needed for each SFC was calculated by applying the designated SFC into the linear model, which is shown in Table 3.1. Linearity for each replicate of the isothermal curves was good (R^2>0.99, except for two replicates in the PNO systems with R^2>0.98). This indicates little interaction between CB-S and liquid oils in the chocolate at room temperature. Comparing the formulations in four different liquid oil systems, PNO, SFO, and CNO systems had no significant difference in all three SFC systems while less liquid oil was needed in the CSO system than the other three oil systems to make chocolate model systems

of 45% and 55% SFC. This matches the isothermal diagrams at 20°C since the CSO curve is slightly lower than that for PNO, SFO and CNO.

4.1.2 DSC

Melting curves obtained from DSC were used to identify the polymorph of cocoa butter under different conditions. Figure 4.10 shows the melting curves from DSC for chocolate model systems made with cottonseed oil and cocoa butter stearin with 65% SFC that were freshly under-tempered, freshly well-tempered and well-tempered chocolate after 4-week storage.

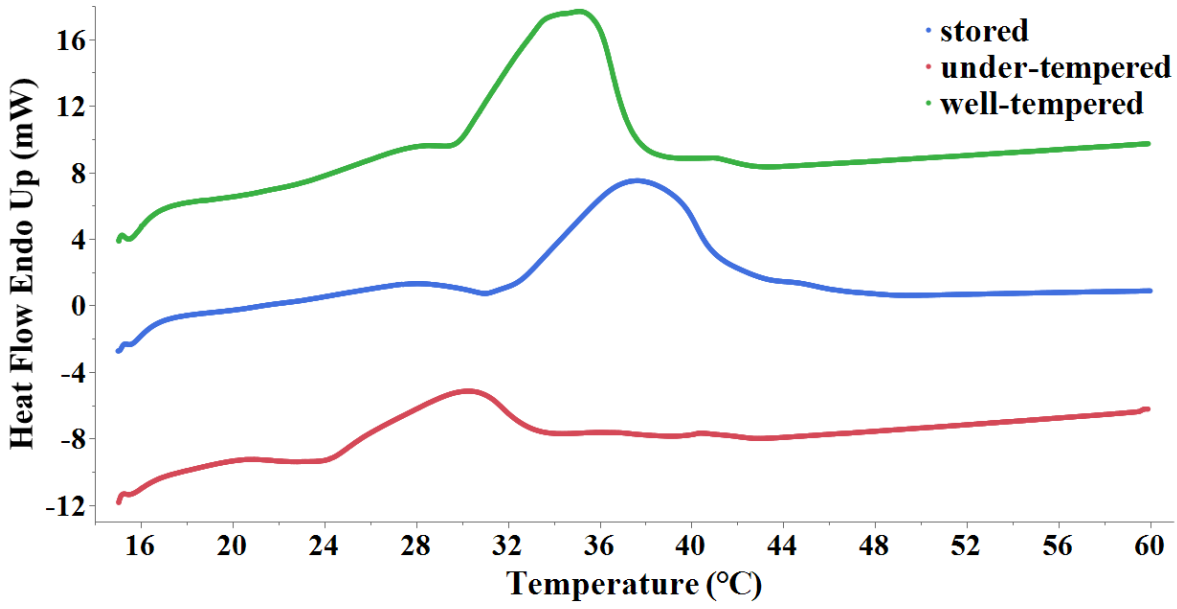


Figure 4. 10 Differential scanning calorimetry (DSC) curves for chocolate model systems made with cottonseed oil and cocoa butter stearin with 65% solid fat content that were freshly under-tempered, freshly well-tempered and well-tempered chocolate after 4-week storage.

As described in Section 2.1.2.2, cocoa butter has six different polymorphs. According to Wille and Lutton (1966), the melting points of the six different polymorphs of cocoa butter are: 17.3°C for Form I, 23.3°C for Form II, 25.5°C for Form III, 27.3°C for Form IV, 33.8°C for Form V, and 36.3°C for Form VI. Form IV usually appears in poorly-tempered chocolate, well-tempered chocolate is structured with Form V, and bloomed chocolate due to storage always consists of Form VI polymorph. As we can see from Figure 4.10, under-tempered chocolate had an average melting point at 29.9°C, indicating that β'IV was the predominant polymorph present, which matches previous results. Moreover, well-tempered chocolate had an average melting point at 34.6°C, indicating a βV polymorph. This also confirms that the tempering process was successful. Chocolate model system after 4-week storage had an average melting point at 37.0°C, as an indication of a βVI polymorph. The DSC results confirm that the expected polymorphic transition of cocoa butter from βV to βVI did occur in the chocolate model system after 4-week storage in this study.

4.1.3 Whiteness index

During storage, fat bloom gradually appeared as whitish spots on the surface of the chocolate in all model systems. The chocolate surface became whiter and whiter over time, resulting in higher whiteness index (WI) values. Figures 4.11 to 4.14 show the progression of bloom represented by WI values in chocolate model systems with four different oil types and three different SFC levels (45, 55 and 65%). The trends in the WI increase over time in four different oil systems were very similar. In general, bloom appeared in the first several days,

increased rapidly and then plateaued. Fat bloom in most model systems reached equilibrium after 28 days of storage.

On the aspect of SFC, chocolate model systems with 45% SFC bloomed first, within only one day, and reached equilibrium within one or two weeks. The initial bloom rates and the WI values over time were the highest among the three SFC systems. Chocolate model systems with 55% SFC began to show visual bloom at day 7, and reached equilibrium within one or two weeks. The initial bloom rates and the WI values over time were midway among the three SFC systems. Chocolate model systems with 65% SFC did not show visual bloom until day 14, with very slow and small increase in WIs until day 28. The initial bloom rates and the WI values over time were the lowest among the three SFC systems. In general, chocolate model systems with lower SFC started to bloom faster, with higher bloom rates and WI values during storage, resulting in higher final WI values. Figure 4.15 shows representative pictures of the chocolate surface with 45% or 65% SFC after 28-day storage, where a significant difference in color can be identified visually.

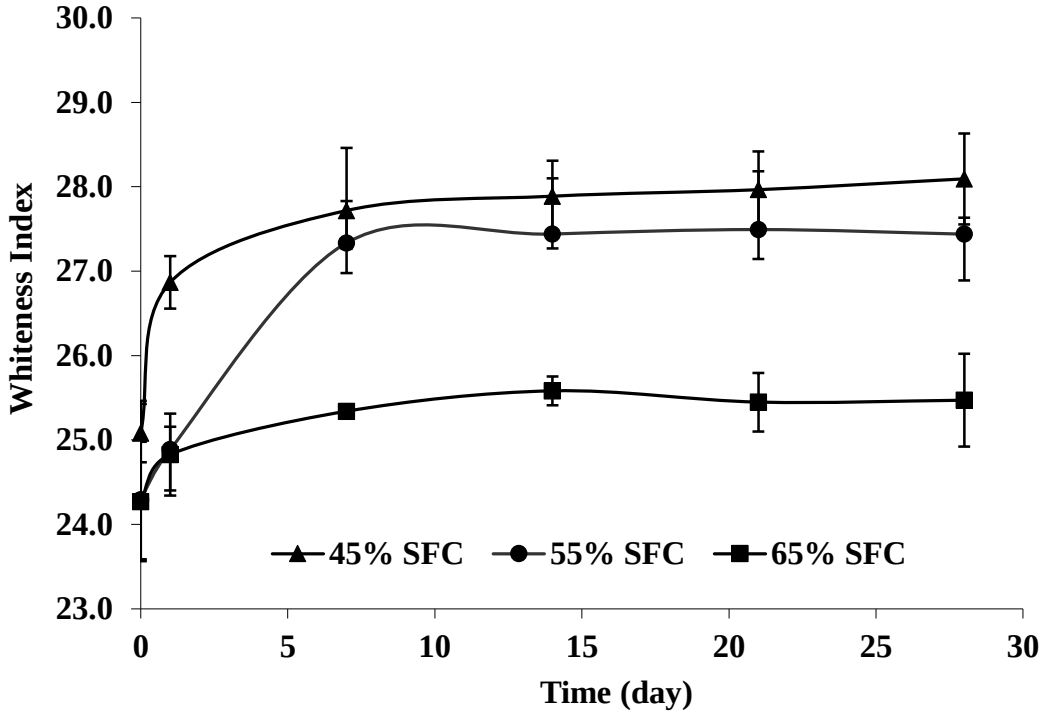


Figure 4. 11 Bloom progression during 28-day storage represented by whiteness index of chocolate model systems made from cocoa butter stearin and peanut oil with solid fat content (SFC) of 45, 55, and 65%. Error bars represent standard deviations.

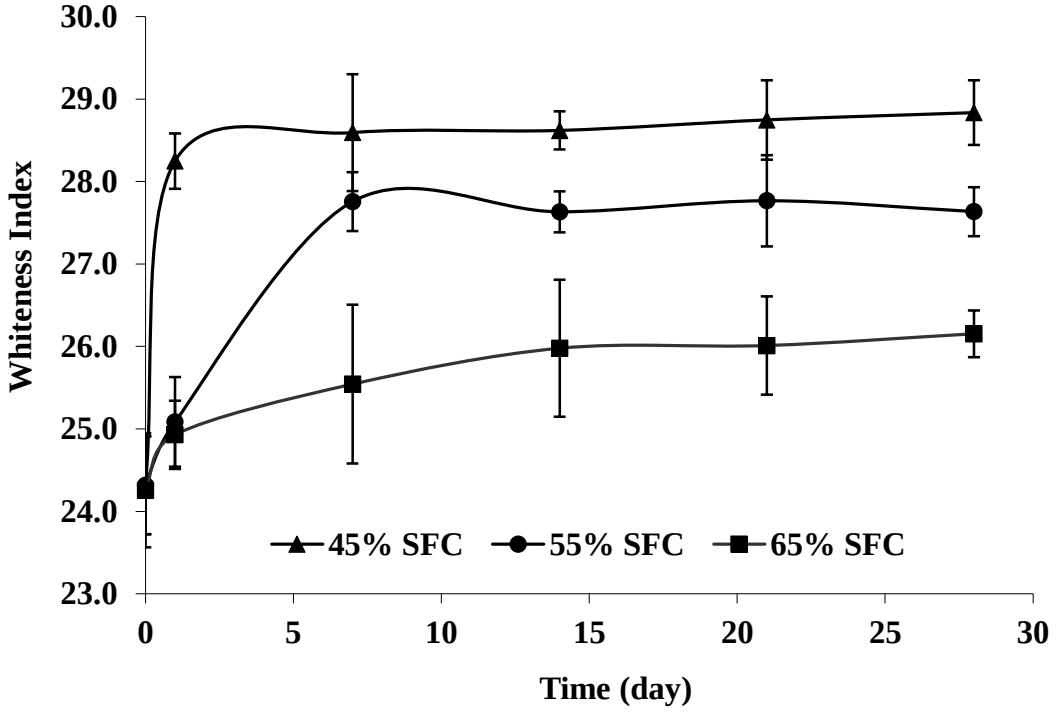


Figure 4. 12 Bloom progression during 28-day storage represented by whiteness index of chocolate model systems made from cocoa butter stearin and cottonseed oil with solid fat content (SFC) of 45, 55, and 65%. Error bars represent standard deviations.

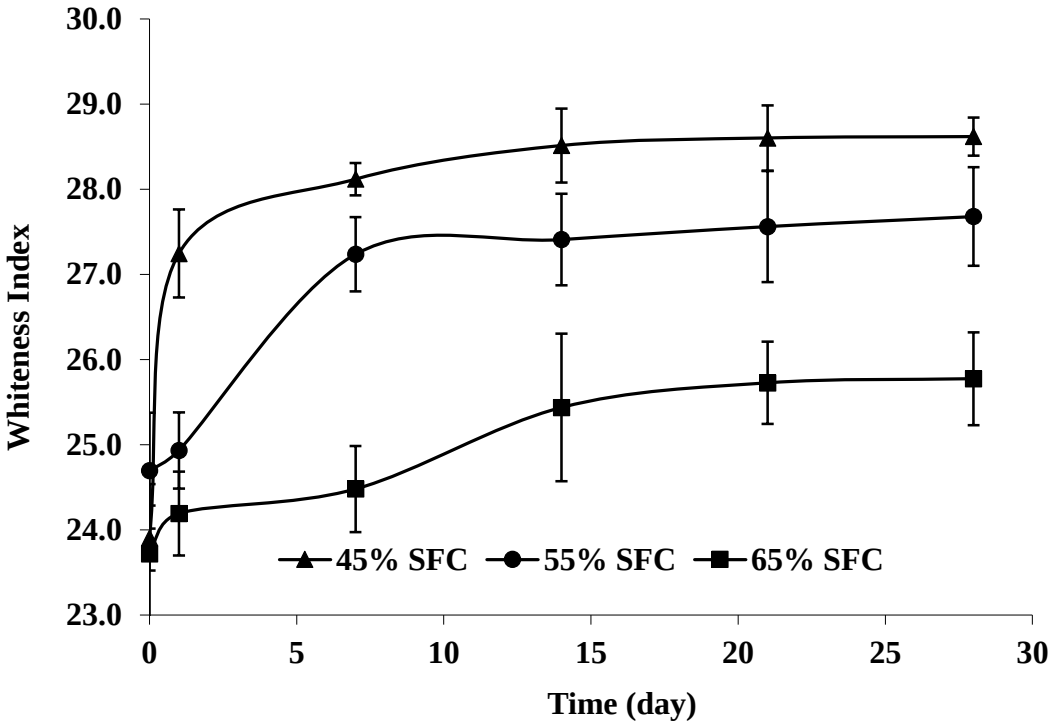


Figure 4. 13 Bloom progression during 28-day storage represented by whiteness index of chocolate model systems made from cocoa butter stearin and sunflower oil with solid fat content (SFC) of 45, 55, and 65%. Error bars represent standard deviations.

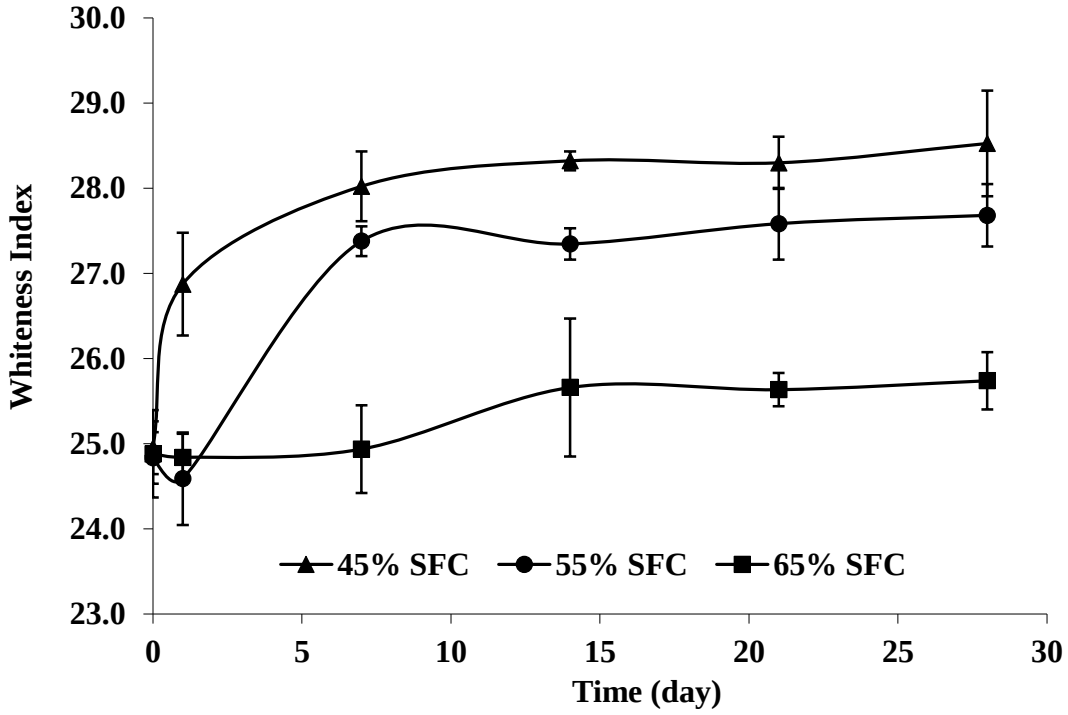


Figure 4. 14 Bloom progression during 28-day storage represented by whiteness index of chocolate model systems made from cocoa butter stearin and canola oil with solid fat content (SFC) of 45, 55, and 65%. Error bars represent standard deviations.

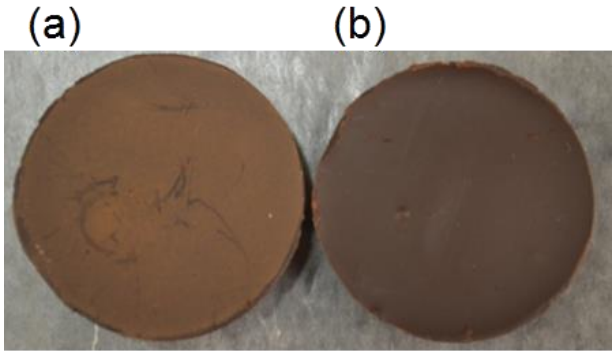


Figure 4. 15 Representative pictures of chocolate surface after 28-day storage with: (a) 45% solid fat content (whiteness index=29); (b) 65% solid fat content (whiteness index=25).

Statistical analyses were conducted on final WI values at day 28 in all model systems, as shown in Table 4.1. Two-way ANOVA on the final WI values at day 28 with 3 levels of SFC and 4 levels of oil type showed that SFC had a significant effect on the final WI values ($p<0.0001$). Tukey HSD showed that chocolate model systems with 45, 55 or 65% SFC were all significantly different from each other. On the other hand, two-way ANOVA showed that oil type did not have a significant effect on the final WI results at day 28 ($p=0.1223$). Tukey HSD showed that model systems with PNO, CSO, SFO, or CNO showed no significant difference from each other. There were no significant effects from the interaction of SFC and oil type ($p=0.8737$), as analyzed by two-way ANOVA.

Table 4. 1 Whiteness Index (WI) values of chocolate model systems with different solid fat content (SFC) and different oil type at day 28. Data represent means ± standard deviations.

SFC (%)	Oil Type			
	SFO	CNO	CSO	PNO
45	28.62 ± 0.22^{ab}	28.53 ± 0.62^{ab}	28.84 ± 0.39^{a}	28.09 ± 0.54^{abc}
55	27.68 ± 0.58^{bc}	27.68 ± 0.37^{bc}	27.64 ± 0.30^{bc}	27.44 ± 0.20^{c}
65	25.78 ± 0.55^{d}	25.74 ± 0.34^{d}	26.15 ± 0.28^{d}	25.47 ± 0.55^{d}

$^{a,b,c,d.}$ Means not connected with the same letter are significantly different ($\alpha=0.05$).
PNO: Peanut oil; CSO: Cottonseed oil; SFO: Sunflower oil; CNO: Canola oil.

On the whole, bloom trends in different liquid oil systems were similar while SFC played an important role in bloom progression during storage. This result agrees with the fat migration and recrystallization theory (Hartel, 1999; Matsuda et al., 2001) as described before, and several previous studies on the effect of SFC (Ramsom-Painter et al., 1997; Ali et al., 2001) as they reported that liquid fat migration level as well as its induction time were negatively correlated with SFC in both chocolate and compound coatings. According to the bloom theory, lower SFC provides more liquid fats in chocolate model systems, which could be available for fat migration. Also, more high-melting fats (CB-S) could be dissolved in the liquid fats in the system as the total solute amount (the amount of liquid fat) is increased. As a result, more liquid fats, carrying more dissolved high-melting fats, together move to the chocolate surface. This provides more high-melting fats on the chocolate surface for fat recrystallization, thus causing more visual bloom to occur. As well, lower SFC reduces viscosity in the chocolate matrix, providing less resistance to fat migration, either due to diffusion or capillary force (Guiheneuf et al, 1997; Aguilera et al., 2004 ; Smith et al., 2007).

4.2 Effects of temperature fluctuation frequency on fat bloom during storage

Since the previous phase confirmed that solid fat content (SFC) of chocolate model systems had a significant effect on bloom during storage, this phase looked at different storage temperature fluctuation frequencies (3-hour, 7-hour, and 11-hour). Knowing that there are no significant differences between chocolate model systems with different liquid oil types, this phase only used chocolate model systems of canola oil (CNO) with three different SFCs (45, 55, and 65%).

4.2.1 Whiteness index

4.2.1.1 Chocolate model system

As the formulation of chocolate model system in this phase was identical to the CNO systems in the previous phase (50% fat, 50% cocoa particles), bloom behaviors were very similar to the previous phase. Figures 4.16 to 4.18 show the progression of bloom represented by whiteness index (WI) values in chocolate model systems of CNO and cocoa butter stearin (CB-S) with three different SFC levels (45, 55 and 65%) when the storage temperature was cycled between 20 and 30°C at different frequencies (3-hour, 7-hour, and 11-hour). The trends in the WI increase over time when chocolate model systems were stored under different temperature fluctuation frequencies were similar, though the bloom extents were different. When cycled at 3-hour frequency, the model system with 45% SFC began to show visual bloom at day 1 and reached equilibrium within one week, whereas 55 and 65% SFC model systems did not show clear visual bloom even at day 28. When cycled at 7-hour frequency, the model system with 45% SFC began to show visual bloom within the first three days and reached

equilibrium within one week; 55% SFC model system started to show clear visual bloom at day 7, reached equilibrium at day 14 and the WI increase in the first week was close to linear; and the 65% SFC model system did not show clear visual bloom until day 7, but the bloom level at day 28 was still very low. When cycled at 11-hour frequency, the model system with 45% SFC began to show visual bloom at day 1 and reached equilibrium within one week; 55% SFC model system started to show clear visual bloom at day 7, and reached equilibrium at day 14; and the 65% SFC model system did not start to bloom until day 7, but the bloom level was still very low after 28-day storage. Generally speaking, chocolate model systems with lower SFC started to bloom earlier, with higher bloom rate and bloom level during storage, resulting in higher final WI values.

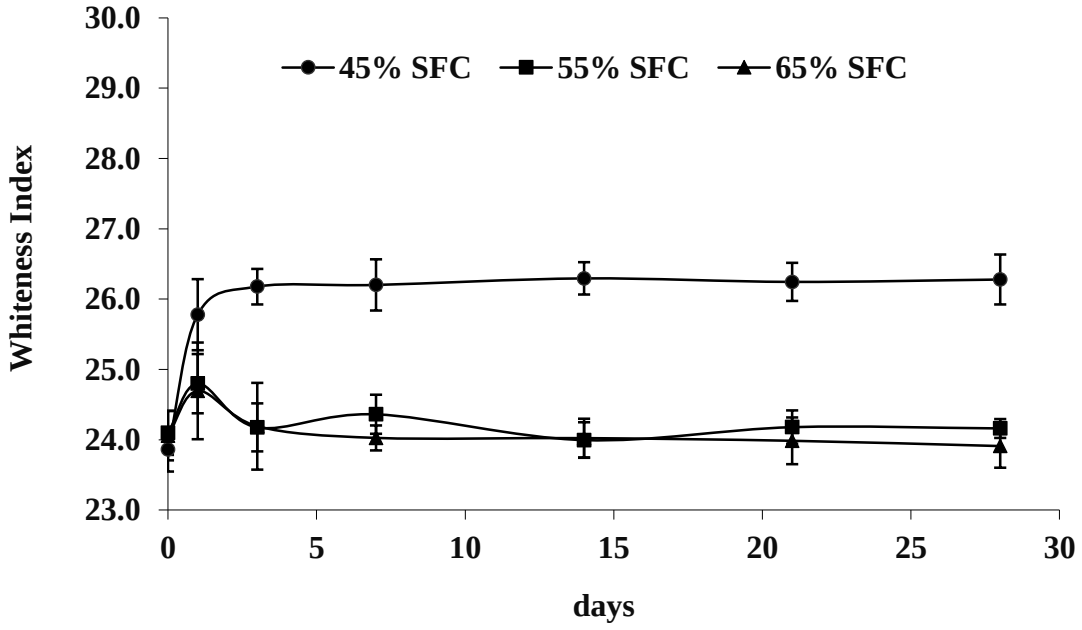


Figure 4. 16 Bloom progression during 28-day storage represented by whiteness index of chocolate model systems made from cocoa butter stearin and canola oil with solid fat content (SFC) of 45, 55, and 65% when temperature was fluctuated between 20 and 30°C every 3 hours. Error bars represent standard deviations.

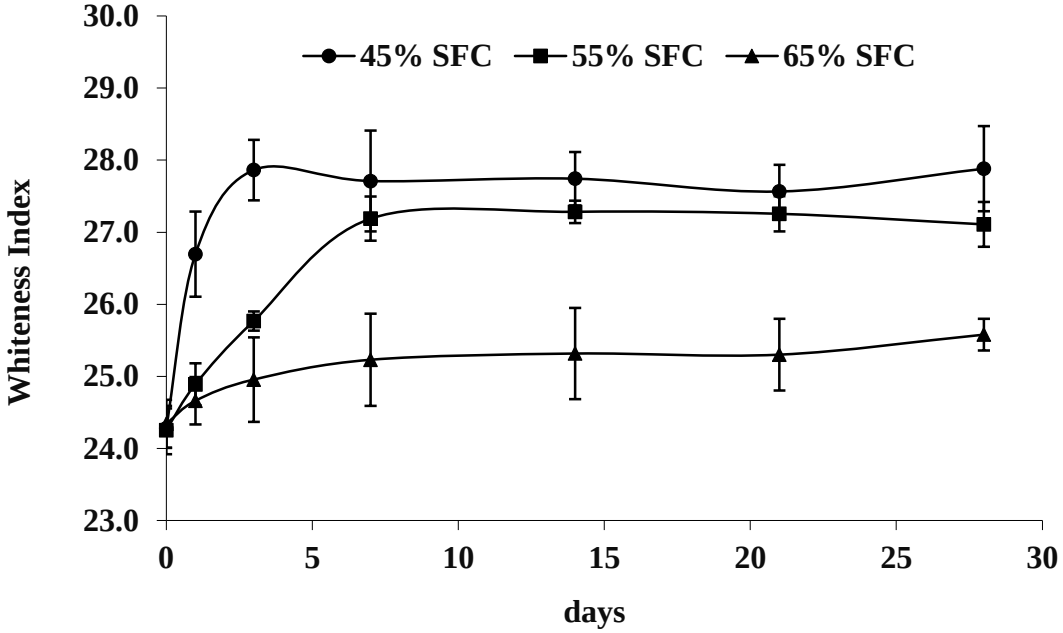


Figure 4. 17 Bloom progression during 28-day storage represented by whiteness index of chocolate model systems made from cocoa butter stearin and canola oil with solid fat content (SFC) of 45, 55, and 65% when temperature was fluctuated between 20 and 30°C every 7 hours. Error bars represent standard deviations.

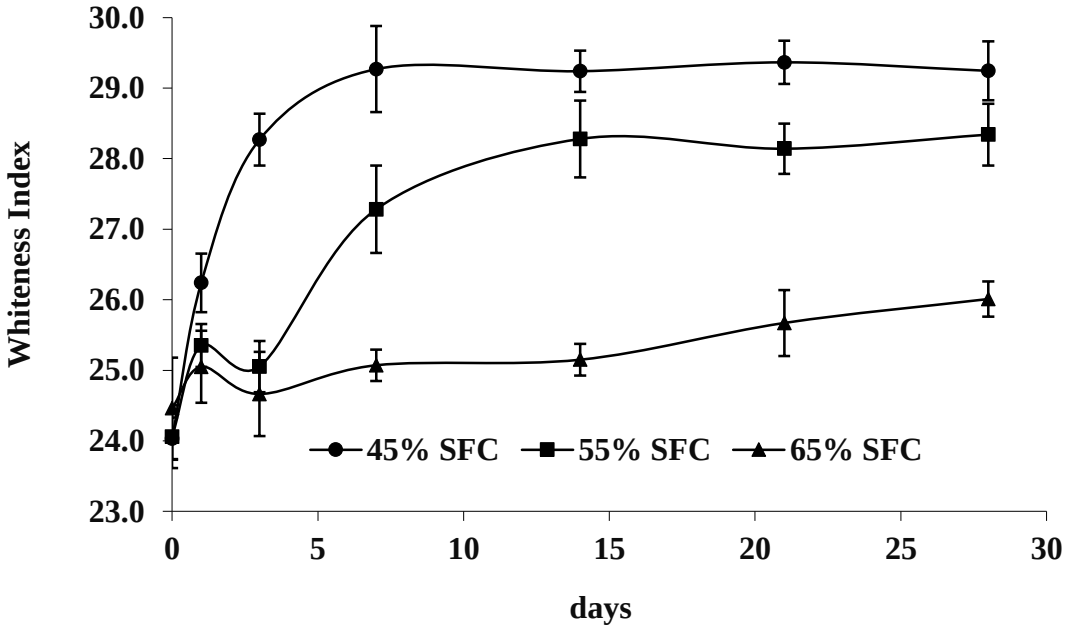


Figure 4. 18 Bloom progression during 28-day storage represented by whiteness index of chocolate model systems made from cocoa butter stearin and canola oil with solid fat content (SFC) of 45, 55, and 65% when temperature was fluctuated between 20 and 30°C every 11 hours. Error bars represent standard deviations.

Temperature fluctuation frequency also showed significant effect on the bloom progression during storage. Reducing temperature fluctuation frequency (increasing hours per cycle) resulted in faster bloom with higher bloom extent. In the 65% SFC model systems, the effect of temperature fluctuation frequency was weaker. With 3-hour cycling, it did not bloom during 28 days, whereas it showed low bloom extent for 7-hour and 11-hour cycling and the differences between them were very subtle. On the other hand, the 45 and 55% SFC model system showed greater difference in WI values with different temperature fluctuation frequency. On the whole, higher temperature fluctuation frequency (lower hours per cycle) reduced bloom extent in these model systems. This trend matches the results in a study on filled dark chocolate where it was concluded that increasing temperature fluctuation frequency from every 7 days to every 2.5 days would reduce WI values (Rothkopf et al., 2017).

Statistical analyses were conducted on final WI values at day 28 in all model systems, as shown in Table 4.2. Two-way ANOVA on the final WI values at day 28 with 3 levels of SFC and 3 levels of temperature fluctuation frequency showed that SFC had a significant effect on the final WI values ($p<0.0001$). Tukey HSD on SFC showed that chocolate model systems with 45% SFC were significantly different from 55 and 65% SFC systems, whereas there was no significant difference between 55 and 65% SFC systems. On the other hand, two-way ANOVA showed that temperature fluctuation frequency had a significant effect on the final WI results at day 28 ($p<0.0001$) as well. Tukey HSD on temperature fluctuation frequency showed that model systems with 3-hour, 7-hour, and 11-hour frequency systems were all significantly different from each other. Further, there was also a significant effect from the interaction of SFC and temperature fluctuation frequency ($p<0.0001$), as analyzed by two-way ANOVA.

Table 4. 2 Whiteness Index (WI) values of chocolate model systems with different solid fat content (SFC) and temperature fluctuation frequency at day 28. Data represent means ± standard deviations.

SFC (%)	Temperature Fluctuation Frequency		
	3-hour frequency	7-hour frequency	11-hour frequency
45	26.28 ± 0.36^{d}	27.88 ± 0.59^{b}	29.24 ± 0.42^{a}
55	24.16 ± 0.13^{f}	27.11 ± 0.31^{c}	28.34 ± 0.44^{b}
65	23.91 ± 0.31^{f}	25.58 ± 0.22^{e}	26.01 ± 0.25^{de}

$^{a,b,c,d,e,f.}$ Means not connected with the same letter are significantly different (α=0.05).

4.2.1.2 Commercial chocolate

The effect of temperature fluctuation frequency on the bloom progression in commercial chocolate during storage was opposite to that in chocolate model systems. Reducing temperature fluctuation frequency (increasing hours per cycle) resulted in slower bloom with smaller bloom extent. Figure 4.19 shows the bloom progression represented by WI values in commercial chocolate (Yucatan BR SEMI-SWEET chocolate buttons) when the storage temperature was cycled between 20 and 30°C at different frequencies (3-hour, 7-hour, and 11-hour). With 11-hour cycling, it did not bloom in the first week, started to show clear visual bloom at day 14, and did not reach equilibrium at day 28; with 7-hour cycling, it did not bloom in the first week either, started to bloom at day 14 and reached equilibrium between day 21 and day 28; and with 3-hour cycling, it started to bloom between day 1 and day 3 and reached equilibrium at day 14. In general, the chocolate with higher temperature fluctuation frequency (lower hours per cycle) started to bloom earlier, with higher bloom rate and higher final WI values. To compare this result with the chocolate model system, Figure 4.20 shows the bloom progression represented by WI values in chocolate model systems made of CB-S and CNO with 45% SFC when the storage temperature was cycled between 20 and 30°C at different

frequencies (3-hour, 7-hour, and 11-hour). It is very clear that in these model systems, without sucrose crystals, higher temperature fluctuation frequency (lower hours per cycle) promoted bloom faster with bloom extent, which was the opposite of the trend in commercial chocolate.

Statistical analyses were conducted on final WI values at day 28 for the commercial chocolate, as shown in Table 4.3. One-way ANOVA on the final WI values at day 28 with 3 levels of temperature fluctuation frequency showed that temperature fluctuation frequency had a significant effect on the final WI values ($p<0.0001$). Tukey HSD on temperature fluctuation frequency showed that bloom on the commercial chocolate stored at 3-hour frequency was significantly different from 7-hour and 11-hour systems, whereas there was no significant difference between 7-hour and 11-hour systems.

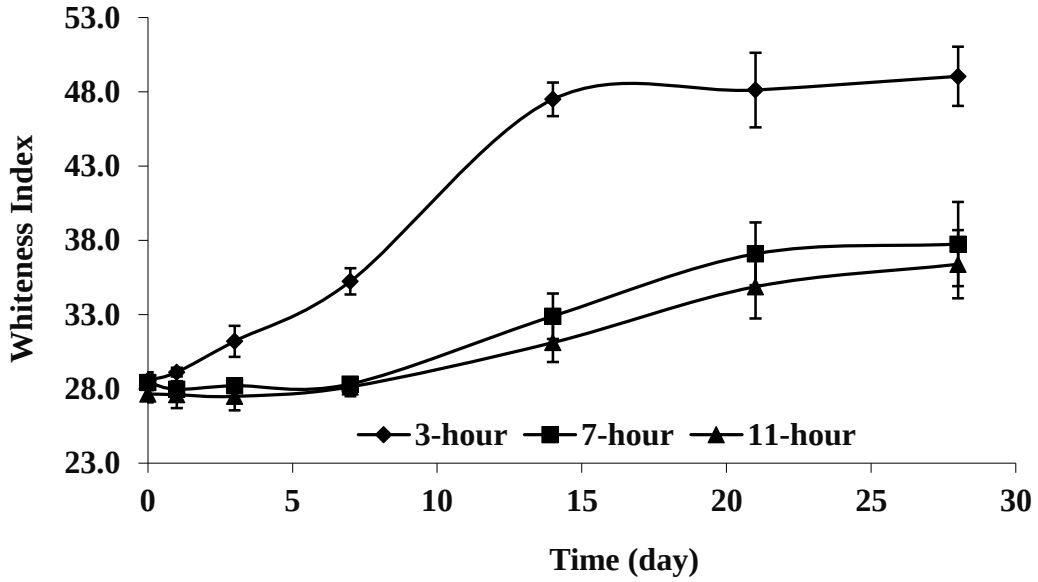


Figure 4. 19 Bloom progression during 28-day storage represented by whiteness index of commercial chocolate (Yucatan BR SEMI-SWEET chocolate buttons) when temperature was fluctuated between 20 and 30°C every 3, 7 or 11 hours. Error bars represent standard deviations.

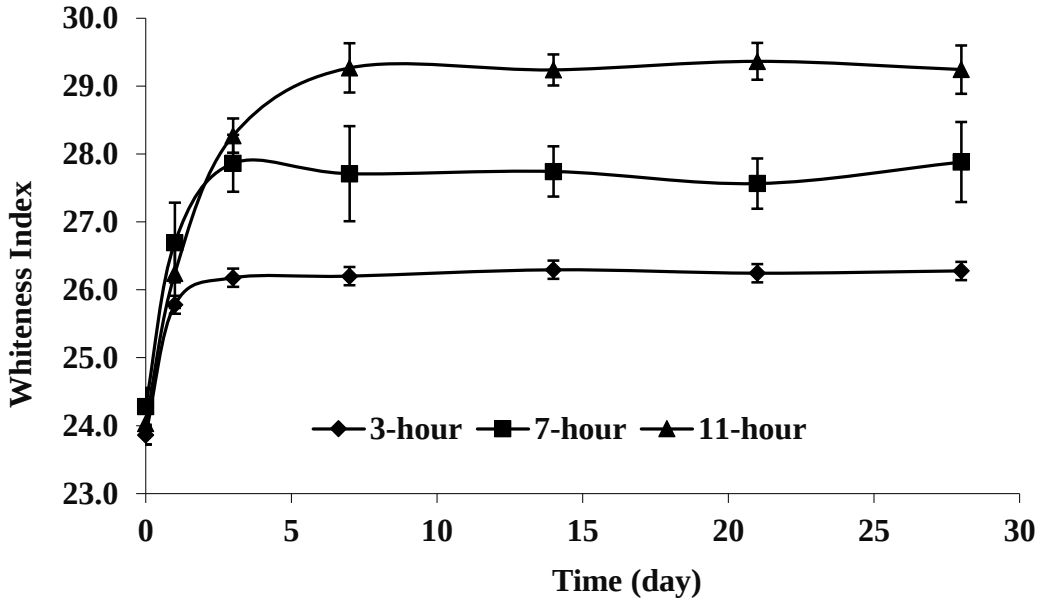


Figure 4. 20 Bloom progression during 28-day storage represented by whiteness index of chocolate model systems made from cocoa butter stearin and canola oil with solid fat content (SFC) of 45%, when temperature was fluctuated between 20 and 30°C every 3, 7 or 11 hours. Error bars represent standard deviations.

Table 4. 3 Whiteness Index (WI) values of commercial chocolates after 28-day storage with different temperature fluctuation frequency. Data represent means ± standard deviations.

Temperature Fluctuation Frequency	WI
3-hour	49.04 ± 1.99^{a}
7-hour	37.75 ± 2.83^{b}
11-hour	36.39 ± 2.29^{b}

[a,b.] Means not connected with the same letter are significantly different (α=0.05).

4.2.2 SFC during storage

When temperature fluctuates, SFC fluctuates accordingly in order to maintain the phase equilibrium shown in Figures 4.6 to 4.9. As temperature increases, the SFC of model systems goes down as higher temperature melts some solid fats to liquid in the system. Table 4.4 shows the changes in SFC (ΔSFC) in CNO and CB-S system with SFC of 45, 55, and 65% when temperature changed between 20 and 30°C. One-way ANOVA showed that ΔSFC in different

SFC groups had no significant difference from each other (p=0.1024). This means the changes during each temperature cycle between 20°C and 30°C in model systems with different SFCs were the same statistically, assuming thermal and phase volume equilibrium were reached in each cycle. Thermal equilibration in chocolate discs was confirmed as the real-time temperature in the oven was reached within 7 min, whether going up or down. Further, in order to ensure equilibration of SFC during each temperature cycle, samples made of canola oil and cocoa butter stearin with different SFC (45, 55, and 65%) at 20°C were prepared in NMR tubes and SFC was tracked at the end of each temperature cycle (20 and 30°C) during storage, as shown in Table 4.5. One-way ANOVA showed that there was no significant difference from different temperature fluctuation frequency systems at 20°C (p=0.9919) or 30°C (p=0.9904), indicating that SFC equilibration was reached within 3 hours. Even though there might be a slight difference in the thermal transfer rate between NMR tubes and chocolate discs, it was assumed that SFC equilibrium was reached in all temperature fluctuation frequency systems. Thus, it is concluded that ΔSFC was the same regardless of the initial SFC at 20°C or the temperature cycling frequency; therefore, the change in SFC is probably not the main cause for the difference in the bloom extent in these chocolate model systems.

Table 4. 4 Changes in solid fat content (ΔSFC) in canola oil and cocoa butter stearin system with solid fat content (SFC) of 45, 55, and 65% when temperature changes between 20 and 30°C. Data represent means ± standard deviations.

SFC (%) at 20°C	ΔSFC (%)
45	21.07 ± 0.72[a]
55	21.59 ± 1.26[a]
65	19.62 ± 1.46[a]

[a.] Means not connected with the same letter are significantly different (α=0.05).

Table 4. 5 Measured solid fat content (SFC) of canola and cocoa butter stearin systems with different SFC at 20°C, at the end of each temperature cycle (20 and 30°C) during storage. Standard deviations are shown below each mean value in the brackets.

	20°C			30°C		
SFC	45%	55%	65%	45%	55%	65%
3-hour frequency	45.83^{a} (0.49)	55.11^{b} (0.44)	64.49^{c} (0.27)	22.47^{A} (0.48)	28.30^{B} (0.39)	38.81^{C} (0.41)
7-hour frequency	45.53^{a} (0.24)	55.73^{b} (0.27)	63.99^{c} (0.16)	22.17^{A} (0.39)	27.97^{B} (0.33)	38.71^{C} (0.57)
11-hour frequency	46.12^{a} (0.55)	55.87^{b} (0.28)	64.60^{c} (0.37)	21.45^{A} (0.35)	28.15^{B} (0.18)	38.54^{C} (0.43)

$^{a, b, c; A, B, C.}$ Means not connected with the same letter are significantly different.

4.2.3 Discussion

In CNO – CB-S model systems made with only cocoa powder, higher temperature fluctuation frequency reduced bloom rate and final bloom extent. This seemed to go against the theory of fat bloom during storage based on fat migration. Increasing temperature fluctuation frequency would increase the frequency of fat migration during bloom formation within certain storage period. Therefore, greater bloom extent would be expected in chocolate when temperature fluctuates more frequently, and this is reported in a study on commercial chocolate based on surface roughness results (Quevedo et al., 2005). The WI results in commercial chocolate in this study also confirmed this.

In order to explain the discrepancy in the WI trend between commercial chocolate and chocolate model system, the hypothesis of storage bloom mechanisms based on fat migration

and recrystallization need to be considered. According to the fat migration and recrystallization theory, when temperature fluctuates, the SFC of the system changes and so does the solubility of high-melting fats in low melting fats. As described before, the temperature increasing stage during the fluctuation will provide more liquid fat together with more dissolved solid fats in the system available for fat migration. Due to the difference in the heat transfer between the surface and center of chocolate, the temperature fluctuation on the surface is larger than the center, resulting in liquid fats pumping to the chocolate surface by either capillary force or diffusion (Guiheneuf et al., 1997; Aguilera et al., 2004). When the temperature goes down during the cycle, high-melting fats recrystallize on the surface as visual bloom as a result of SFC and solubility changes. To note, recrystallization itself is not sufficient to cause visual bloom (Bricknell and Hartel, 1998) as certain shape of fat crystals, which is usually spike- or needle-like, need to be formed during recrystallization to cause clear visual bloom. The recrystallized fats need to poke out from the surface to give light reflection resulting in visual bloom and WI value changes (Hartel et al., 2016). Thus, the morphology of the recrystallized in bloom progression is also important. Any factor that influences the previous steps in bloom formation would lead to different bloom trends.

As shown in Section 4.2.2, change in SFC is not significantly different in different model systems; thus, it is not an essential factor for the discrepancy in bloom extent between commercial chocolate and model systems. The potential mechanism might be more related to the difference in the microstructure of the two systems. In commercial chocolate, the nonfat particulate level is around 67% while there are only 50% nonfat particles in the model system in this phase. Also, the average SFC in commercial chocolate is around 75%, while the SFCs

in the model systems were much lower (45, 55, and 65%). Further, there is around 0.5% lecithin in commercial chocolate, which was reported to have an influence on the particle network in the chocolate system (Johansson and Bergenstahl, 1992a), while no lecithin was added in the model system in this study. All of these factors would contribute to significant differences in the microstructure of the chocolate, thus influencing the migration pathway, kinetics and even the nature of migration during bloom formation (Dahlenborg et al., 2015). On the other hand, the recrystallization step might also be different in the two systems. There is about 50% crystalline sugar in commercial chocolate, which could potentially act as nucleation sites for fat recrystallization during bloom formation, whereas no sugar was added in the chocolate model system in this study, and this could change the recrystallization kinetics during bloom formation. Moreover, Bricknell and Hartel (1998) reported that chocolate with crystalline sugar had sharp fat crystals on the surface leading to clear visual bloom, whereas chocolate without crystalline sugar (with amorphous particles) only had recrystallized fat with a smoother shape leading to no visual bloom. The difference in the fat crystal shape on the chocolate surface may lead to different bloom results as sharp crystals protruding out from surface reflect lights more effectively than flat particles. Thus, the nature of recrystallization during bloom might also be different between commercial chocolate and chocolate model systems in this study.

Even though both of the bloom trends in model systems and commercial chocolate from this study were observed in literature (Quevedo et al., 2005; Rothkopf et al., 2017), the real mechanism behind the discrepancy between the two systems is still unclear. Further investigation is needed to control the factors mentioned above to better understand the causality between temperature fluctuation frequency and visual bloom extent.

4.3 Effects of different nonfat particles on fat bloom during storage

Since the previous phases illustrated the effects of the fat phase on bloom formation during storage, this phase continued to study the effects of other ingredients in chocolate, especially sugar particles and lecithin, on storage bloom. Sugar particles, as well as lecithin, might influence the particle interactions in the chocolate system and the microstructure of the chocolate network, thus having an effect on the migration and recrystallization steps during bloom formation (Johansson and Bergenstahl, 1992a). Therefore, the effects of sugar types, sugar concentration, and the presence of lecithin were studied by three different bloom evaluation methods (whiteness index, stereomicroscopy, and visual analysis). The interactions between particles were also investigated by three different methods (rheology, sedimentation, and tensiometry). Correlations between the particle interaction and bloom extent were investigated.

4.3.1 Bloom evaluation

In this phase, bloom was evaluated by three different evaluation methods (whiteness index, stereomicroscopy, and visual analysis) and three factorial designs were conducted in each bloom evaluation: (1) effect of sugar particle types in replacement of cocoa powder, (2) effect of lecithin with different sugar fraction, and (3) effect of lecithin with different types of sugar particles.

4.3.1.1 Effect of sugar particle types in replacement of cocoa powder

Four types of sugar including two crystalline sugars (sucrose and maltitol) and two amorphous sugars (corn syrup solids (CSS, moisture content = 6.12%) and polydextrose (PD, moisture content = 4.71%)), were added in the model systems. Sugar concentrations of 0, 25, 50, and 75% on a volume basis in the particle phase were used to gradually replace cocoa powder while still keeping the particle volume consistent. Crystalline sugars, especially with sharp surfaces, were expected to show higher bloom extents as the sharp edges were hypothesized to provide nucleation sites for cocoa butter recrystallization (Bricknell and Hartel, 1998). On the other hand, amorphous sugars, with smooth and rounder surfaces, were expected to have lower bloom extents as they could change or block the migration pathway for fat migration during bloom or reduce nucleation sites for cocoa butter recrystallization. Figures 4.21 to 4.23 show the progression of bloom represented by changes in whiteness index (ΔWI), white area percentage and visual rate values in chocolate model systems of cocoa butter (CB) and particles, respectively, where cocoa powder was gradually replaced by sugar particles. To better illustrate the chocolate surfaces, Figures 4.24 to 4.27 show representative pictures from stereomicroscope on the surfaces of these chocolate model systems at day 28 and Figures 4.28 to 4.31 show pictures taken directly by digital camera of these chocolate samples at day 28.

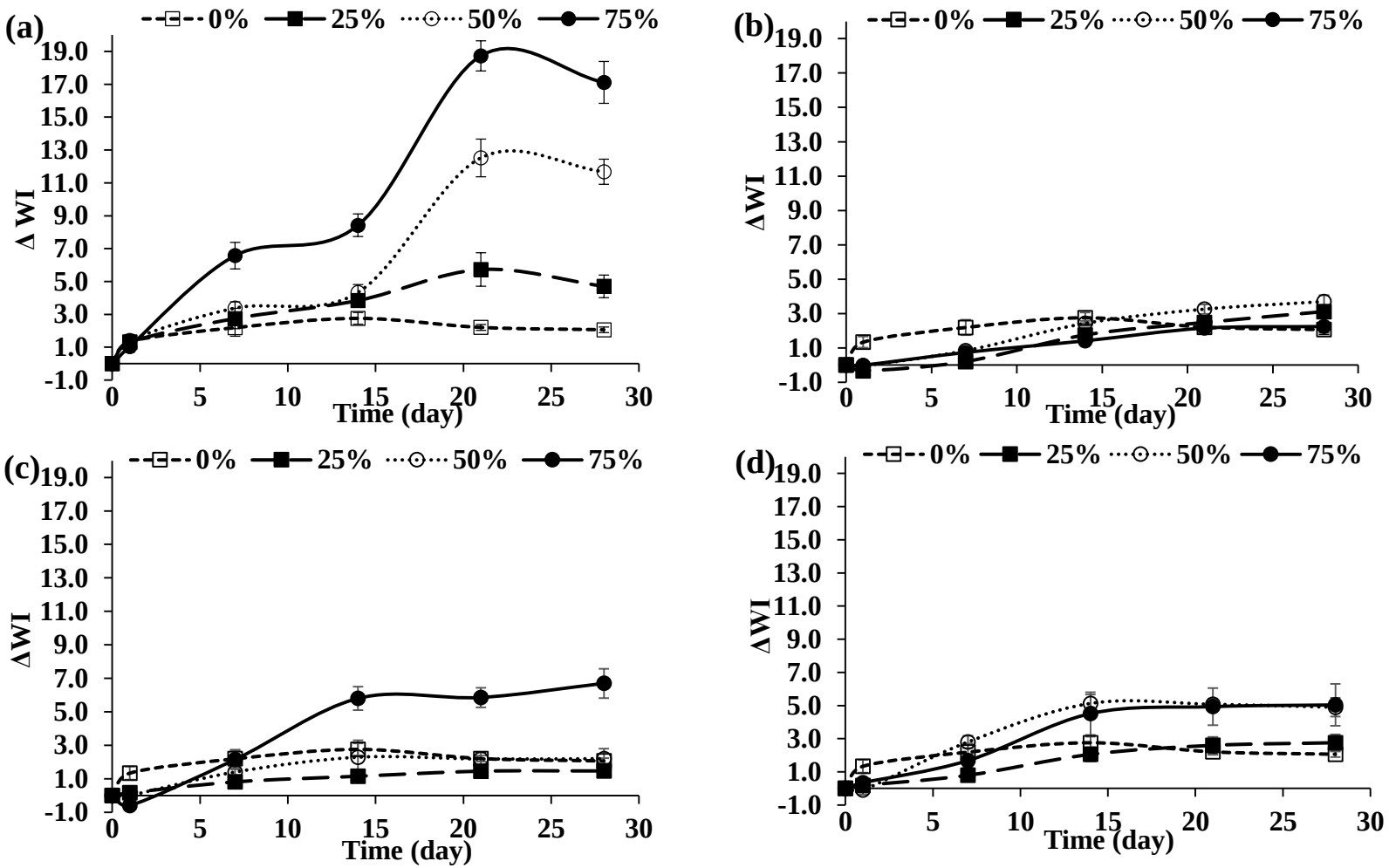


Figure 4. 21 Bloom progression represented by changes in whiteness index (ΔWI) values in chocolate model systems of 50% cocoa butter and 50% particles, where cocoa powder was gradually replaced by: (a) sucrose, (b) maltitol, (c) corn syrup solids, (d) polydextrose particles, at different levels (0, 25, 50, and 75%) on a volume basis.

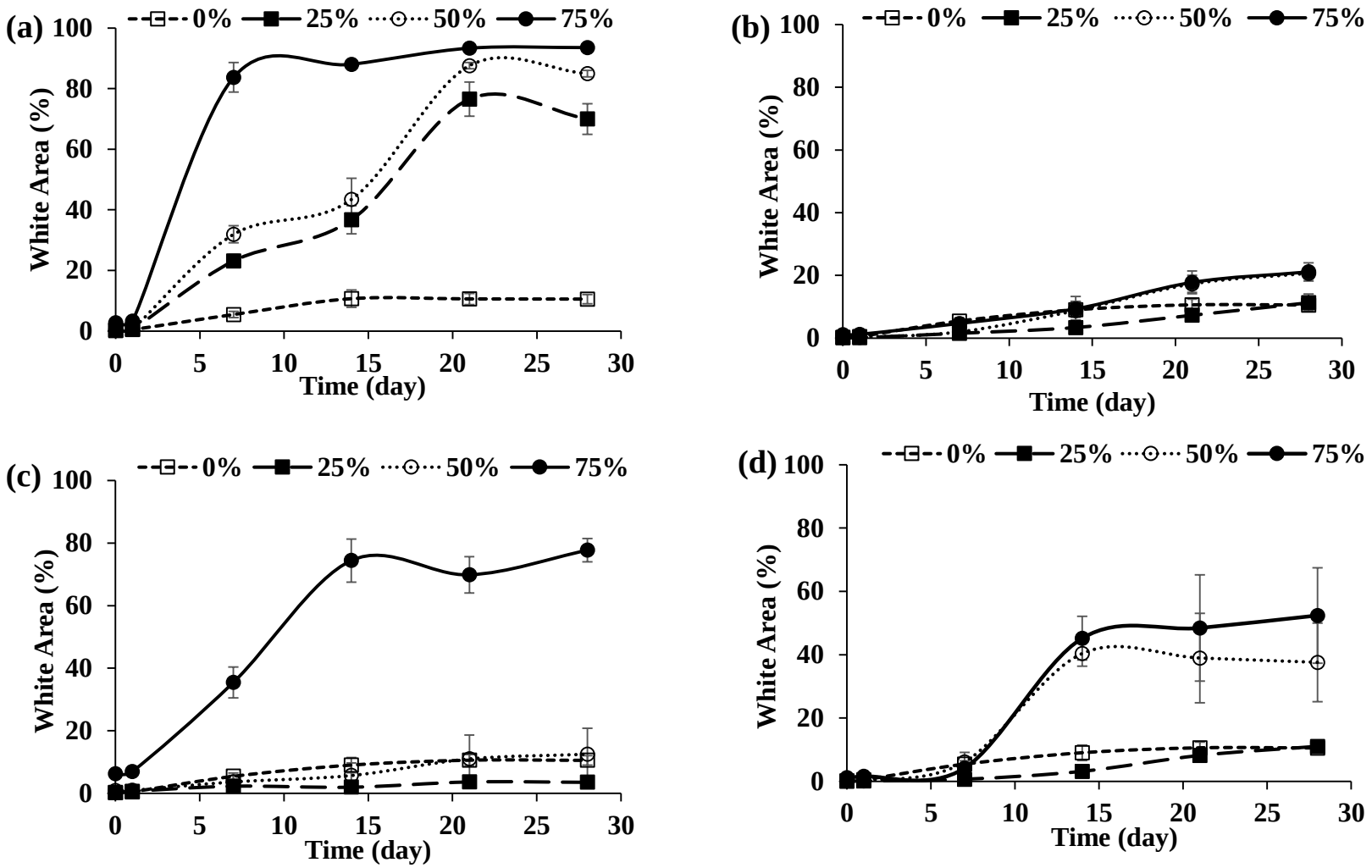


Figure 4. 22 Bloom progression represented by changes in whiteness index (ΔWI) values in chocolate model systems of 50% cocoa butter and 50% particles, where cocoa powder was gradually replaced by: (a) sucrose, (b) maltitol, (c) corn syrup solids, (d) polydextrose particles, at different levels (0, 25, 50, and 75%) on a volume basis.

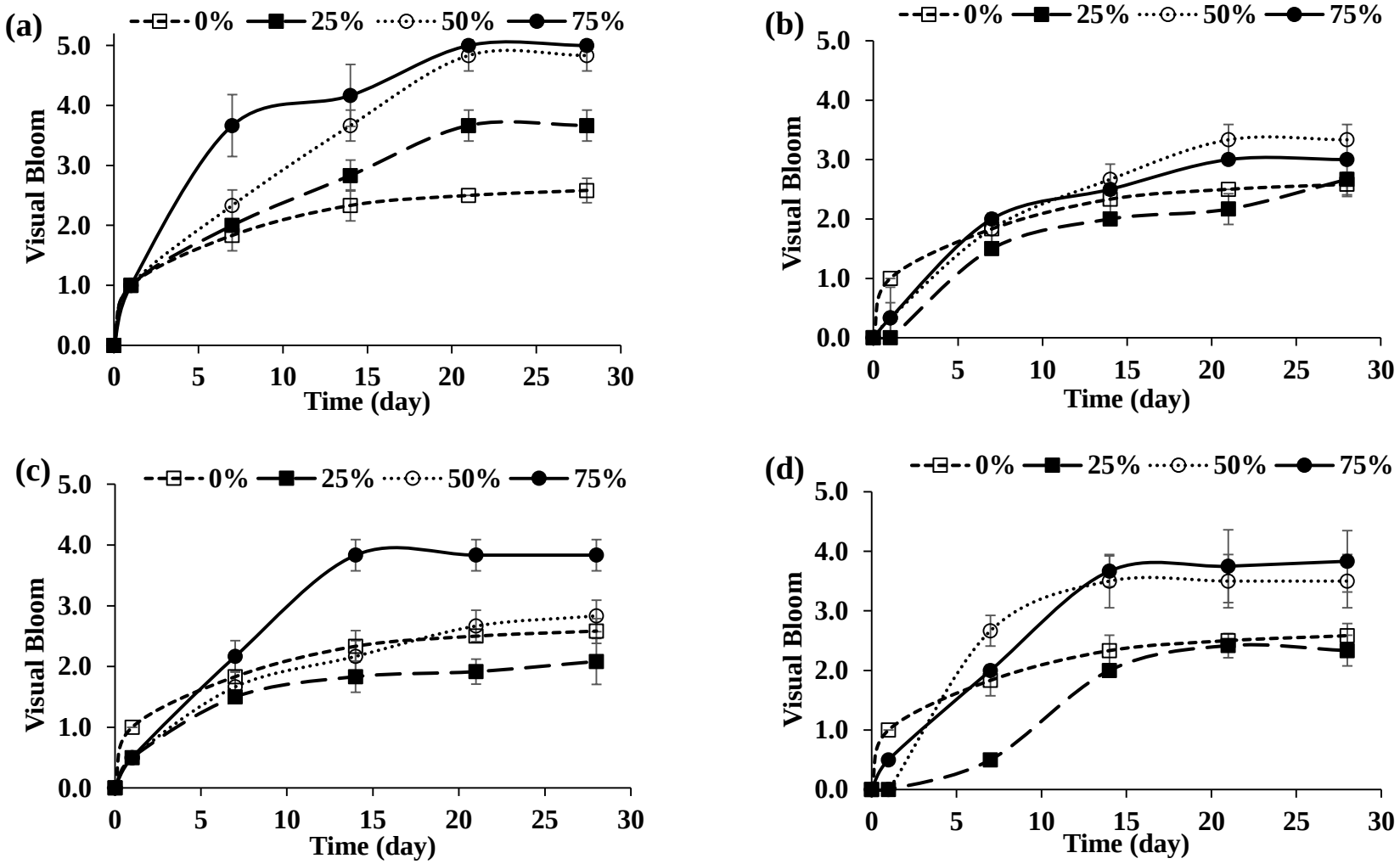


Figure 4. 23 Bloom progression represented by visual bloom in chocolate model systems of 50% cocoa butter and 50% particles, where cocoa powder was gradually replaced by: (a) sucrose, (b) maltitol, (c) corn syrup solids, (d) polydextrose particles, at different levels (0, 25, 50, and 75%) on a volume basis.

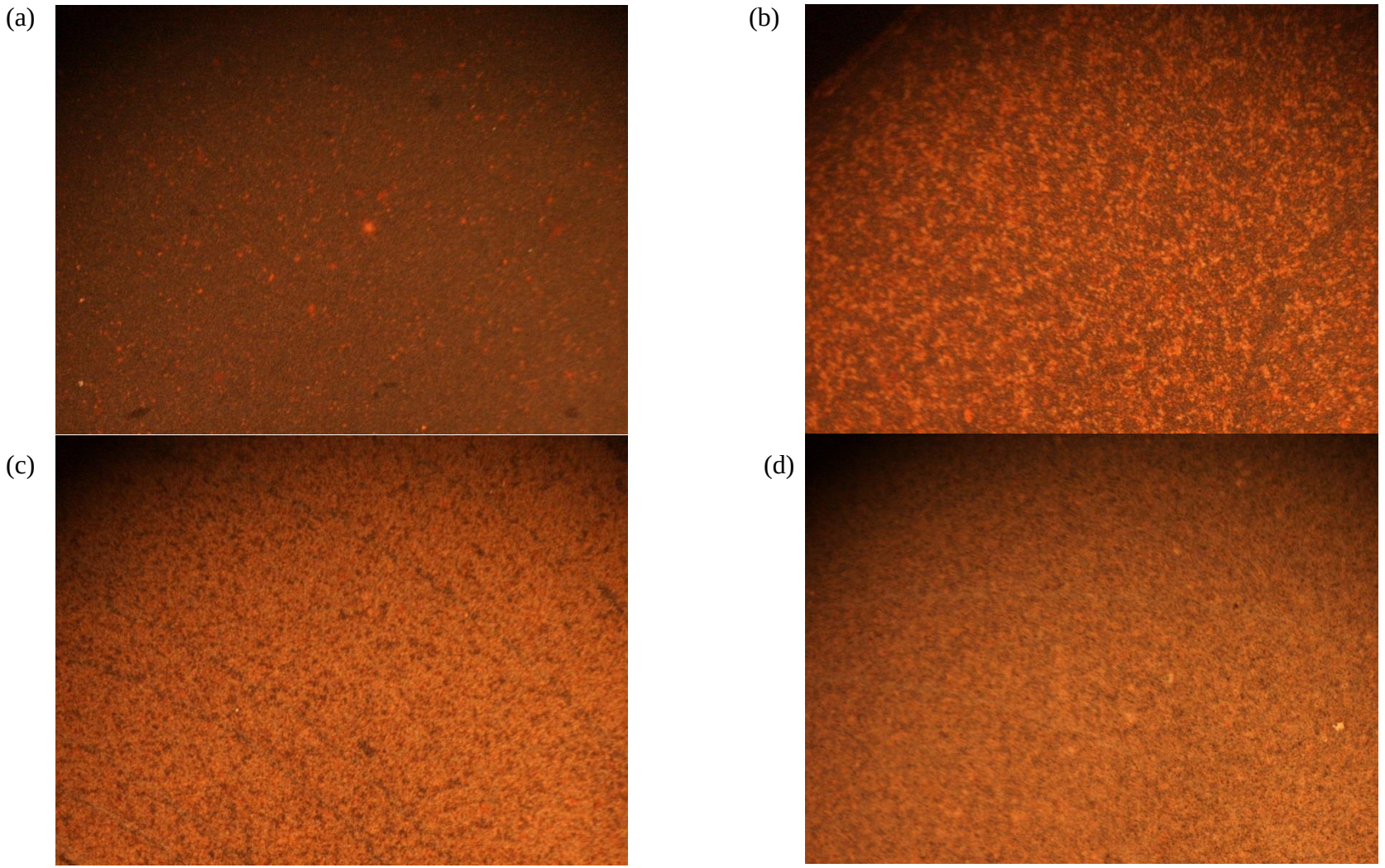


Figure 4. 24 Representative pictures from stereomicroscope at day 28 on the surfaces of chocolate model systems of 50% cocoa butter and 50% particles, where cocoa powder was gradually replaced by sucrose particles at different levels on a volume basis: (a) 0%, (b) 25%, (c) 50%, (d) 75%.

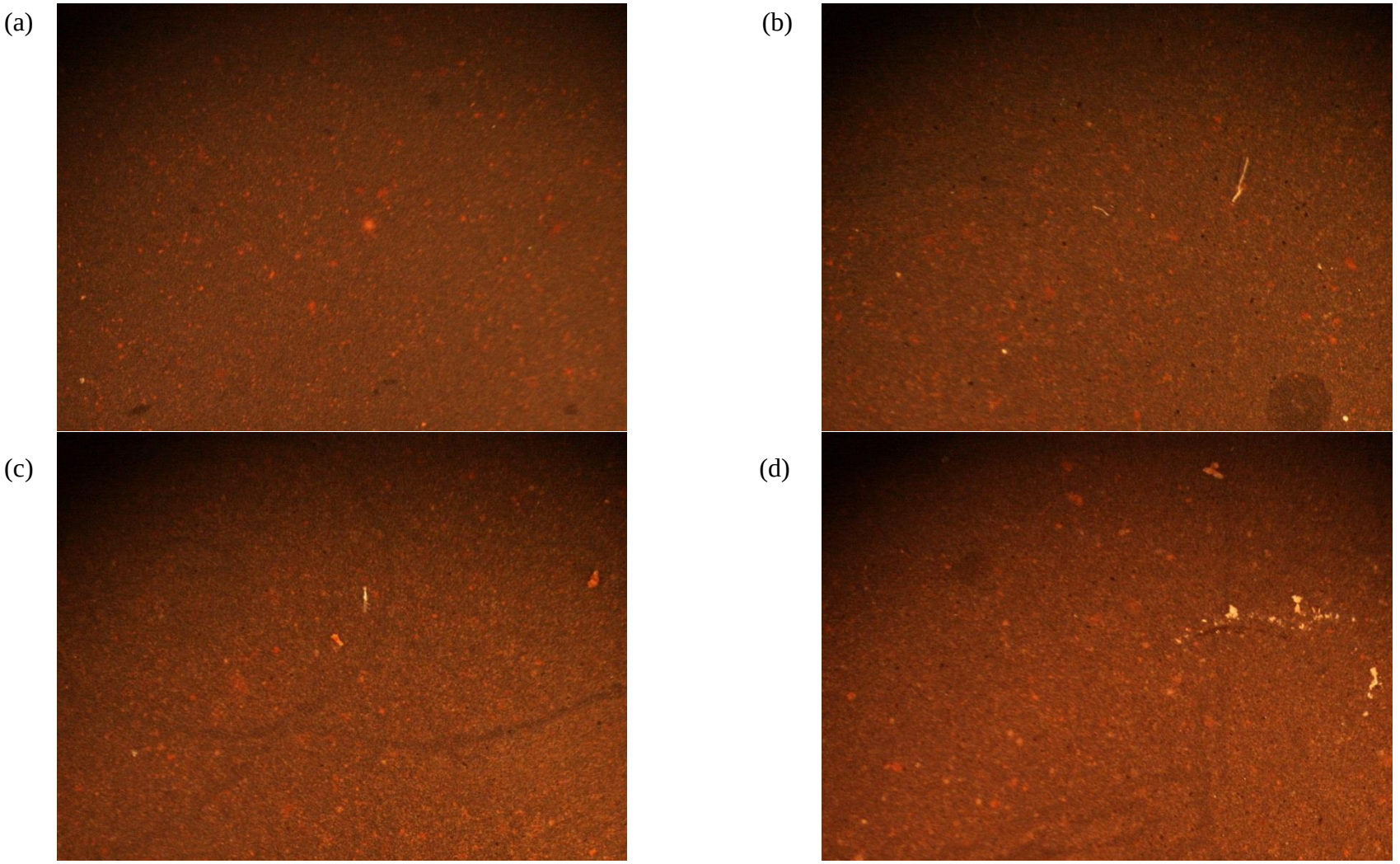


Figure 4. 25 Representative pictures from stereomicroscope at day 28 on the surfaces of chocolate model systems of 50% cocoa butter and 50% particles, where cocoa powder was gradually replaced by maltitol particles at different levels on a volume basis: (a) 0%, (b) 25%, (c) 50%, (d) 75%.

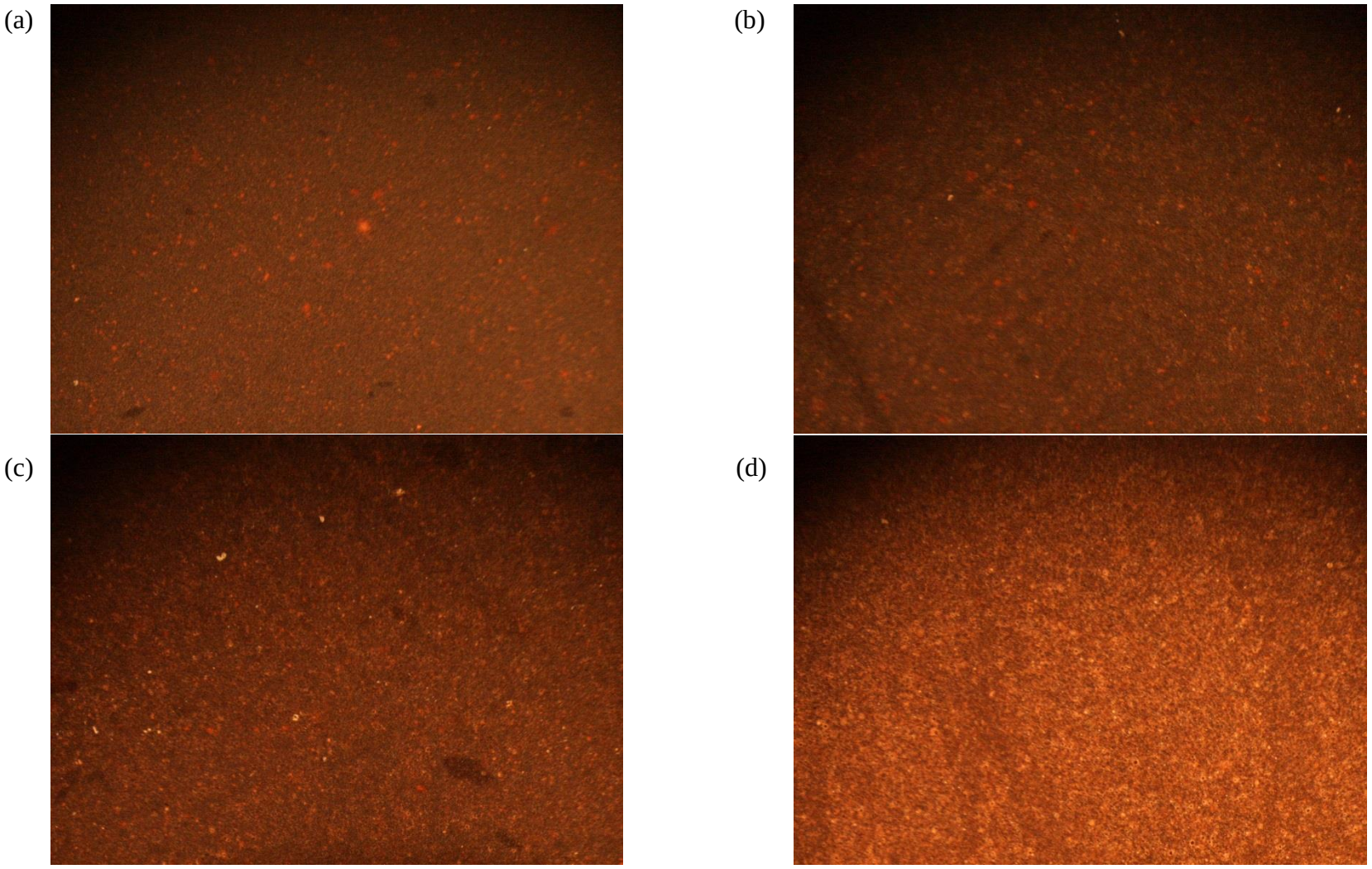


Figure 4. 26 Representative pictures from stereomicroscope at day 28 on the surfaces of chocolate model systems of 50% cocoa butter and 50% particles, where cocoa powder was gradually replaced by corn syrup solids at different levels on a volume basis: (a) 0%, (b) 25%, (c) 50%, (d) 75%.

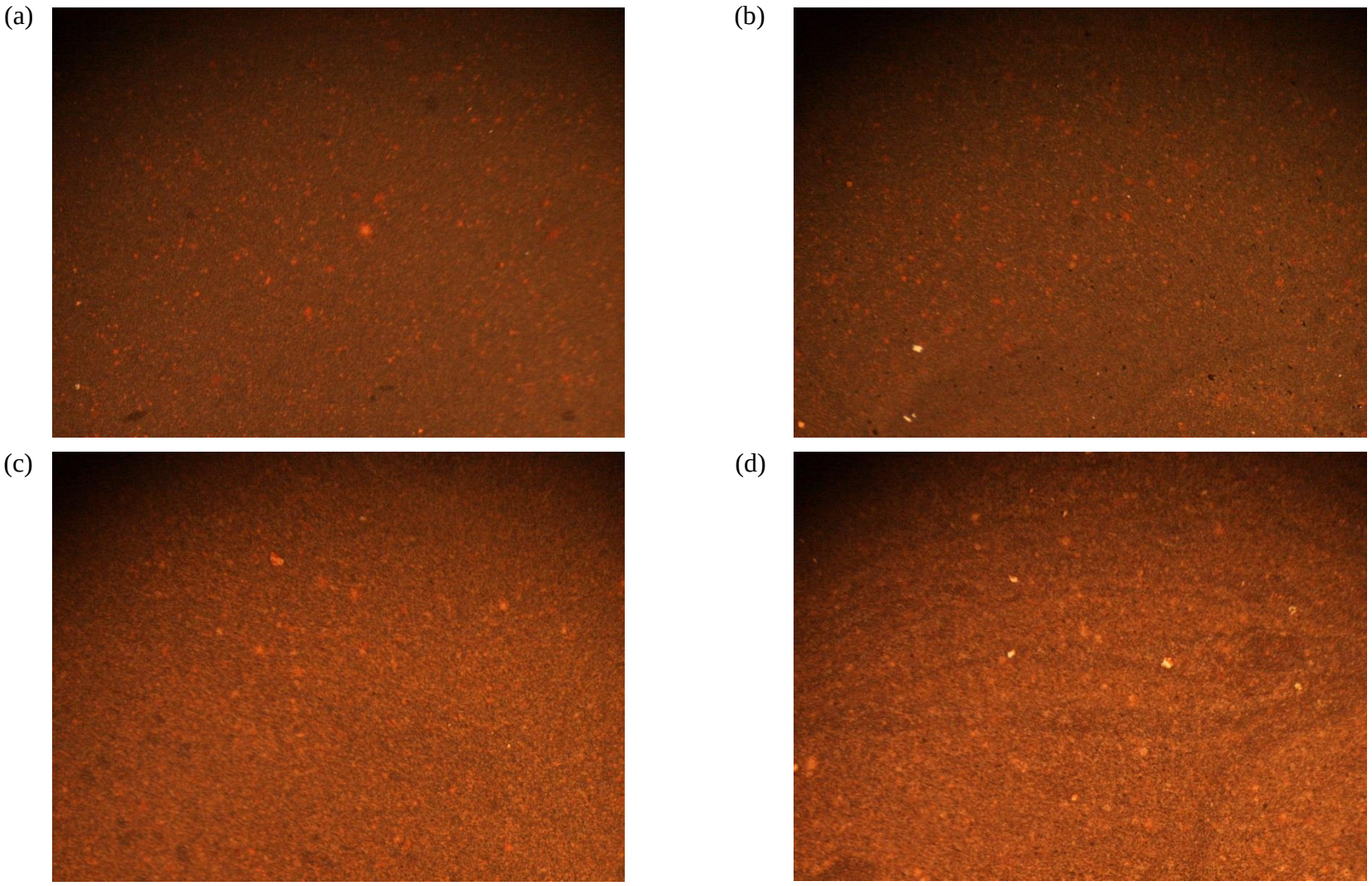


Figure 4. 27 Representative pictures from stereomicroscope at day 28 on the surfaces of chocolate model systems of 50% cocoa butter and 50% particles, where cocoa powder was gradually replaced by polydextrose at different levels on a volume basis: (a) 0%, (b) 25%, (c) 50%, (d) 75%.

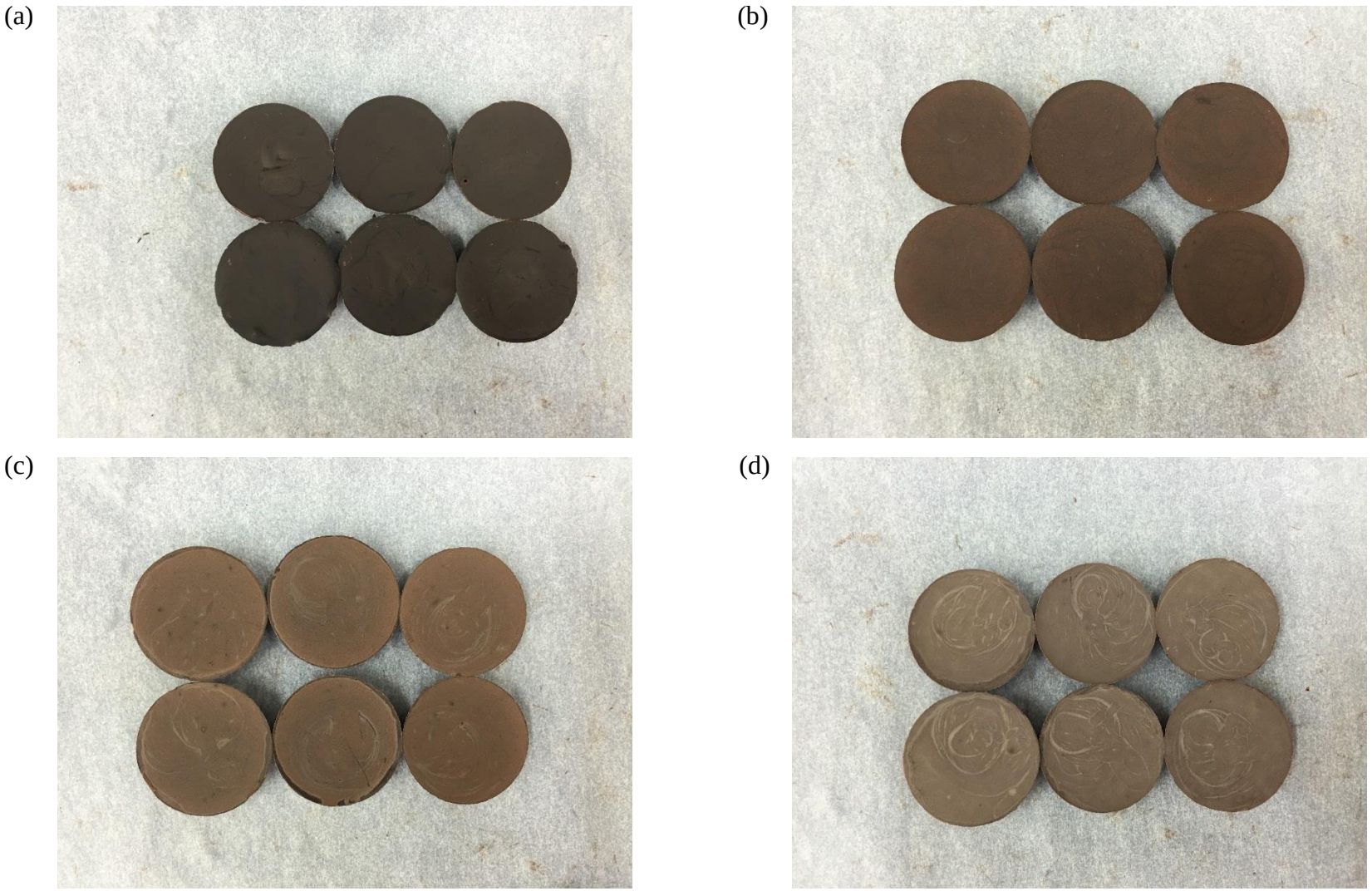


Figure 4. 28 Pictures at day 28 of the surfaces of chocolate model systems of 50% cocoa butter and 50% particles, where cocoa powder was gradually replaced by sucrose particles at different levels on a volume basis: (a) 0%, (b) 25%, (c) 50%, (d) 75%.

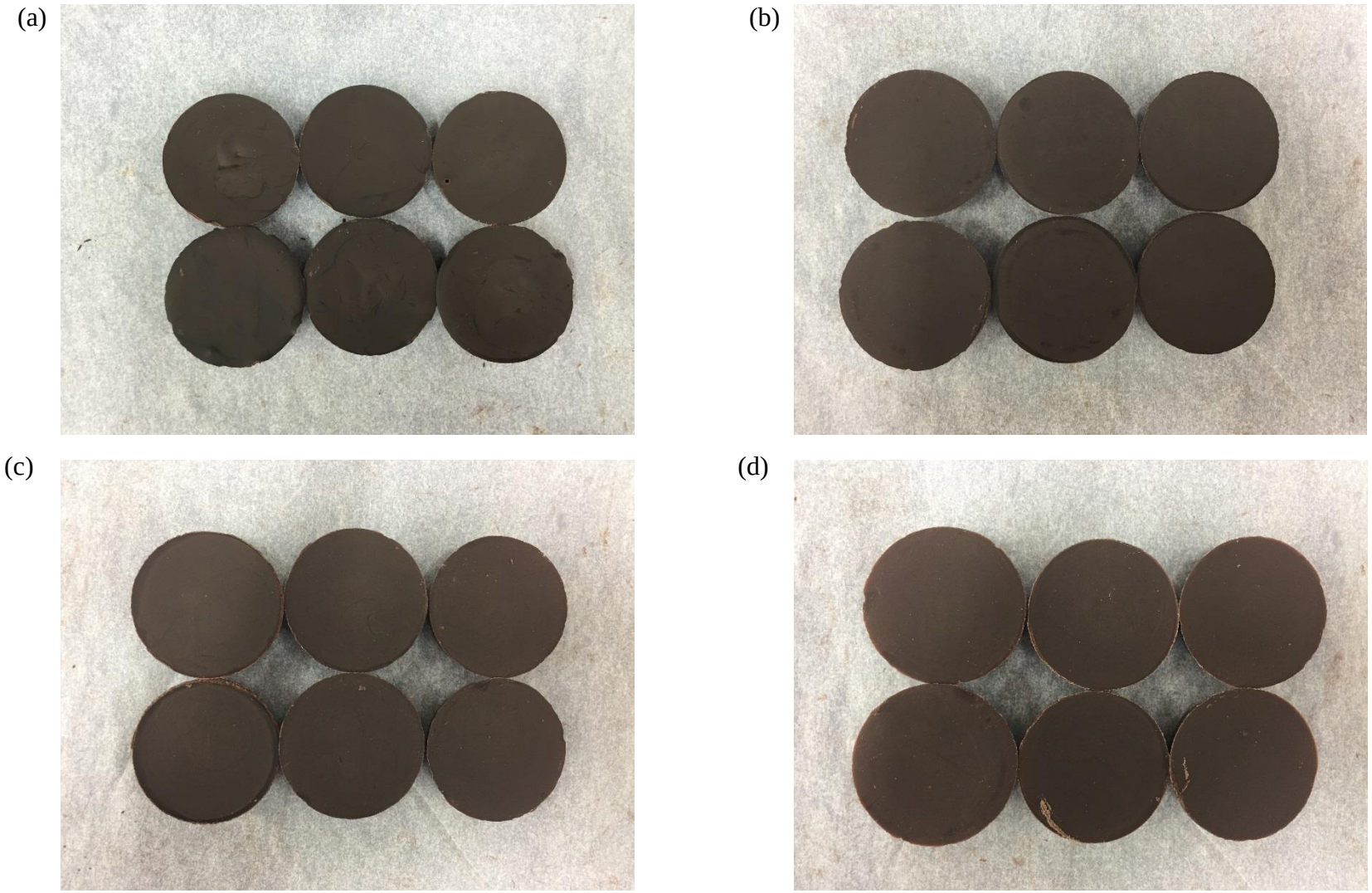


Figure 4. 29 Pictures at day 28 of the surfaces of chocolate model systems of 50% cocoa butter and 50% particles, where cocoa powder was gradually replaced by maltitol particles at different levels on a volume basis: (a) 0%, (b) 25%, (c) 50%, (d) 75%.

(a)

(b)

(c)

(d)

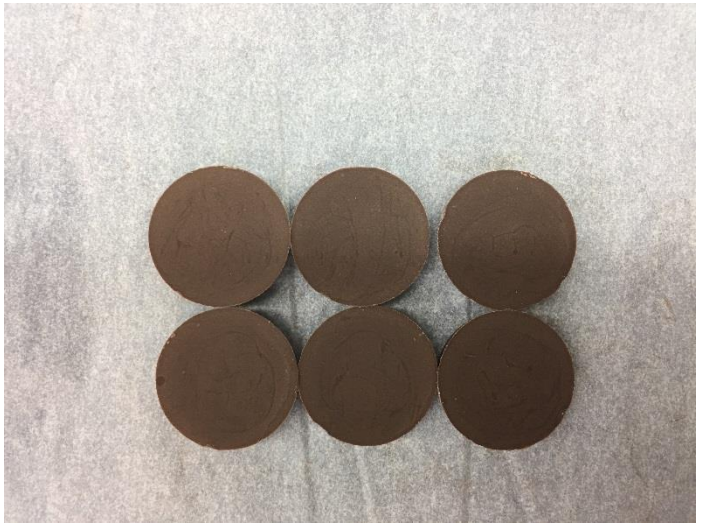
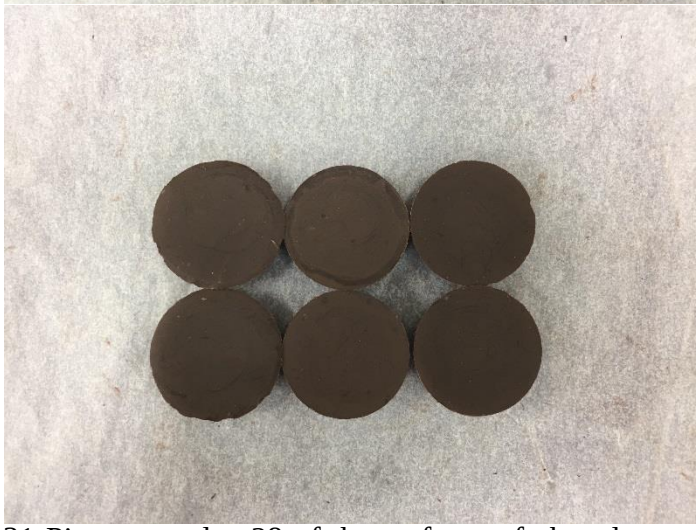


Figure 4. 30 Pictures at day 28 of the surfaces of chocolate model systems of 50% cocoa butter and 50% particles, where cocoa powder was gradually replaced by corn syrup solids at different levels on a volume basis: (a) 0%, (b) 25%, (c) 50%, (d) 75%.

(a)

(b)

(c)



(d)

Figure 4. 31 Pictures at day 28 of the surfaces of chocolate model systems of 50% cocoa butter and 50% particles, where cocoa powder was gradually replaced by polydextrose at different levels on a volume basis: (a) 0%, (b) 25%, (c) 50%, (d) 75%.

From the ΔWI results, among model systems with different type of sugar, the control samples (100% cocoa powder) were the same. They all showed a slight increase in ΔWI at day 1 and reached a plateau with a very low ΔWI (about 2) within two to three weeks.

The effects of different sugars in model systems with different sugar concentrations on ΔWI were very different (Figure 4.21). With sucrose, the system with 25% (v/v, referenced to the total particle volume) cocoa powder replacement began to show an obvious rise in ΔWI at day 1 and reached a plateau at about 5 ΔWI at day 28 (Figure 4.21a). The initial bloom rate and the ΔWI values over time were lowest among the three systems, but still higher than the control system (0% cocoa powder replacement. The system with 50% (v/v, referenced to the total particle volume) cocoa powder replacement began to show an obvious rise in ΔWI at day 1 and reached a plateau at about 12 ΔWI at day 28. The initial bloom rate and the ΔWI values over time were midway among between 25% and 50% cocoa powder replacement systems. The system with 75% (v/v, referenced to the total particle volume) cocoa powder replacement began to show an obvious rise in ΔWI at day 1 and reached a plateau at about 17 ΔWI at day 28. The initial bloom rate and the ΔWI values over time were the highest among the three sucrose addition levels. In general, sucrose promoted bloom at all levels with higher sucrose concentrations showing faster bloom, higher bloom rate and ΔWI values during storage, resulting in higher final ΔWI values.

With maltitol, the system with 25% (v/v, referenced to the total particle volume) cocoa powder replacement began to show an obvious rise in ΔWI at day 7, bloomed slowly over time and had not reached a plateau at day 28 (Figure 4.21b). The final ΔWI value (around 3) was midway among 50% and 75% cocoa powder replacement systems. The system with 50% (v/v,

referenced to the total particle volume) cocoa powder replacement began to show an obvious rise in ΔWI at between day 7 and 14, bloomed slowly over time and had not reached a plateau at day 28, with the highest final ΔWI value (around 4). The system with 75% (v/v, referenced to the total particle volume) cocoa powder replacement did not show obvious rise in ΔWI until day 14, bloomed slowly over time and reached a plateau at about 2 ΔWI at day 28. The final ΔWI value was smallest among the three system with maltitol and close to that of the control sample. The differences in the bloom behavior and the final ΔWI values among different systems were extremely small. In general, systems with any level of maltitol crystals did not show significant bloom over time.

With CSS, the system with 25% (v/v, referenced to the total particle volume) cocoa powder replacement began to show an obvious rise in ΔWI at day 7, bloomed slowly over time and reached a plateau at about 1.5 ΔWI at day 28 (Figure 4.21c). The final ΔWI value was smallest among the three systems with CSS, and was even lower than the control system. The system with 50% (v/v, referenced to the total particle volume) cocoa powder replacement began to show an obvious rise in ΔWI at day 7, bloomed slowly over time and reached a plateau about 2 ΔWI at day 21. The final ΔWI value was midway among 25% and 75% replacement systems and was similar to the ΔWI level in the control system. The system with 75% (v/v, referenced to the total particle volume) cocoa powder replacement began to show an obvious rise in ΔWI at day 7, bloomed quickly over time and had not reached a plateau at day 28, with the highest final ΔWI value (about 7). In sum, systems with low CSS levels (25% and 50% cocoa powder replacement level) did not show significant bloom over time (25% level system even showed

a bloom reduction effect); whereas 75% level system showed significant bloom, but at a relatively lower extent compared to sucrose.

With PD model systems, the system with 25% (v/v, referenced to the total particle volume) began to show an obvious rise in ΔWI at day 7, bloomed slowly over time and reached a plateau at about 3 ΔWI at day 28 (Figure 4.21d). The final ΔWI value was lowest among three systems with PD and was slightly higher than the control system. The system with 50% (v/v, referenced to the total particle volume) began to show an obvious rise in ΔWI at day 7 and reached a plateau at about 5 ΔWI at day 21. The model system with 75% (v/v) PD began to show obvious rise in ΔWI at day 7, bloomed quickly over time and reached a plateau at about 5 ΔWI at day 28. In general, systems with higher PD concentrations started to bloom faster, with higher bloom rate and ΔWI values during storage, resulting in higher final ΔWI values. All three PD replacement levels had higher final ΔWI values than the control. The 25% cocoa powder replacement system did not show significant bloom, and the bloom extent was similar to the control; whereas, the 50% and 75% cocoa powder replacement systems, with similar final ΔWI values, had more significant bloom extents, but they were still relatively small compared to the sucrose systems.

The white area percentage (Figure 4.22) and visual bloom (Figure 4.23) results showed almost the same trends. Comparing bloom results in model systems with different sugar types, sucrose had a significant promotion effect in all model systems with the highest bloom extent. Higher CSS or PD concentrations (75% level in CSS systems as well as 50 and 75% levels in PD systems) showed some significant bloom, but the bloom extents were small compared to

sucrose systems. Systems with maltitol had no significant bloom at any replacement level, with the lowest bloom extent of all particles tested.

Statistical analyses were conducted on final ΔWI, white area percentage, and visual bloom values at day 28 in all model systems, as shown in Table 4.6. Two-way ANOVA on the final ΔWI values, white area percentage, and visual rate, respectively, with 4 levels of cocoa powder replacement level and 4 levels of sugar types showed that cocoa powder replacement level had a significant effect on the final bloom values ($p<0.0001$ for all of the three tests). Tukey HSD showed that chocolate model systems with 0, 25, 50 and 75% cocoa powder replacement levels were all significantly different from each other, with higher replacement levels having higher ΔWI, white area percentage or visual bloom values.

Also, two-way ANOVA showed that sugar type had a significant effect on the final bloom extents at day 28 ($p<0.0001$ for all of the three tests) as well. Tukey HSD on ΔWI results showed that model systems with sucrose and PD had significantly higher ΔWI values than CSS and maltitol systems and ΔWI values in sucrose systems were significantly higher than PD systems. CSS and maltitol systems presented the lowest ΔWI values and there was no significant difference between them. Tukey HSD on white area percentage showed that model system with sucrose had the highest final whitish area, whereas maltitol had the lowest final whitish area among the systems with four sugar types. White area in CSS and PD systems were in the midway and there was no significant difference between them. Tukey HSD on visual bloom results showed that model system with sucrose had the highest final visual level, PD had the second highest, whereas CSS had the lowest final visual bloom level among the systems with four sugar types. Visual bloom levels in maltitol were midway among CSS and PD

systems. There were no significant differences between CSS and maltitol systems, as well as between maltitol and PD systems.

Table 4. 6 Changes in whiteness index (ΔWI), white area percentage, and visual bloom values of chocolate model systems (50% cocoa butter and 50% particles) with different cocoa powder replacement levels (0, 25, 50, and 75% on a volume basis) and different sugar types (sucrose, maltitol, CSS and PD) at day 28. Data represent means ± standard deviations.

Bloom Evaluation	Sugar type	Cocoa powder replacement level (%)			
		0	25	50	75
ΔWI	Sucrose	2.1 ± 0.2^{gh}	4.7 ± 0.7^{de}	11.7 ± 0.8^{b}	17.1 ± 1.3^{a}
	Maltitol	2.1 ± 0.2^{gh}	3.1 ± 0.1^{fg}	3.7 ± 0.4^{ef}	2.2 ± 0.5^{gh}
	CSS	2.1 ± 0.2^{gh}	1.5 ± 0.3^{h}	2.2 ± 0.6^{gh}	6.7 ± 0.9^{c}
	PD	2.1 ± 0.2^{gh}	2.8 ± 0.5^{fgh}	4.9 ± 0.6^{de}	5.0 ± 1.3^{d}
White Area (%)	Sucrose	10.5 ± 1.5^{FG}	70.0 ± 5.0^{C}	85.0 ± 1.1^{AB}	93.5 ± 0.6^{A}
	Maltitol	10.5 ± 1.5^{FG}	11.3 ± 2.8^{FG}	20.7 ± 1.8^{F}	21.1 ± 2.9^{F}
	CSS	10.5 ± 1.5^{FG}	3.5 ± 0.9^{G}	12.5 ± 8.3^{FG}	77.7 ± 3.7^{BC}
	PD	10.5 ± 1.5^{FG}	11.1 ± 1.9^{FG}	37.6 ± 12.4^{E}	52.4 ± 15.1^{D}
Visual Bloom	Sucrose	$2.6 \pm 0.2^{\varepsilon\zeta\eta}$	$3.7 \pm 0.3^{\beta}$	$4.8 \pm 0.3^{\alpha}$	$5.0 \pm 0.0^{\alpha}$
	Maltitol	$2.6 \pm 0.2^{\varepsilon\zeta\eta}$	$2.7 \pm 0.3^{\varepsilon\zeta}$	$3.3 \pm 0.3^{\beta\gamma\delta}$	$3.0 \pm 0.0^{\gamma\delta\varepsilon}$
	CSS	$2.6 \pm 0.2^{\varepsilon\zeta\eta}$	$2.1 \pm 0.4^{\eta}$	$2.8 \pm 0.3^{\delta\varepsilon\zeta}$	$3.8 \pm 0.3^{\beta}$
	PD	$2.6 \pm 0.2^{\varepsilon\zeta\eta}$	$2.3 \pm 0.3^{\zeta\eta}$	$3.50 \pm 0.4^{\beta\gamma}$	$3.8 \pm 0.5^{\beta}$

$^{a,b,c,d,e,f,g,h;\ A,B,C,D,E,F,G;\ \alpha,\beta,\gamma,\delta,\varepsilon,\zeta,\eta}$ Means not connected with the same letter within each evaluation method across both cocoa butter replacement level and sugar type are significantly different (α=0.05).
CSS: corn syrup solids; PD: polydextrose.

Further, there was also a significant effect from the interaction of cocoa powder replacement level and sugar type ($p<0.0001$ for all of the three tests), as analyzed by two-way ANOVA. This means that the effect of cocoa powder replacement level depends on the sugar type and might be different under different scenarios of sugar type.

4.3.1.2 Effect of lecithin with different sucrose fraction

Lecithin was added at 0.5% (referenced to total mass in the control system) level in model systems with sucrose, and different sucrose concentrations (0, 25, 50, and 75% on a volume basis) in the particle phase were used to gradually replace cocoa powder while still keeping the particle volume consistent. Lecithin was expected to have a reduction effect on bloom as it changes the microstructure of chocolate model system (Johansson and Bergenstahl, 1992a) as well as influencing CB crystallization (Lonchampt and Hartel, 2004). Figure 4.32 shows the progression of bloom represented by changes in whiteness index (ΔWI), white area percentage, and visual rate values in chocolate model systems of 50% CB, 50% particles and 0.5% lecithin (referenced to total mass in the control model system), where cocoa powder was replaced by sucrose at different levels (0, 25, 50, and 75%) on a volume basis. Figure 4.33 shows representative pictures from stereomicroscope on the surfaces of these chocolate model systems at day 28 and Figure 4.34 shows pictures taken directly by digital camera of these chocolate samples at day 28.

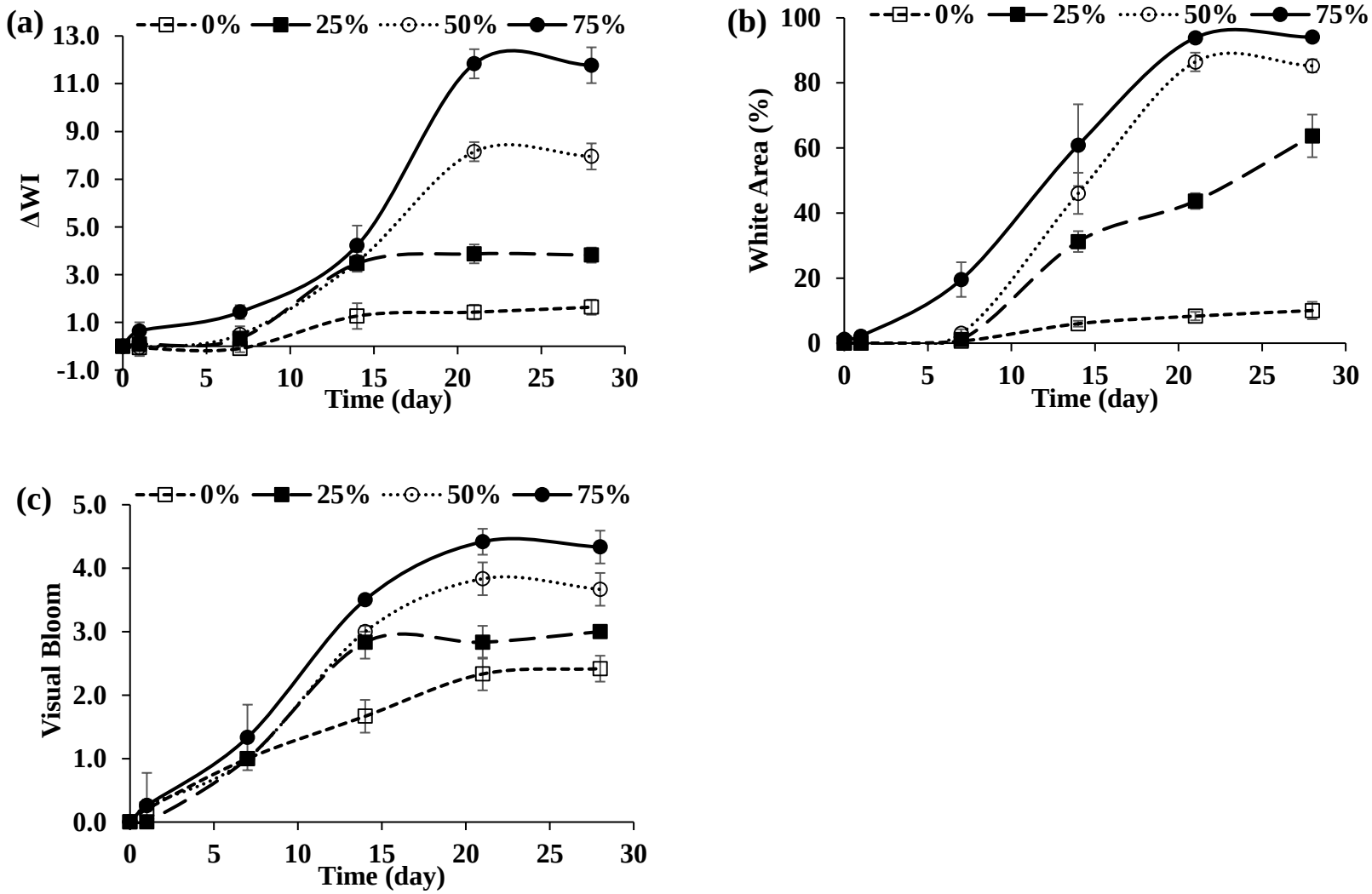


Figure 4. 32 Bloom progression represented by changes in: (a) whiteness index (ΔWI), (b) white area percentage, (c) visual bloom levels in chocolate model systems of 50% cocoa butter, 50% particles and 0.5% lecithin (referenced to total mass in the control model system system), where cocoa powder was replaced by sucrose at different levels (0, 25, 50, and 75%) on a volume basis.

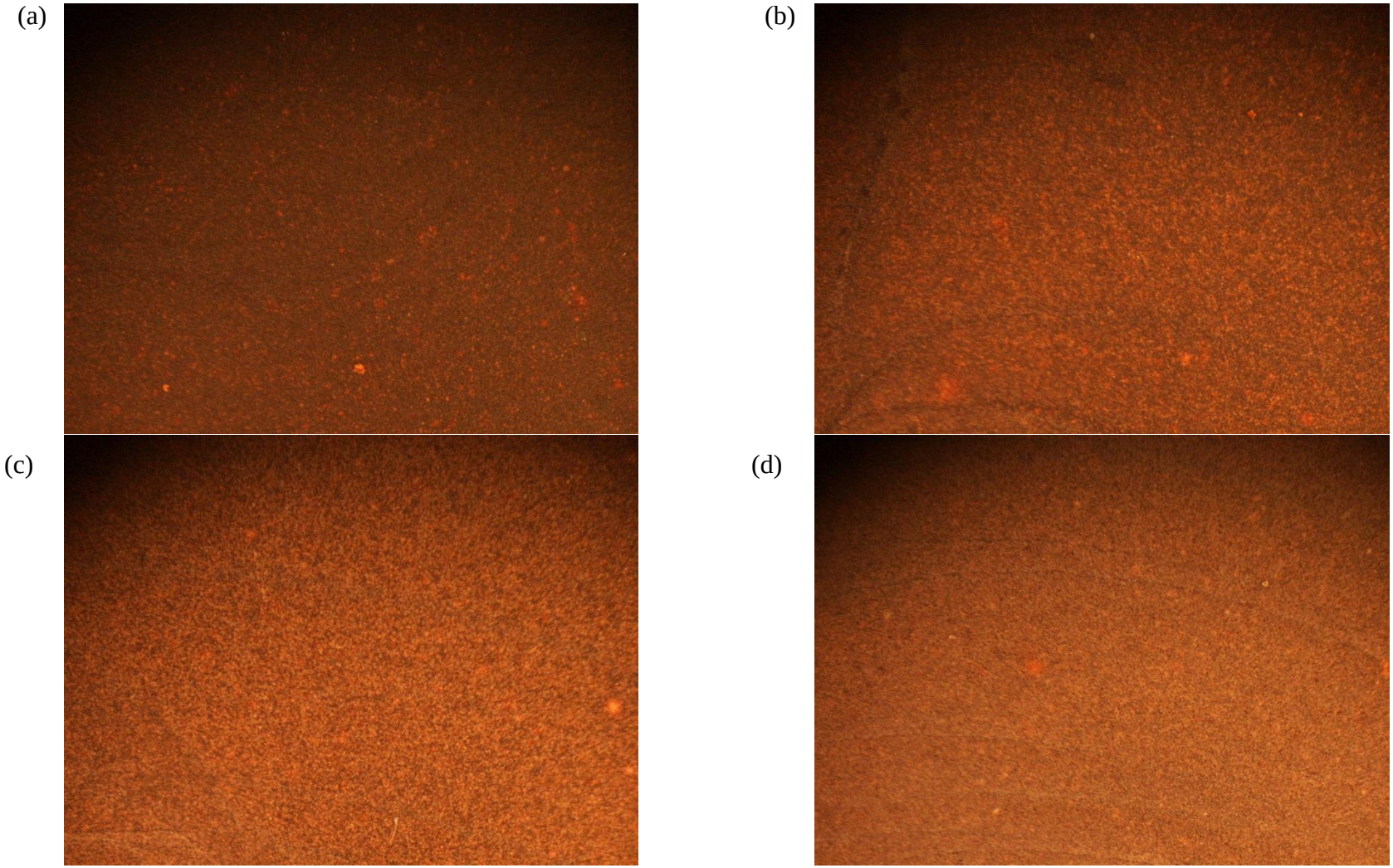


Figure 4. 33 Representative pictures from stereomicroscope at day 28 on the surfaces of chocolate model systems of 50% cocoa butter, 50% particles and 0.5% lecithin (referenced to total mass in the control model system system), where cocoa powder was replaced by sucrose at different levels on a volume basis: (a) 0%, (b) 25%, (c) 50%, (d) 75%.

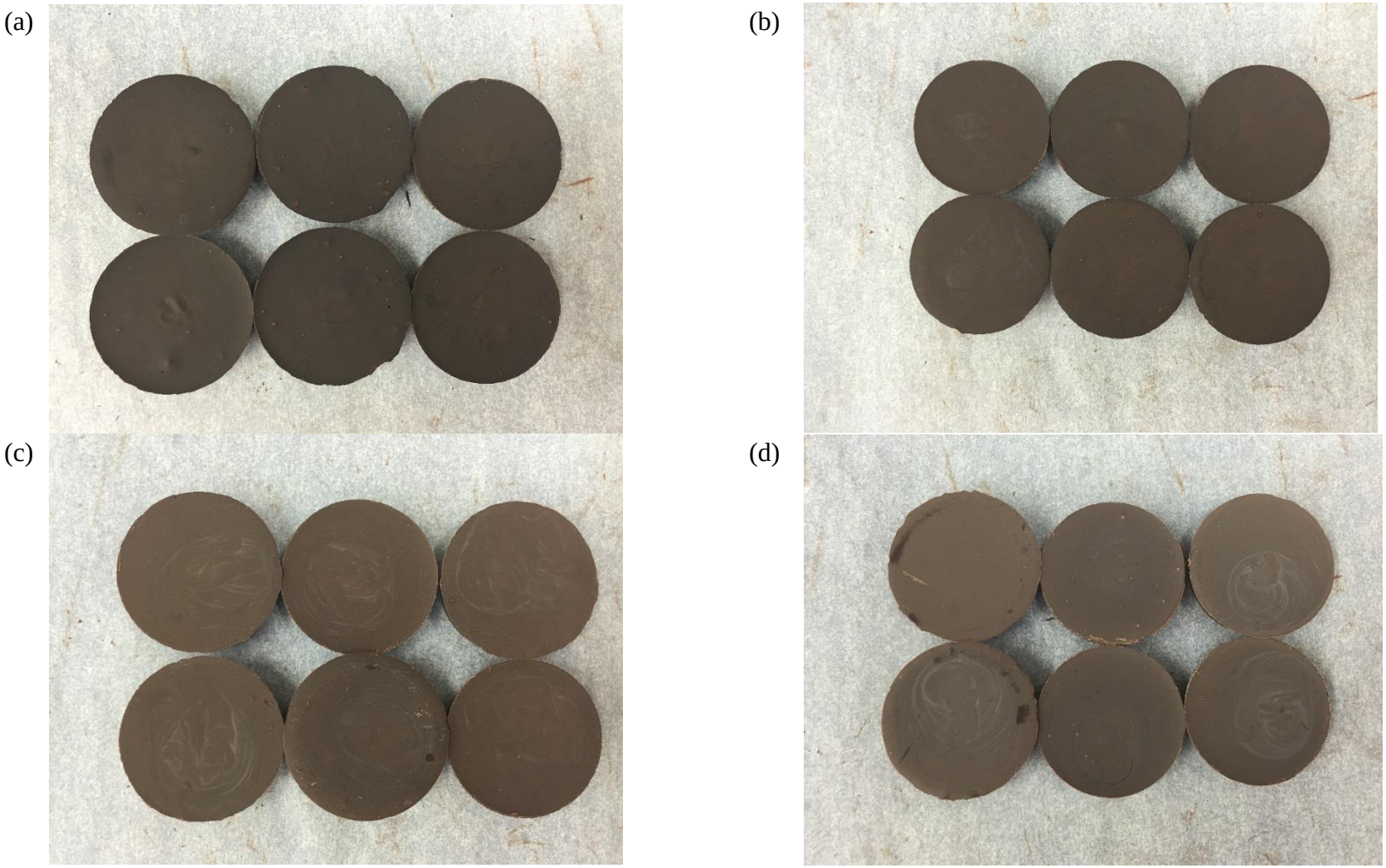


Figure 4. 34 Pictures at day 28 on the surfaces of chocolate model systems of 50% cocoa butter, 50% particles and 0.5% lecithin (referenced to total mass in the control model system system), where cocoa powder was replaced by sucrose at different levels on a volume basis: (a) 0%, (b) 25%, (c) 50%, (d) 75%.

With 0.5% lecithin, the system with 0% (v/v) sucrose (referenced to the total particle volume) began to show obvious rise in ΔWI at day 14, bloomed slowly over time and reached a plateau at about 1.5 ΔWI at day 28. This control had the lowest initial bloom rate and ΔWI values compared to the systems with different cocoa powder replacement level (0, 25, 50, and 75%) and 0.5% lecithin. The system with 25% (v/v) sucrose began to show an obvious rise in ΔWI at day 7, bloomed moderately over time and reached a plateau at about 4 ΔWI at day 21, with ΔWI values higher than the 0% system. The system with 50% (v/v) sucrose began to show an obvious rise in ΔWI at day 7, bloomed quickly over time and reached a plateau at about 8 ΔWI at day 28, with ΔWI values higher than the 25% system. The initial bloom rates in the 25% and 50% cocoa powder replacement systems were similar. The system with 75% (v/v) sucrose began to show an obvious rise in ΔWI at day 1, bloomed quickly over time and reached a plateau at about 12 ΔWI at day 28, with the highest initial bloom rate and ΔWI values of the samples. The white area percentage (Figure 4.32b) and visual bloom (Figure 4.32c) values showed quite similar results.

As in the case with no lecithin, addition of sucrose promoted bloom in all model systems; chocolate model systems with higher sucrose concentrations started to bloom faster, with higher bloom rate and bloom extent during storage, resulting in higher final ΔWI, white area percentage and visual bloom values. However, comparing to the model systems with sucrose where no lecithin was added, lecithin had a reduction effect on bloom formation in all model systems with different sugar concentrations (Table 4.7). Statistical analyses were conducted on final ΔWI, white area percentage and visual bloom values at day 28 in all model systems, as shown in Table 4.7. Two-way ANOVA on the final ΔWI values at day 28 with 2 levels of

Table 4. 7 Changes in whiteness index (ΔWI), white area percentage and visual bloom values at day 28 in chocolate model systems (50% cocoa butter and 50% particles), where cocoa powder was replaced by sucrose at 0, 25, 50, and 75% level (v/v) and with 0 or 0.5% lecithin (referenced to total mass in the control system). Data represent means ± standard deviations.

Bloom Evaluation	Cocoa powder replacement level (%)	Lecithin concentration (%)	
		0	0.5
ΔWI	0	2.1 ± 0.2^{e}	1.6 ± 0.3^{e}
	25	4.7 ± 0.7^{d}	3.8 ± 0.3^{d}
	50	11.7 ± 0.8^{b}	8.0 ± 0.5^{c}
	75	17.1 ± 1.3^{a}	11.8 ± 0.8^{b}
White Area (%)	0	10.5 ± 1.5^{E}	10.0 ± 2.7^{E}
	25	70.0 ± 5.0^{C}	63.7 ± 6.6^{D}
	50	85.0 ± 1.1^{B}	85.3 ± 2.0^{B}
	75	93.5 ± 0.6^{A}	94.1 ± 0.4^{A}
Visual Bloom	0	$2.6 \pm 0.2^{\varepsilon}$	$2.4 \pm 0.2^{\varepsilon}$
	25	$3.7 \pm 0.3^{\gamma}$	$3.0 \pm 0.0^{\delta}$
	50	$4.8 \pm 0.3^{\alpha}$	$3.7 \pm 0.3^{\gamma}$
	75	$5.0 \pm 0.0^{\alpha}$	$4.3 \pm 0.3^{\beta}$

$^{a,b,c,d,e;\ A,B,C,D,E;\ \alpha,\beta,\gamma,\delta,\varepsilon.}$ Means not connected with the same letter within each evaluation method across both cocoa butter replacement level and lecithin concentration are significantly different (α=0.05).

lecithin concentration and 4 levels of sucrose fraction showed that the presence of lecithin had a significant effect on the final ΔWI values ($p<0.0001$). Tukey HSD showed that chocolate model systems with 0.5% lecithin were significantly different from systems without lecithin. Model systems with 0.5% lecithin had significantly lower final ΔWI values. The same statistical test on visual bloom showed the same result ($p<0.0001$ respectively). But the test on white area percentage did not show significant effect from addition of 0.5% lecithin ($p=0.1153$). On the other hand, two-way ANOVA showed that sugar concentration had a significant effect on the final ΔWI, white area percentage and visual rate results at day 28 ($p<0.0001$ for all of the three tests) as well. Tukey HSD showed that chocolate model systems with 0, 25, 50 and

75% cocoa powder replacement levels were all significantly different from each other; higher replacement levels presented higher ΔWI, white area percentage and visual rate values. Further, there was also a significant effect from the interaction of lecithin concentration and sugar concentration ($p<0.0001$ for tests on ΔWI and visual rate; $p=0.0425$ for the test on white area percentage), as analyzed by two-way ANOVA. This means the effect of sugar concentration is dependent on the effect of lecithin level and would be different with or without lecithin.

4.3.1.3 Effect of lecithin with different types of sugar particles

Four types of sugar (including sucrose, maltitol, CSS and PD) were added in the model systems with 50% CB and 50% particles where cocoa powder was replaced by sugar at 50% level (v/v), and two different lecithin concentrations (0 and 0.5% referenced to total mass in the control system) were used to study the effect of lecithin with different sugar type. Again, lecithin was expected to have a reduction effect on bloom extents. Figures 4.35 to 4.37 show the progression of bloom represented by changes in whiteness index (ΔWI), white area percentage, and visual bloom values, respectively, in these chocolate model systems. To note, the curves for sucrose systems were the same data from Section 4.3.1.1 and Section 4.3.1.2 and were included for better comparison. Figure 4.38 shows representative pictures from stereomicroscope on the surfaces of these chocolate model systems at day 28 and Figure 4.39 show pictures taken directly by digital camera of these chocolate samples at day 28.

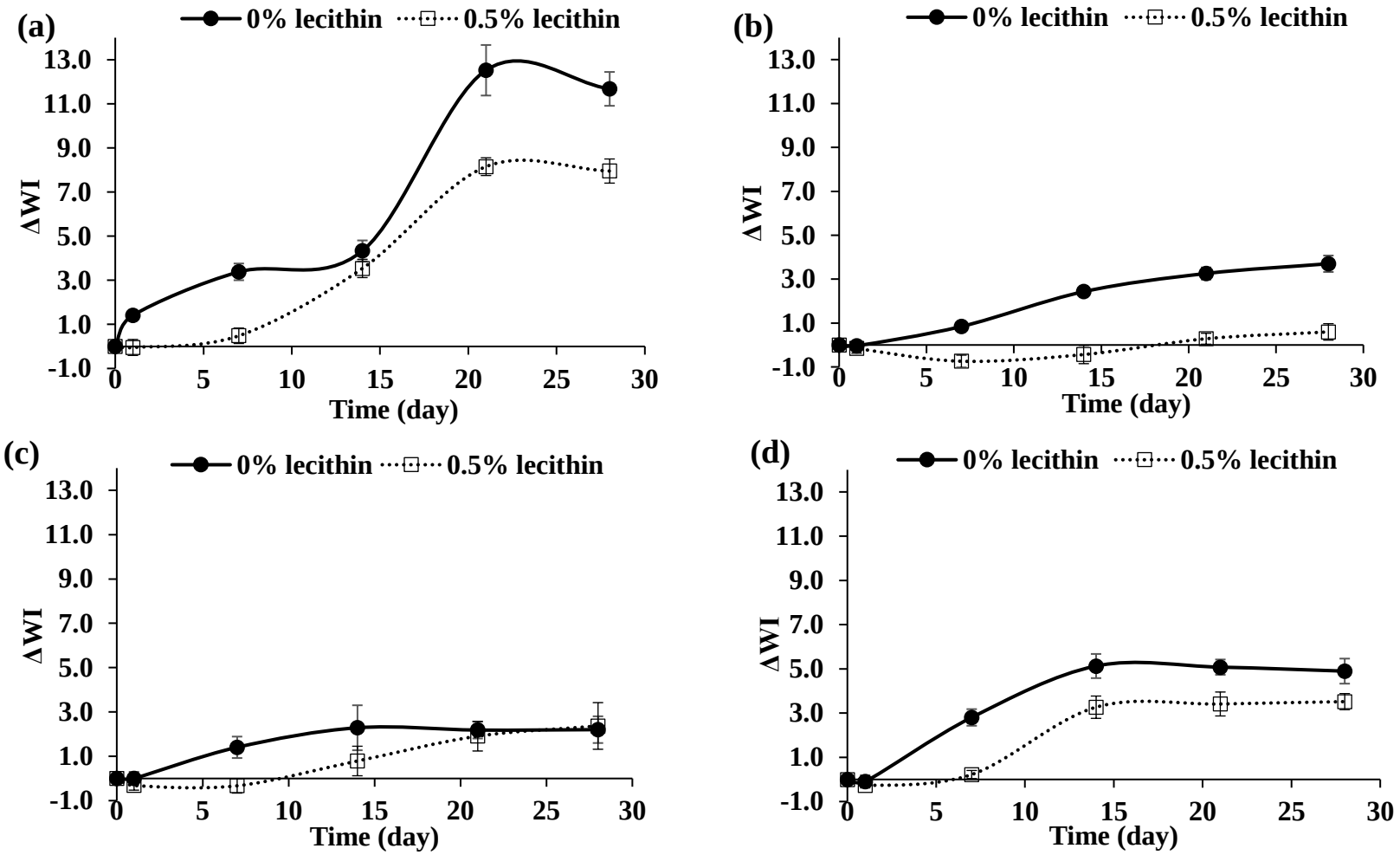


Figure 4. 35 Bloom progression represented by changes in whiteness index (ΔWI) values in chocolate model systems of 50% cocoa butter and 50% particles, where cocoa powder was replaced by: (a)sucrose, (b) maltitol, (c) corn syrup solids, (d) polydextrose at 50% level (v/v) and different lecithin concentrations (0 and 0.5% referenced to total mass in the control system).

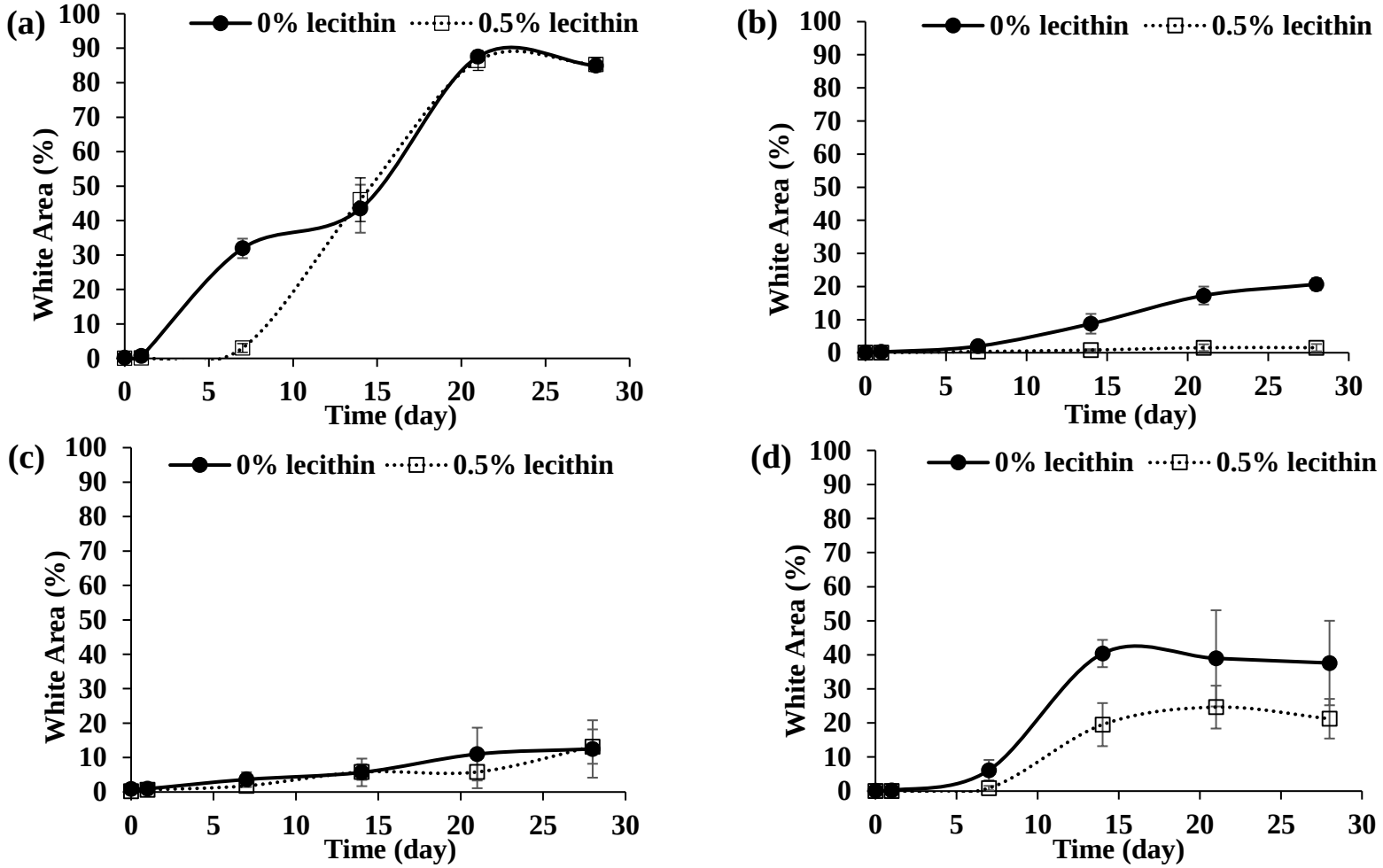


Figure 4. 36 Bloom progression represented by white area percentage on the surface of chocolate model systems of 50% cocoa butter and 50% particles, where cocoa powder was replaced by: (a) sucrose, (b) maltitol, (c) corn syrup solids, (d) polydextrose at 50% level (v/v) and different lecithin concentrations (0 and 0.5% referenced to total mass in the control system).

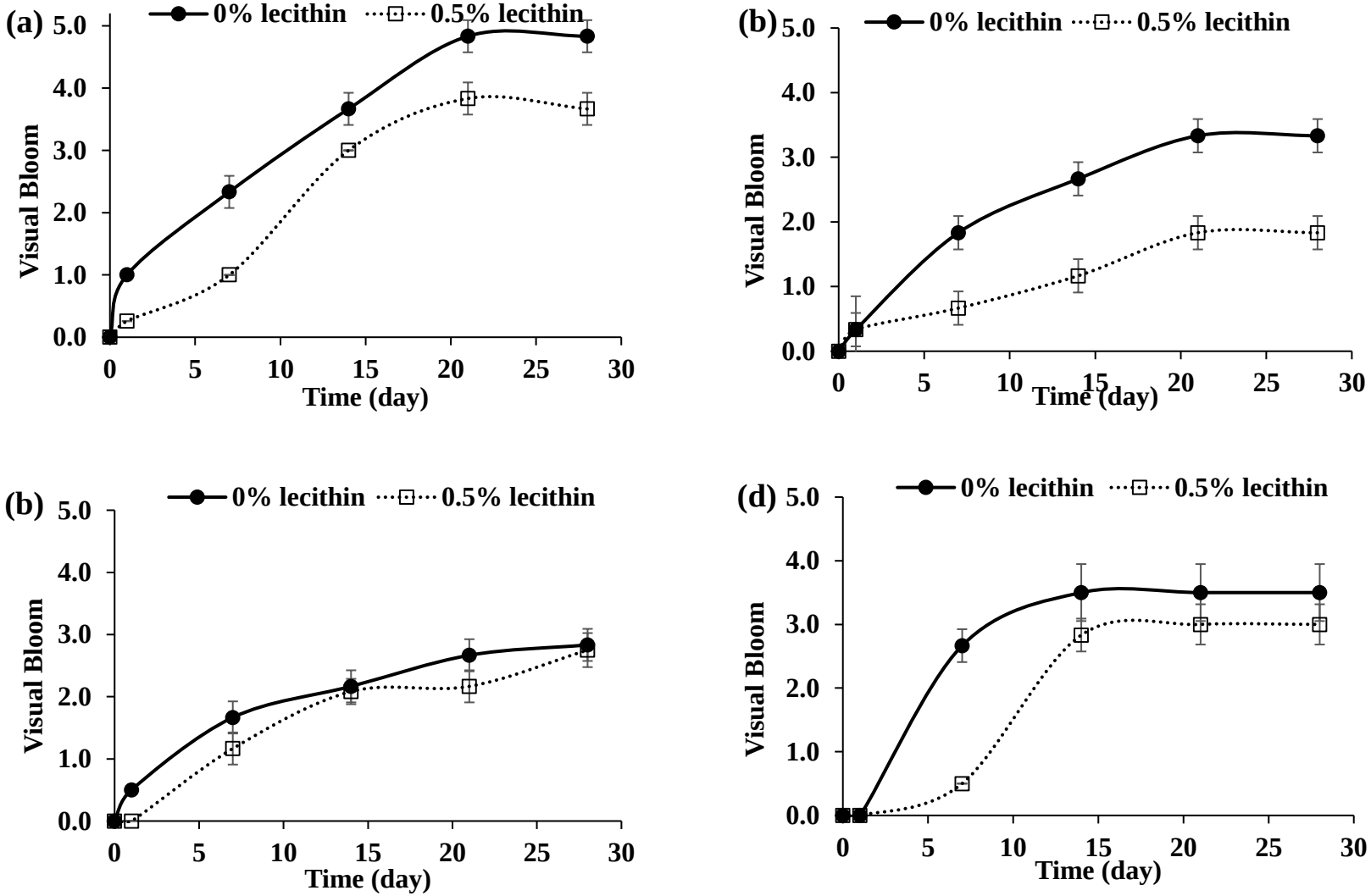


Figure 4. 37 Bloom progression represented by visual bloom of the surface of chocolate model systems of 50% cocoa butter and 50% particles, where cocoa powder was replaced by: (a) sucrose, (b) maltitol, (c) corn syrup solids, (d) polydextrose at 50% level (v/v) and different lecithin concentrations (0 and 0.5% referenced to total mass in the control system).

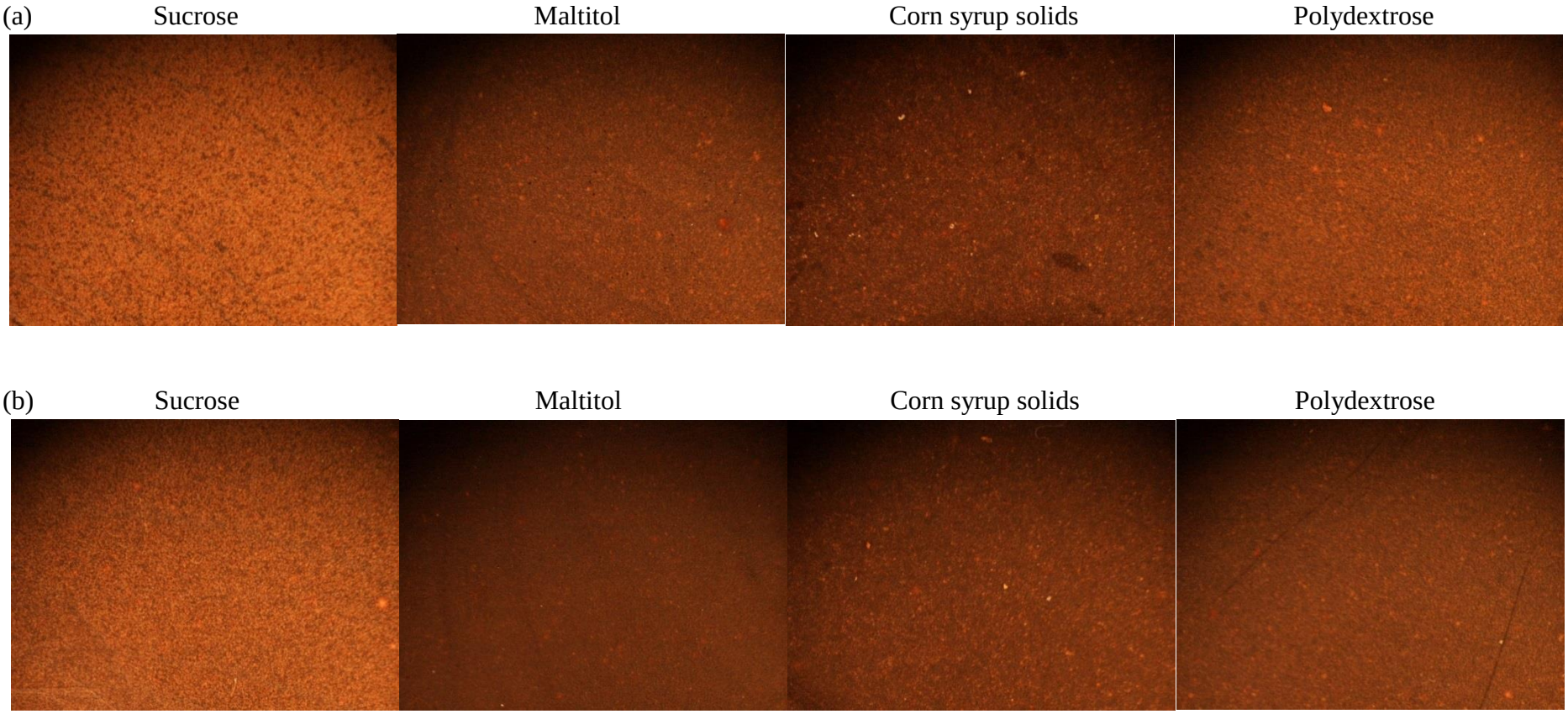


Figure 4. 38 Representative pictures from stereomicroscope at day 28 on the surfaces of chocolate model systems of 50% cocoa butter, 50% particles, and different lecithin concentrations (referenced to total mass in the control system): (a) 0% and (b) 0.5%, where cocoa powder was replaced by sucrose, maltitol, corn syrup solids, or polydextrose by 50% on a volume basis.

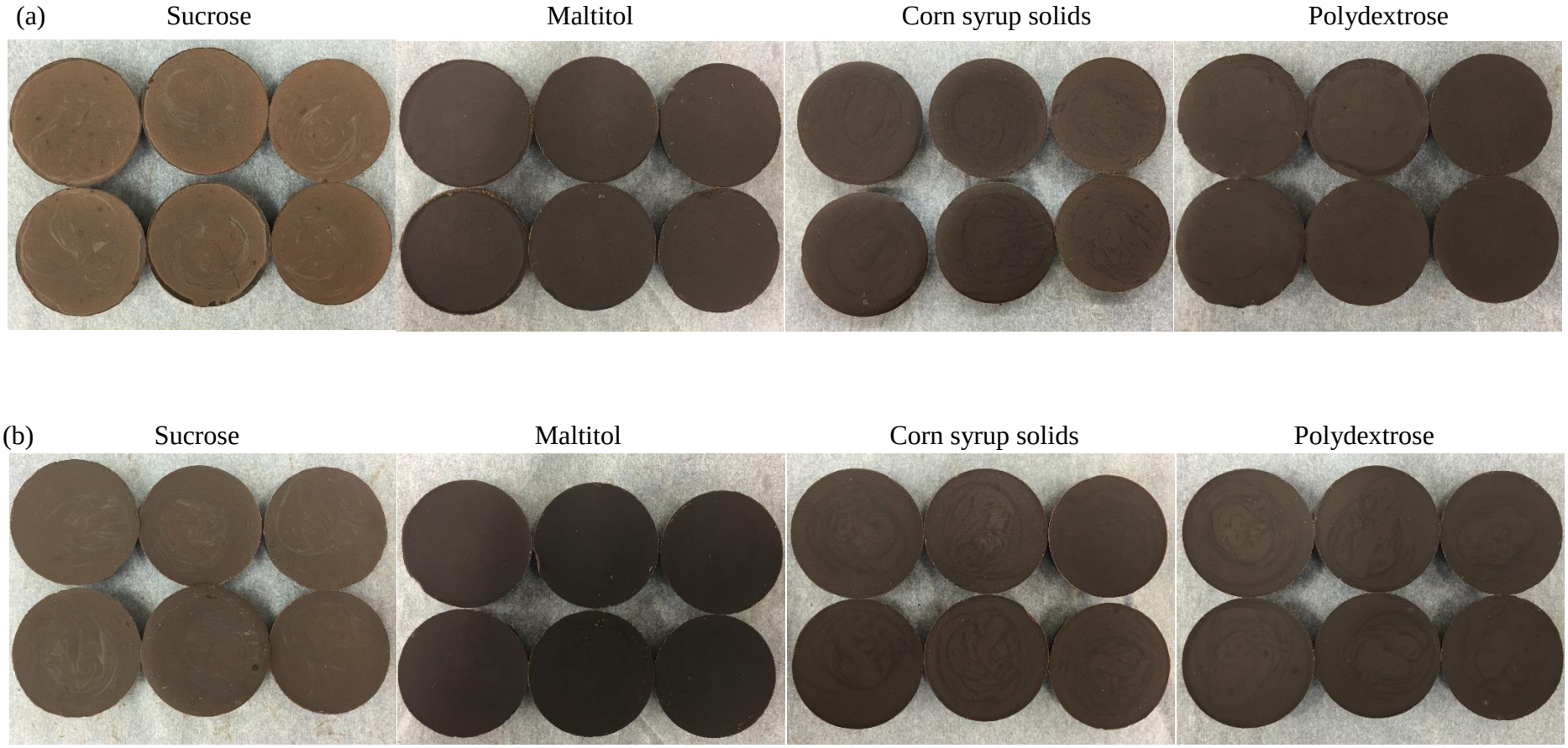


Figure 4. 39 Pictures at day 28 on the surfaces of chocolate model systems on the surfaces of chocolate model systems of 50% cocoa butter, 50% particles, and different lecithin concentrations (referenced to total mass in the control system): (a) 0% and (b) 0.5%, where cocoa powder was replaced by sucrose, maltitol, corn syrup solids, or polydextrose by 50% on a volume basis

For sucrose, the system with no lecithin began to show an obvious rise in ΔWI at day 1, bloomed quickly over time and reached a plateau at about 12 ΔWI at day 28. The sucrose system with 0.5% lecithin began to show an obvious rise in ΔWI at day 7, bloomed moderately over time and reached a plateau at about 8 ΔWI at day 28, with a lower initial bloom rate and the ΔWI values than the lecithin-free system. For maltitol, the system with no lecithin began to show an obvious rise in ΔWI at day 7, bloomed slowly over time and had not plateaued by day 28. The final ΔWI was around 4. The maltitol system with 0.5% lecithin generally did not show an obvious rise in ΔWI over time. For CSS, the system with no lecithin began to show an obvious rise in ΔWI at day 7, bloomed quickly over time and reached a plateau at about 2 ΔWI at day 21. The CSS system with 0.5% lecithin began to show an obvious rise in ΔWI at day 14, bloomed slowly over time and had not reached a plateau at day 28, with a lower initial bloom rate than the lecithin-free system. The final WI values in the two CSS systems, either with or without 0.5% lecithin, were almost the same. For PD, the system with no lecithin began to show an obvious rise in ΔWI at day 7, bloomed moderately over time and reached a plateau at about 5 ΔWI at day 21. The system with 0.5% lecithin began to show an obvious rise in ΔWI at day 14, bloomed moderately over time and reached a plateau at about 3.5 ΔWI at day 21, with a lower initial bloom rate and ΔWI values than the lecithin-free system. In sum, systems with no lecithin, compared to systems with 0.5% lecithin, started to bloom faster, had higher bloom rates and ΔWI values during storage, resulting in higher final ΔWI values, except for CSS systems. Generally speaking, expect for the model system with sucrose, bloom extents in model systems with the other three sugar types with 0.5% lecithin were all very low. The white area percentage and visual rate results showed similar trends.

Statistical analyses were conducted on final ΔWI, white area percentage and visual bloom values at day 28 in all model systems, as shown in Table 4.8. Two-way ANOVA on the final ΔWI, white area percentage, and visual bloom values at day 28 with 2 levels of lecithin concentration and 4 levels of sugar types showed that the presence of lecithin had a significant effect on the final bloom results ($p<0.0001$ for all three tests). Tukey HSD showed that, overall, chocolate model systems with 0.5% lecithin were significantly different from systems without lecithin. Overall, model systems with 0.5% lecithin had significantly lower bloom extents. This agreed with the results of the lecithin effect in Section 4.3.1.2.

Two-way ANOVA showed that sugar type had a significant effect on the final ΔWI , white area percentage and visual bloom results at day 28 ($p<0.0001$ for all three tests) as well. Tukey HSD of ΔWI showed that model systems with sucrose and PD had significantly higher ΔWI values than CSS and maltitol systems and ΔWI values in sucrose systems were significantly higher than PD systems. CSS and maltitol systems presented the lowest ΔWI values and there was no significant difference between them. Tukey HSD of white area percentage showed that model systems with sucrose and PD had significantly higher final white area percentage than CSS and maltitol systems; final white area percentage in sucrose systems were significantly higher than PD systems. CSS and maltitol systems resulted in the lowest final white area percentage and there was no significant difference between them. Tukey HSD of visual bloom showed that model systems with sucrose had the highest final visual bloom levels whereas PD had second highest; CSS and maltitol systems had the lowest final visual bloom levels and there was no significant difference between them.

Further, there was also a significant effect from the interaction of lecithin concentration and sugar type ($p<0.0001$ for tests on ΔWI and visual rate; $p=0.0002$ for the test on white area percentage), as analyzed by two-way ANOVA. This indicates that the effect of lecithin is dependent on the effect of sugar type and might be different among different sugars.

Table 4. 8 Changes in whiteness index (ΔWI), white area percentage, and visual bloom values at day 28 in chocolate model systems (50% cocoa butter and 50% particles), where cocoa powder was replaced by different sugars (sucrose, maltitol, CSS and PD) at 50% level (v/v) and with 0 or 0.5% lecithin (referenced to total mass in the control system). Data represent means ± standard deviations.

Bloom Evaluation	Sugar type	Lecithin concentration (%)	
		0	0.5
ΔWI	Sucrose	11.7 ± 0.8^{a}	8.0 ± 0.5^{b}
	Maltitol	3.7 ± 0.4^{d}	0.6 ± 0.4^{g}
	CSS	2.2 ± 0.6^{f}	2.4 ± 1.1^{ef}
	PD	4.9 ± 0.6^{c}	3.5 ± 0.4^{de}
White Area (%)	Sucrose	85.0 ± 1.1^{A}	85.3 ± 2.0^{A}
	Maltitol	20.7 ± 1.8^{D}	1.5 ± 1.1^{G}
	CSS	12.5 ± 8.3^{C}	13.2 ± 5.0^{D}
	PD	37.6 ± 12.4^{CD}	21.2 ± 5.8^{CD}
Visual Bloom	Sucrose	$4.8 \pm 0.3^{\alpha}$	$3.7 \pm 0.3^{\beta}$
	Maltitol	$3.3 \pm 0.3^{\beta\gamma\delta}$	$1.8 \pm 0.3^{\zeta}$
	CSS	$2.8 \pm 0.3^{\delta\varepsilon}$	$2.8 \pm 0.3^{\varepsilon}$
	PD	$3.5 \pm 0.4^{\beta\gamma}$	$3.0 \pm 0.3^{\gamma\delta\varepsilon}$

$^{a,b,c,d,e,f,g;\ A,B,C,D,E,F,G;\ \alpha,\beta,\gamma,\delta,\varepsilon,\zeta.}$ Means not connected with the same letter within each bloom evaluation across both lecithin level and sugar type are significantly different ($\alpha=0.05$).
CSS: corn syrup solids; PD: polydextrose.

4.3.1.4 Discussion on bloom results

It has been suggested that crystalline sugar tends to promote more visual bloom because the shape edge of sugar surface assists in nucleation step during fat recrystallization to form sharp crystals with protrusions (Bricknell and Hartel, 1998). Also, replacing cocoa powder with sucrose, which is in more regular shape, might also increase bloom since the migration rate may be enhanced as the more regular shape of sucrose compared to cocoa powder reduces tortuosity of migration pathway during bloom (Smith, 1998; Hartel et al., 2016).

All three methods of bloom analysis, including white index measurements, stereomicroscopy and visual analysis, showed that increasing sucrose level significantly increased the rate of bloom and the final bloom extent at day 28. This matches our hypothesis that more sugar crystals promote visual bloom. On the other hand, adding 0.5% lecithin to the model systems significantly reduced the level of bloom and the final bloom extent at day 28. As described in the previous sections, emulsifier may act as a bloom inhibitor, as shown in literature (Easton et al., 1952; DuRoss and Knightly, 1965; Weyland, 1994; Lonchampt and Hartel, 2004). The addition of emulsifier decreases chocolate viscosity, affects cocoa butter crystallization and may retard polymorphic transition in chocolate during bloom formation (Nakae, 2000; Lonchampt and Hartel, 2004; Miyasaki, 2016). On the other hand, the interaction between emulsifiers, sugar particles and fats in these systems may change the microstructure of the matrix, thus changing fat migration and recrystallization during bloom (Johansson and Bergenstahl, 1992a).

The bloom extents in the other three sugar types (maltitol, corn syrup solids and polydextrose) were not as high as in sucrose systems. For corn syrup solids, only the 75% level

led to significant surface change and bloom level was very low compared to 75% sucrose model system. Polydextrose system had similar results, with only 50 and 75% levels showing obvious change in bloom extents, but still having significantly lower values compared to sucrose systems. These were as expected and agreed with previous research where the bloom levels in chocolate with amorphous sugar were much lower than that with sucrose (Bricknell and Hartel, 1998). The potential mechanism could be that amorphous sugar may change the shape of the recrystallized fats on the chocolate surface; thus, those fats no longer poke out from the surface to reflect light to give the white color (Bricknell and Hartel, 1998; Hartel et al., 2016). Also, amorphous sugars were thought to improve heat stability in chocolate (Niedieck, 1981; Krüger, 2017). It is possible that amorphous sugar may change the migration during bloom as its round shape would force the matrix to be packed firmly together, blocking the migration pathway (Lonchampt and Hartel, 2004).

Unexpectedly, maltitol, which is a crystalline sugar, also did not promote substantial bloom. Change in bloom extents for model systems with different maltitol levels were all very similar and very low. This agreed with previous research showing that chocolate systems with maltitol did not have obvious bloom (Son et al., 2018). The potential explanation could be related to the effect of maltitol on the migration and recrystallization steps during bloom since the shape of maltitol is different from sucrose, which changes tortuosity of the chocolate matrix for fat migration as well as the surface energy of particles for fat recrystallization. Also, the interaction between maltitol, lecithin and fats in the system might be different, and this might change the microstructure of chocolate influencing bloom.

Further, adding 0.5% lecithin had a significant reduction on bloom in all sugar systems, except for the corn syrup solids system (where bloom levels in 50% corn syrup solids model system were already extremely low). The potential mechanism for the effects of lecithin is similar to that in the chocolate systems with sucrose as described before.

The three different bloom evaluation methods showed almost the same trends in bloom results. Comparing them, whiteness index from colorimeter worked best especially in higher bloom samples. The standard deviation from colorimeter is around 0.5, which would make differences at lower bloom levels less significant, as shown in data from preliminary experiments (Hartel et al., 2016). Stereomicroscope is perfect for predicting medium and low bloom levels with a whitish area percentage from 10 to 80%. Noise from the dark background contributes to the whitish area when the software is analyzing the picture, which makes it harder to compare model systems where visual bloom is not obvious (whitish area % < 10), while systems with extremely high bloom levels (whitish area % > 90) all resulted in whitish bloom occurring in most surface area. Even though the whiteness levels were different, the area percentages were similar, which was also difficult to compare. Visual analysis seems to have a good combination of evaluations on both whiteness and whitish area, which makes it a good method. Its disadvantage is that the scale is too narrow and for some extreme cases when bloom is not that clear or total surface turns white, the difference becomes too close to compare. In conclusion, whiteness index is a good method for systems with obvious visual bloom, stereomicroscopy works fine for samples with low to medium bloom, and visual analysis is good to compare bloom levels with smaller differences when visual bloom is present, but not good after the total chocolate surface turns white.

4.3.2 Particle interactions

Chocolate is a system made of numerous solid particles in a cocoa butter continuous phase (Hartel et al., 2016). Since some of the previous bloom results cannot be fully explained, a focus on the interactions within the system may provide clues about the changes in the chocolate matrix during bloom. Different particle interactions may indicate different microstructures in the chocolate system as the distance and arrangement between particles vary with different ingredients (Johansson and Bergenstahl, 1992a; Barbin et al., 2005). In this section, three different methods, including rheology, gravity sedimentation and tensiometry, were used to quantify the interactions between particles in the chocolate system, with the aim of building a correlation between bloom and particle interaction.

4.3.2.1 Rheology

A flow sweep method was used on the rheometer to determine the viscosity of different dispersion systems. Figures 4.40 to 4.43 show average curves from flow sweeps on sugar-in-CB dispersion systems with different sugar types at different sugar level (low, medium, or high; referenced to the same sugar concentration in CB as chocolate system with cocoa powder replacement level of 25, 50, or 75%). In these dispersion systems, the particle size ranges from 45 to 90 μm. All flow sweep curves presented a shear thinning behavior, where the viscosity decreases as shear rate increases, which is very similar to the chocolate system (Beckett, 2000; Barbin et al., 2005). Yield stress values in all systems were close to 0, which is much smaller than the chocolate system. This might be due to the lack of cocoa powder in the dispersed phase, which increases the surface area of particles in the system and leads to more particle adsorption

thus promoting more particulate interactions in the system (Johansson and Bergenstahl, 1992b; Wolf, 2017). It is also obvious that increasing sugar level increases shear stress values, which is reasonable since higher amounts of particles in the system promote more interactions (higher viscosity).

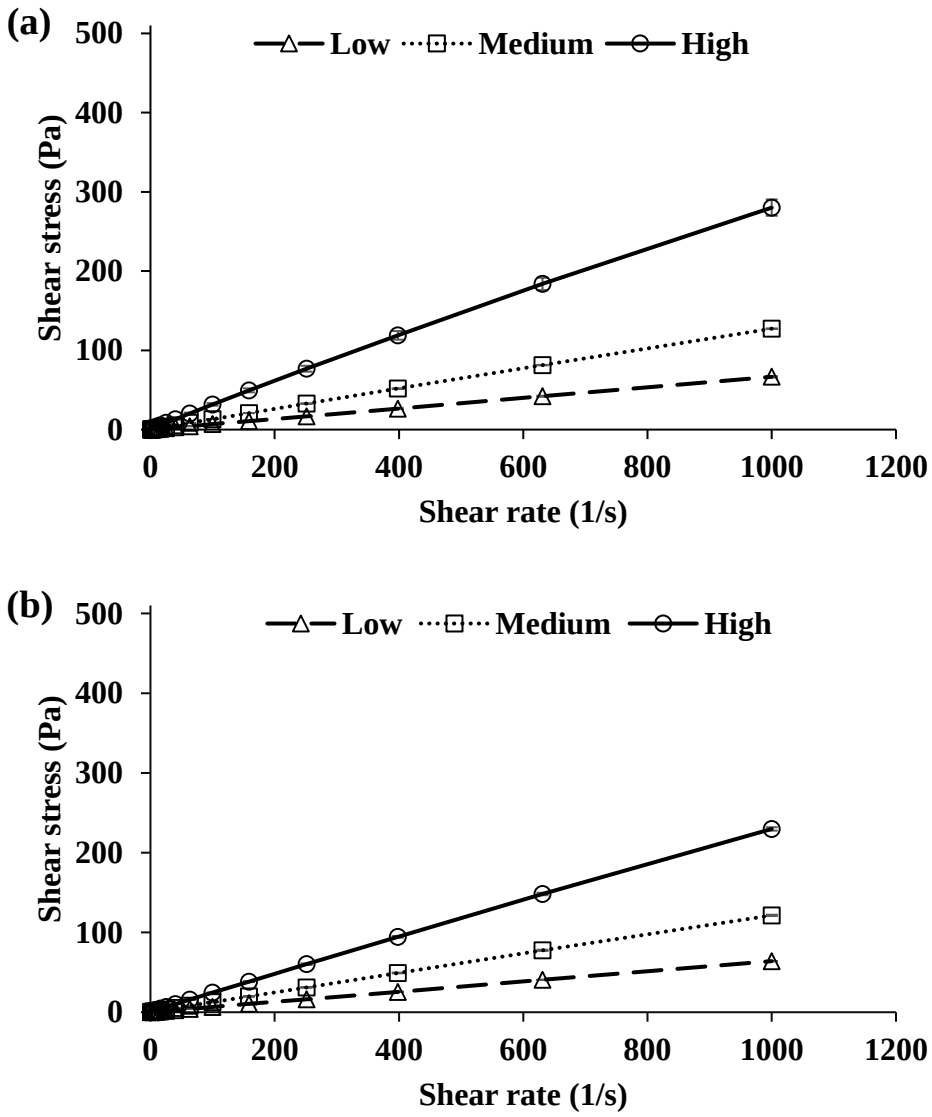


Figure 4. 40 Average flow sweeps for sugar-in-fat dispersion systems made from cocoa butter and sucrose (particle size: 45-90 μm) according to the composition in Table 3.10, with three different sugar levels (low, medium, or high; referenced to the same sugar concentration in CB as chocolate system with cocoa powder volume replacement level of 25, 50, or 75%) and different lecithin concentrations: (a) with no lecithin, (b) with 0.5% lecithin.

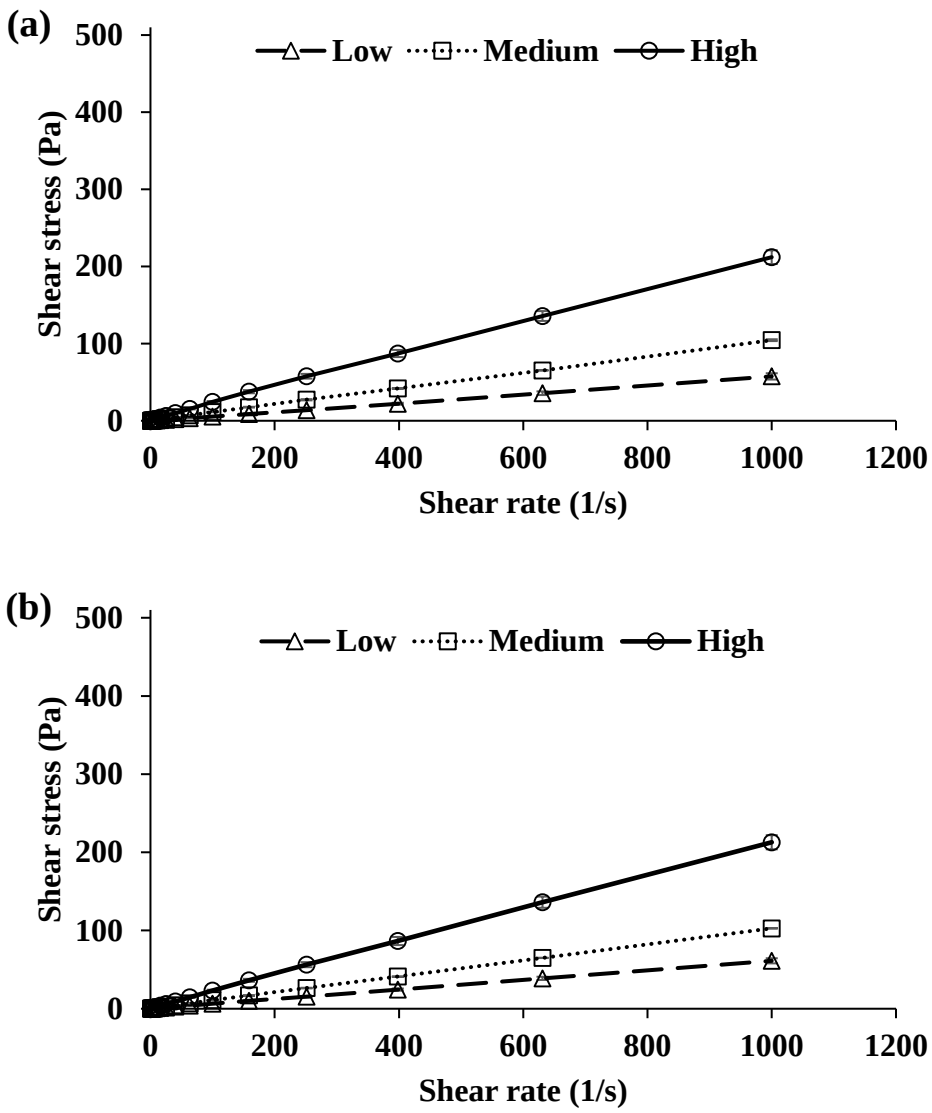


Figure 4. 41 Average flow sweeps for sugar-in-fat dispersion systems made from cocoa butter and maltitol (particle size: 45-90 μm) according to the composition in Table 3.10, with three different sugar levels (low, medium, or high; referenced to the same sugar concentration in CB as chocolate system with cocoa powder volume replacement level of 25, 50, or 75%) and different lecithin concentrations: (a) with no lecithin, (b) with 0.5% lecithin.

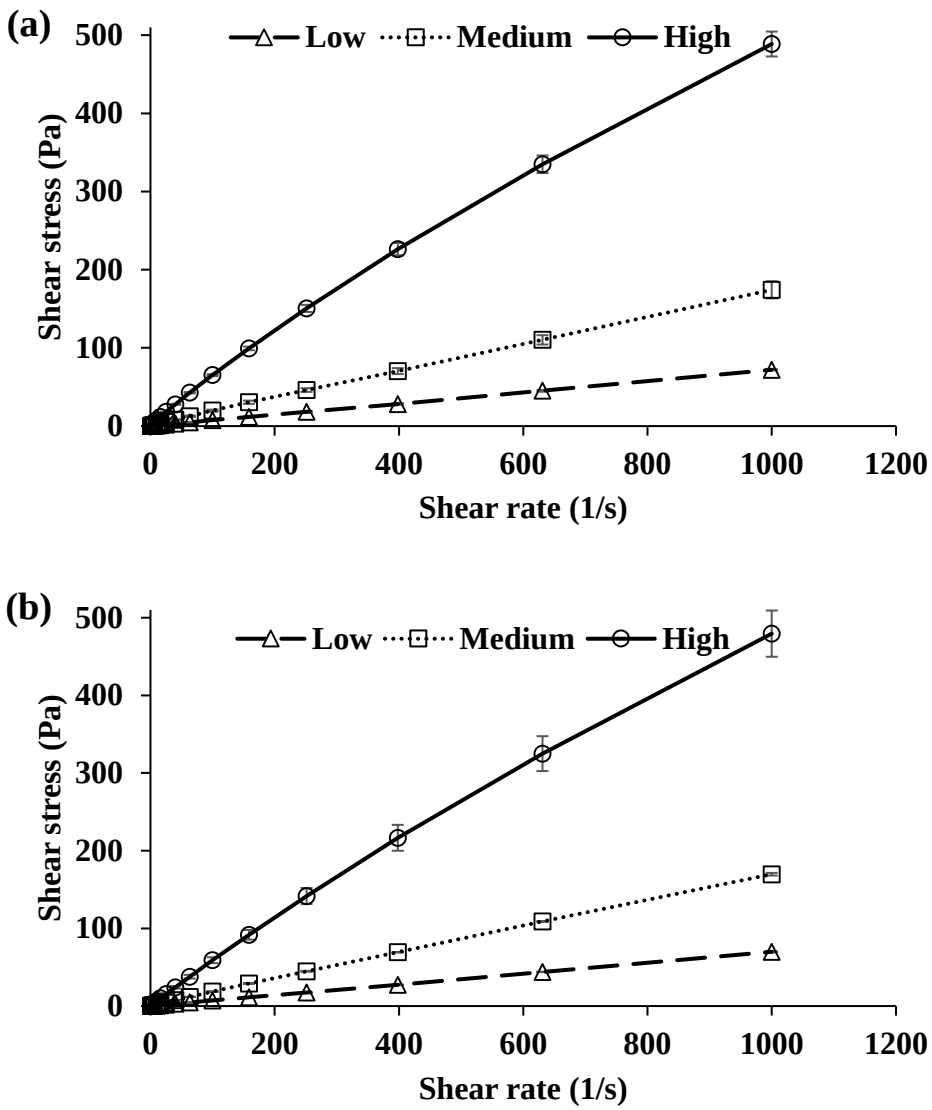


Figure 4. 42 Average flow sweeps for sugar-in-fat dispersion systems made from cocoa butter and corn syrup solids (particle size: 45-90 μm) according to the composition in Table 3.10, with three different sugar levels (low, medium, or high; referenced to the same sugar concentration in CB as chocolate system with cocoa powder volume replacement level of 25, 50, or 75%) and different lecithin concentrations: (a) with no lecithin, (b) with 0.5% lecithin.

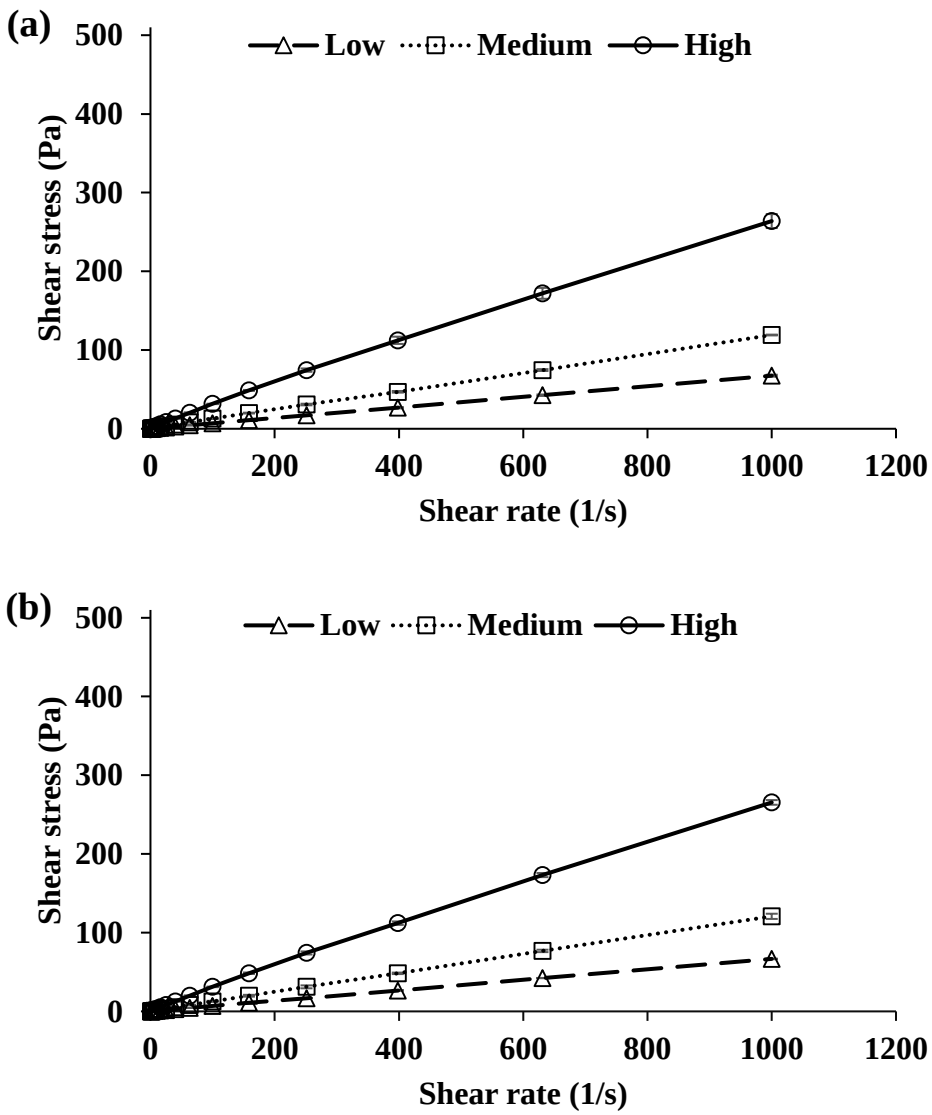


Figure 4. 43 Average flow sweeps for sugar-in-fat dispersion systems made from cocoa butter and polydextrose (particle size: 45-90 μm) according to the composition in Table 3.10, with three different sugar levels (low, medium, or high; referenced to the same sugar concentration in CB as chocolate system with cocoa powder volume replacement level of 25, 50, or 75%) and different lecithin concentrations: (a) with no lecithin, (b) with 0.5% lecithin.

To better quantify the particle interactions in the system, the Casson model was fit to the flow sweep curves to determine Casson viscosity. From Table 4.9, it is clear that higher sugar level led to higher Casson viscosity, indicating greater interactions, as expected. In the lecithin-free samples, the Casson viscosity was the highest for CSS, followed by sucrose, PD and then maltitol. The trends of the Casson viscosity with different sugar levels matched those in a similar study on sugar-in fat dispersion systems (Barbin et al., 2005).

From the statistical analysis, sugar level had a significant effect on the Casson viscosity as shown from two-way ANOVA with 3 sugar levels, 2 lecithin levels and 4 sugar types. Tukey HSD showed that systems with different sugar levels were significantly different from each other ($p<0.0001$). Two-way ANOVA also showed that sugar types had a significant effect on Casson viscosity ($p<0.0001$). Sucrose had a significantly higher apparent viscosity than maltitol, as confirmed by Tukey HSD. Unexpectly, PD systems were not significantly different from the sucrose system while CSS system had an even higher Casson viscosity. This was most likely because corn syrup solids and polydextrose have a much higher hygroscopicity than the other sugars (Krüger, 2017), thus having a higher moisture content, 6.12% and 4.71% respectively, as measured by Karl Fischer. The particle network in the dispersed phase is rearranged by water and forms bridges, which are responsible for the strong adhesion in the system (Johansson and Bergenstahl, 1992a; Barbin et al., 2005). This could partially explain higher viscosity in the highly hygroscopic CSS and PD. Moreover, overall, there was no significant effect from lecithin on Casson viscosity ($p=0.1428$). However, Tukey HSD on the interaction between lecithin and sugar types did show that there was a significant reduction effect from lecithin on Casson viscosity in the sucrose system. In the other three sugar systems,

the effects of lecithin on Casson viscosity were not significant. In maltitol, this might due to the low level of Casson viscosity that makes the difference not significant enough to identify. For CSS and polydextrose, as they are both amorphous sugars, it is possible that the nature of particle interactions were different from crystalline sugars.

Table 4. 9 Casson viscosity of sugar-in-fat dispersion systems made from cocoa butter and different sugars (sucrose, maltitol, corn syrup solids and polydextrose) according to the formulation in Table 3.10 with different sugar levels (25, 50 and 75%, referenced to the sugar concentration on a volume basis replacing cocoa powder in the chocolate model systems in this phase), with or without 0.5% lecithin. Standard deviations are shown below each mean value in the brackets.

Lecithin level	Sugar level	Casson viscosity (cP)			
		Sucrose	Maltitol	Corn syrup solids	Polydextrose
0%	25%	66.0^{g} (0.7)	56.9^{g} (3.8)	71.0^{g} (0.0)	66.9^{g} (0.5)
	50%	124.8^{f} (0.2)	101.1^{f} (2.9)	166.2^{e} (10.5)	115.8^{f} (0.8)
	75%	264.8^{b} (8.2)	198.2^{d} (3.9)	434.1^{a} (15.7)	244.3^{bc} (10.1)
0.5%	25%	63.2^{g} (0.4)	60.4^{g} (2.9)	69.5^{g} (2.9)	66.0^{g} (0.9)
	50%	119.1^{f} (0.1)	100.8^{f} (1.0)	163.7^{e} (1.5)	118.2^{f} (1.6)
	75%	222.2^{cd} (1.4)	201.0^{d} (1.2)	439.6^{a} (26.2)	248.1^{bc} (2.0)

$^{a,b,c,d,e,f,g.}$ Means not connected with the same letter across lecithin level, sugar level and sugar type are significantly different.

4.3.2.2 Sedimentation Volume

As another indicator of particle interaction in the dispersion system, sedimentation volume (Table 4.10) was obtained by gravity sedimentation. Statistical analyses were done by two-way ANOVA with two levels of lecithin concentration and four levels of sugar type. Tukey HSD also showed that sucrose system had a significantly higher sedimentation volume than the other three, whereas where was no significant difference between the other three sugar systems. Further, the effect of interaction between lecithin level and sugar type was also significant ($p<0.0001$). This means the effect of lecithin is dependent on the effect of sugar type and might vary in different sugar systems. In sucrose system, the lecithin-free sample had a significantly higher sedimentation volume than the sample with lecithin, but the effect of lecithin in the other three sugar systems was not significant. This is consistent with the Casson viscosity data in Section 4.3.2.1, with either low, medium or high sugar levels, where the reduction effect of lecithin was more significant in the sucrose systems. In general, the sedimentation method confirmed that the interactions in the sucrose system were higher than the other three sugar systems and that lecithin had a reduction effect on the particulate interaction for sucrose (not significant for the other three sugars).

Table 4. 10 Averaged sedimentation volumes (mL) of sugar-in-fat dispersion systems (total volume=14 mL) made from 90% cocoa butter and 10% sugars with different sugar types (sucrose, maltitol, corn syrup solids and polydextrose), with or without 0.5% lecithin.

Lecithin level	Sugar type			
	Sucrose	Maltitol	Corn syrup solids	Polydextrose
0	2.9 ± 0.1^{a}	2.1 ± 0.1^{b}	2.1 ± 0.1^{b}	2.0 ± 0.1^{b}
0.5	2.1 ± 0.1^{b}	2.1 ± 0.1^{b}	2.0 ± 0.0^{b}	2.1 ± 0.1^{b}

[a,b.] Means not connected with the same letter are significantly different.

4.3.2.3 Contact angle

Contact angle, a direct parameter representing the interaction between a particle and liquid fat, was measured by tensiometer. Table 4.11 shows contact angles between compacted sugar powder surface (sucrose, maltitol, CSS and PD) and melted cocoa butter, with the statistical results. One-way ANOVA was conducted on the contact angle with 4 levels of sugar type. The effect of sugar type was significant ($p=0.0007$). Tukey HSD showed that sucrose had a significantly higher contact angle than CSS, whereas maltitol and PD, with almost identical contact angles, had no significant difference from either sucrose or CSS. From this, the interactions between sugar particles and melted cocoa butter were lowest in sucrose system, second lowest in maltitol and PD, and highest in CSS system, although the differences were very small.

Table 4. 11 Contact angles (º) between compacted sugar surfaces (sucrose, maltitol, corn syrup solids, and polydextrose) and melted cocoa butter at 50ºC.

Sugar type	Sucrose	Maltitol	Corn syrup solids	Polydextrose
Contact angle (º)	12.6 ± 1.9^{a}	11.1 ± 2.0^{ab}	9.1 ± 1.5^{b}	10.9 ± 1.2^{ab}

[a,b.] Means not connected with the same letter are significantly different.

In order to eliminate the effects of surface porosity and impurity of the compacted powders, contact angles between melted cocoa butter and different sucrose crystal surfaces were compared as shown in Table 4.12. One-way ANOVA on the contact angle with 3 levels of sucrose surface showed that surface types had a significant effect on contact angle ($p<0.0001$). The washed rock candy had a significantly higher contact angle than compressed powder and

raw rock candy, whereas there was no significant difference between raw rock candy and compressed powder.

Table 4. 12 Contact angles (º) between sucrose with different surface (compressed powder, rock candy, and washed rock candy) and melted cocoa butter at 50ºC.

Surface type	Compressed powder	Rock candy	Washed rock candy
Contact angle (º)	12.6 ± 1.9^{b}	12.8 ± 1.9^{b}	20.7 ± 4.2^{a}

[a,b.] Means not connected with the same letter are significantly different.

The main difference between raw rock candy and compressed sucrose powder was the surface porosity. As there was no significant difference between them, surface porosity was not a significant factor contributing to contact angle in this case. On the other hand, the washing procedure reduced surface impurities on the sucrose crystal surface. As the difference in contact angle before and after washing was significant, surface impurity had a significant effect on contact angle, which agreed with the literature (Yekta-Fard and Ponter, 1992; Reinke et al., 2015). Reducing surface impurities increases contact angle since nonpolar components might attach on the sucrose surface during product processing and the nonpolar components on the sucrose surface might enhance its interaction to the melted cocoa butter. Thus, surface impurity need to be take into account when comparing different sugar types. Further, since surface impurity had an effect on contact angle, the interaction between particles in the chocolate might be changed with different extents of particle impurity. Thus, surface impurity might be another factor for bloom (See Section 4.4).

4.3.2.4 Discussion on particle interaction results

Comparing Casson viscosity in dispersion systems with different sugar particles, CSS system had the highest particulate interaction and maltitol system had the lowest. Sucrose system's averaged viscosity was higher than PD but the difference was not significant. Considering the high hygroscopicity of CSS and PD (Krüger, 2017), the trend in particulate interaction followed the trend in bloom extent except for the CSS system. The effect of lecithin overall was not very significant for the Casson viscosity ($p=0.1428$). Only Casson viscosity in sucrose system showed significant reduction effect from lecithin, which agreed with the literature (Barbin et al., 2005). This is because lecithin can adsorb onto sucrose to decrease interaction energetics (Johansson and Bergenstahl, 1992a), thus smoothly combining sugar and fat in the system to better enable flow (Wolf, 2017). The discrepancy of lecithin's effect on sucrose and other three sugar particle systems might indicate that lecithin does not adsorb as well as sucrose to the other particles.

The sedimentation method showed similar results as the lecithin effect. It was only significant in sucrose system as emulsifier can inhibit hydrophilic adhesion between the particles, resulting in a reduction in sediment volume (Johansson & Bergenstahl, 1992b). The other three sugar systems showed no significant difference in sedimentation volume with or without lecithin. This might be because the effect was too small to differentiate as Barbin et al. (2005) showed that emulsifier's reduction effect in melted cocoa butter system was not that significant compared to other liquid oils including palm kernel oil, soybean oil and milk fat. On the other hand, sucrose had a significantly higher sedimentation volume than the other three sugars, which is consistent with the bloom results.

The contact angle method did not show huge differences among the four sugar particles. Only sucrose had a significantly higher contact angle than CSS, indicating a lower interaction. The results between compacted sucrose powder, rock candy and washed rock candy showed that particle surface impurity might be an important factor for contact angle, a possible explanation for the difference in contact angle of sucrose and CSS. Further, since particle surface impurity might change particle interactions in the chocolate system, it might also be a potential factor for bloom.

To better compare the correlation between bloom and particle interactions, the final ΔWIs in the model systems with sucrose and maltitol were plotted against Casson viscosity or sedimentation volume as shown in Figures 4.44 and 4.45, respectively. CSS and PD systems were not included because the moisture content in these systems was high, and thus could not be strictly compared. It was expected that particle interaction (either Casson viscosity or sedimentation volume) in the chocolate system had a significant and positive correlation with final bloom extents since systems with different particle interaction extents may have different particle arrangements in the matrix that greatly changed the microstructure of the dispersion system (Johansson & Bergenstahl, 1992a). Microstructure in the chocolate has an important effect on bloom since it might give different fat migration pathways and recrystallization energetics might change the migration and recrystallization steps during bloom formation (Bricknell and Hartel, 1998; Hartel et al., 2016). In the Casson viscosity vs. ΔWI plot, there was a positive trend, but the overall correlation was not good ($R^2 = 0.4765$). However, when only comparing the data points in the sucrose system, the correlation was strong and positive ($R^2 = 0.8625$), as expected. Maltitol systems, on the other hand, did not show a clear correlation

because bloom levels were low regardless of the amount of maltitol added. In the sedimentation volume vs. ΔWI plot, the overall correlation was not strong (R^2 = 0.6023). In each system, either sucrose or maltitol, there was only two data points (with and without lecithin). Thus, it is hard to evaluate the significance of the correlation, although the plot that the correlation in the sucrose system was positive. In sum, the expected correlation between particle interaction and bloom was only shown in the sucrose system, and was not strong in the overall plots or in the maltitol system. This indicates that the mechanism of bloom in chocolate might be different with different sugar types.

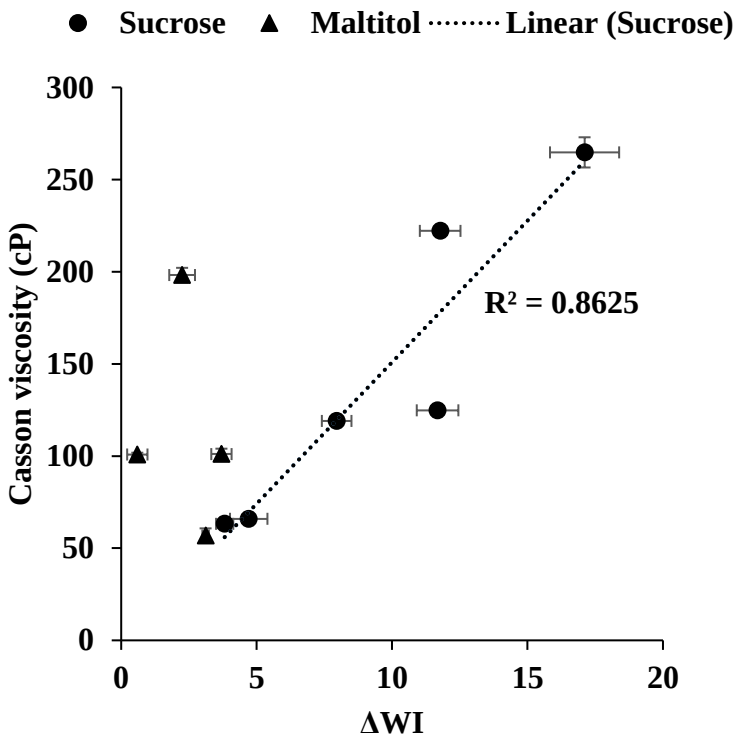


Figure 4. 44 Plots of changes in whiteness index (ΔWI) in chocolate model systems (50% cocoa butter and 50% particles), where cocoa powder was replaced by sucrose or maltitol at 25, 50, and 75% level (v/v) and with 0 or 0.5% lecithin (referenced to total mass in the control system) against Casson viscosity in the dispersion system with the same composition as the chocolate system but without cocoa powder.

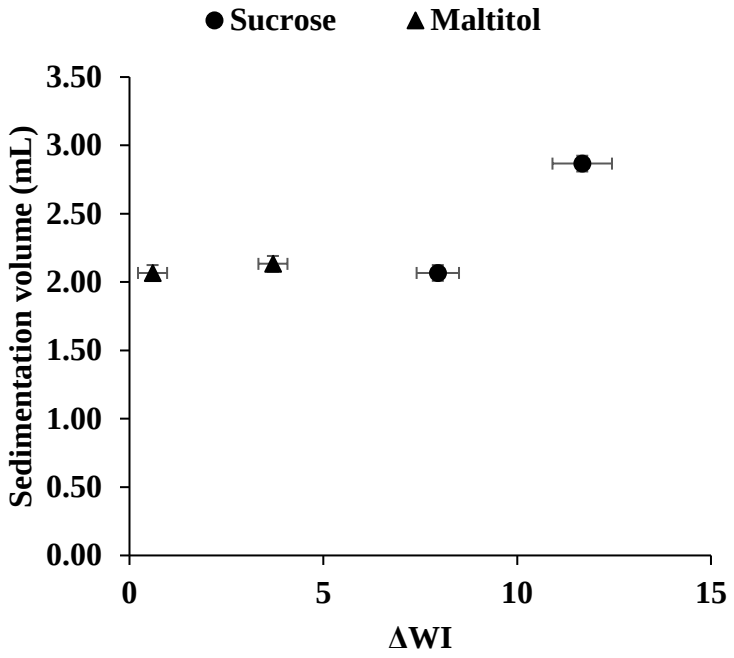


Figure 4. 45 Plots of changes in whiteness index (ΔWI) in chocolate model systems (50% cocoa butter and 50% particles), where cocoa powder was replaced by sucrose or maltitol at 50% level (v/v) and with 0 or 0.5% lecithin (referenced to total mass in the control system) against sedimentation volumes of sugar-in-fat dispersion systems (total volume=14 mL) made from 90% cocoa butter and 10% sucrose or maltitol with or without 0.5% lecithin.

In sum, rheology and sedimentation results showed some similar trends especially in the reduction effect of lecithin in the sucrose system. This agreed with the conclusions from previous studies (Johansson & Bergenstahl, 1992a; Johansson & Bergenstahl, 1992b; Barbin et al., 2005). Contact angle measurements may be influenced by heterogeneity and impurity and the difference were too small to evaluate. The general trend in the particle interaction results is consistent with the bloom results except for the high viscosity of CSS due to the high hygroscopicity as well as less significant reduction effects from lecithin in CSS, PD and maltitol. Thus, particle interactions appear to have some effect on bloom results but not in all cases. Further work is needed to clarify these interactions.

4.4 Effects of particle surface properties on fat bloom during storage

It was concluded in Section 4.3 that particle interactions within the chocolate model system cannot explain everything in the bloom results. The surface properties of sugar particles are another important factor that may affect bloom extent. Literature has showed that the difference in shape of sugar particles may explain the difference in bloom results between crystalline sugar and amorphous sugars, with a hypothesis that sharp surfaces in sucrose are advantageous for cocoa butter (CB) recrystallization (Bricknell and Hartel, 1998; Hartel et al., 2016). However, whether this difference was due to the nature of particles or just the surface circularity, namely shape of particles has not been studied. Thus, this phase was aimed at developing a method to round off crystalline sugar surface and comparing effects of the shape and nature of particle surface on bloom. Shapes of particles were quantified by microscopy and image analysis, while bloom was evaluated by whiteness index (WI), stereomicroscopy and visual analysis.

4.4.1 Sugar surface modification

Surface modification (rounding off the sucrose surface) is the key in this phase. Here, a white chocolate mix was made by adding 40 g melted cocoa butter, 60 g sucrose crystals (size 45-90 μm), and 0.22 g lecithin. To modify the surface, 2.4 g distilled water was added to the white chocolate mix, which was stirred at 60°C at 138 rpm for 24 h. This was followed by addition of 0.28 g lecithin with continued mixing at 60°C for 24 h. The efficiency of this surface modification process can be evaluated by rheology and microscopy. In general, a particle

suspension with modified sucrose (“a white chocolate mix” with smoother surface) would be expected to have lower viscosity than a refined sugar suspension with sharp crystal surfaces.

4.4.1.1 Rheology

Flow sweeps of white chocolate mix with 40 g CB, 60 g sucrose and 0.5 g lecithin before and after the modification process are shown in Figure 4.46. Both of the flow sweeps show a shear-thinning behavior, typical of a chocolate system (Beckett, 2000; Barbin et al., 2005). Similar to the dispersion systems in Section 4.3.2.1, the yield stress was close to 0, due to the lack of cocoa powder. It is also obvious that apparent viscosity of the white chocolate mix was always lower after modification at any shear rate. This was expected since modified particles with smaller and smoother surfaces would have higher ability to flow across each other to assist flow (St. John et al., 1995; Wolf, 2017). One-way ANOVA and student t-test also confirmed that Casson viscosity after modification was significantly lower than that before modification ($p=0.0002$). The average Casson viscosity before surface modification was 540 cP (standard deviation = 10.1 cP), whereas after surface modification, it went down to 470 cP (standard deviation = 1.1 cP). This indicates that the modification process was successful.

4.4.1.2 Optical Microscopy

The shape of the sucrose particles was quantified by optical microscopy and image analysis. Figure 4.47 shows representative pictures from optical microscope of sugar particles before or after the modification process. By visual evaluation, it is clear that the surface roundness was significantly increased and the particle surface looked smoother after

modification. Image J calculated the surface circularity of particles before and after surface modification. The average surface circularity before surface modification was 0.74 (standard deviation = 0.07), whereas after surface modification, it went up to 0.87 (standard deviation = 0.06). One-way ANOVA showed that the modification process had a significant effect on the surface circularity of sugar particles ($p<0.0001$). Student t-test confirmed that circularity of particles after modification was significantly increased compared to before.

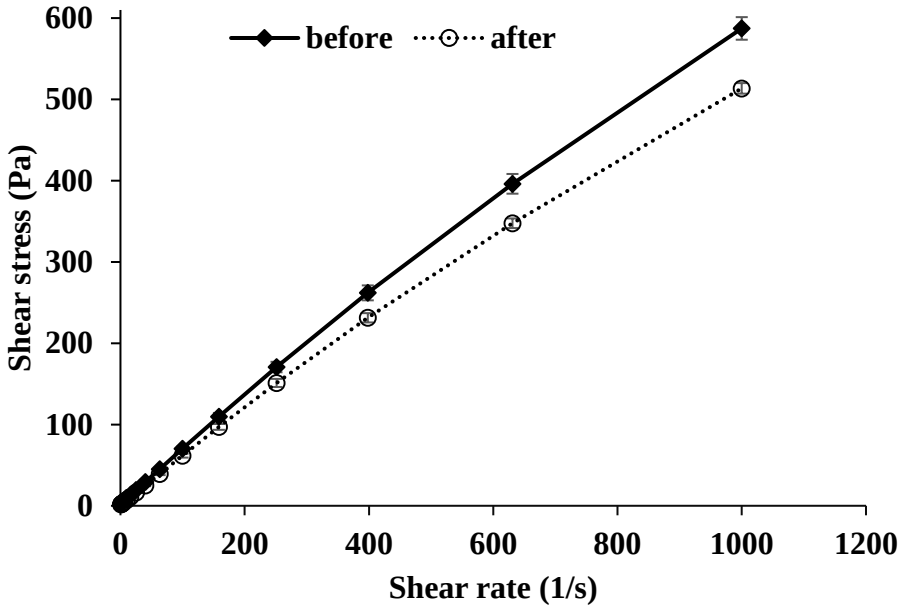
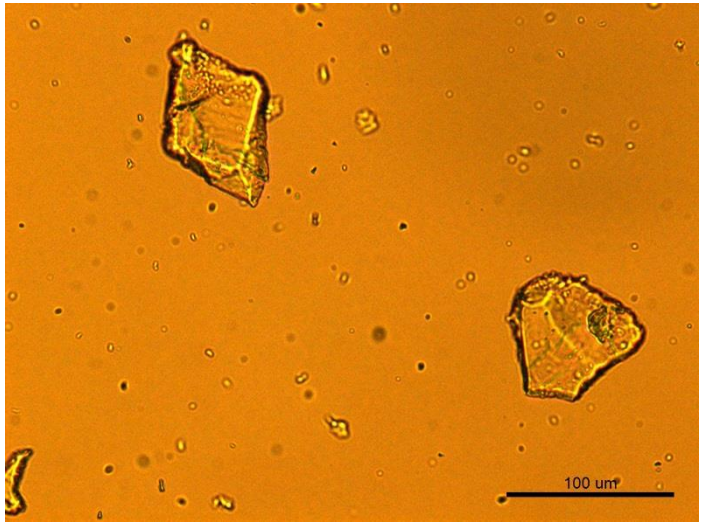
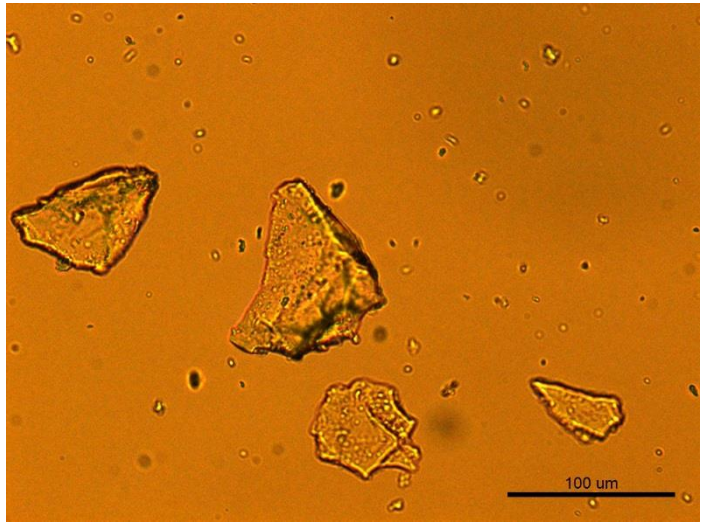


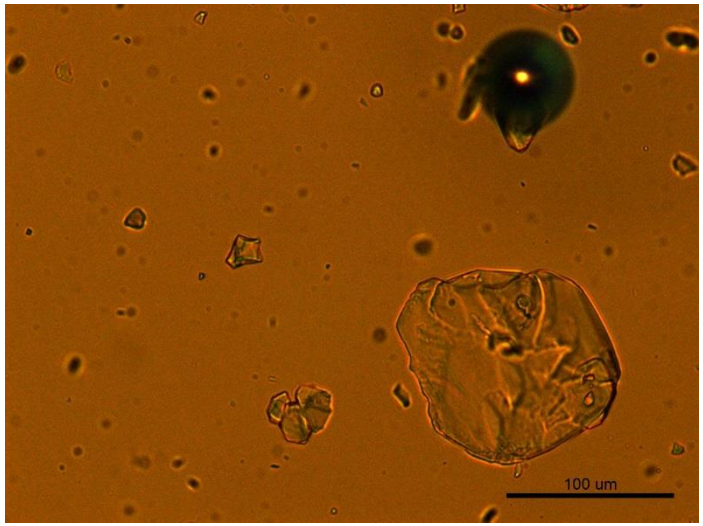
Figure 4. 46 Averaged flow sweeps of melted white chocolate at 40ºC, made from 40 g cocoa butter, 60 g sucrose and 0.5 g lecithin, before or after the surface modification process where the sucrose particle surface was smoothed by adding water and lecithin, and drying at 60ºC for 24 hours.

(a)





(b)



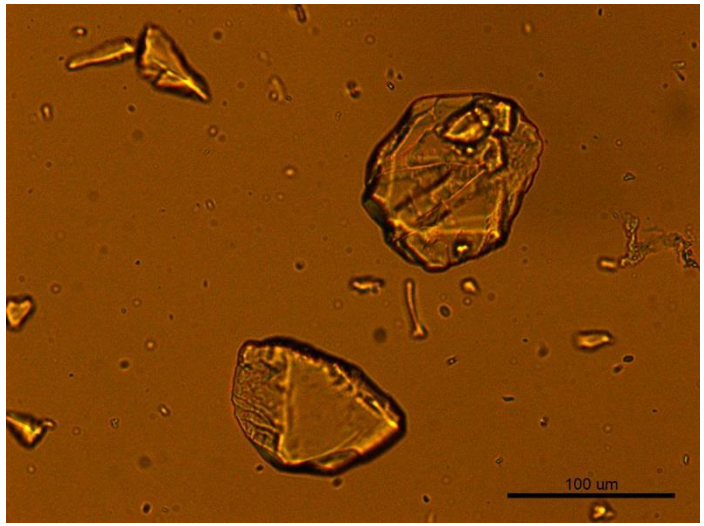


Figure 4. 47 Representative pictures (two pictures for each system) from optical microscope of sugar particles: (a) before or (b) after the modification process where the sucrose particle surface was smoothed by adding water and lecithin, and drying at 60ºC for 24 hours.

4.4.2 Bloom in chocolate with surface modified sugar

As shown in Section 4.4.1, the modification method of washing the surface of sucrose with water and lecithin together with a drying process reduced viscosity of the sucrose particle suspension in melted CB. The particle surface was smoother and rounder, which may influence bloom formation (Bricknell and Hartel, 1998).

To validate this hypothesis, the white chocolate mix after this modification process was mixed with extra CB, lecithin and cocoa powder (formulations shown in Table 3.10) to match the compositions of model systems with untreated sucrose in Section 4.3.1.2. Chocolate was tempered and stored in the same method used in Section 4.3.1.2. Chocolate bloom was tracked at day 0, 1, 7, 14, 21, 28 by three different methods including whiteness index measurement, stereomicroscopy and visual analysis. Figure 4.48 shows the progression of bloom represented by ΔWI, white area percentage, and visual bloom level, respectively, in chocolate model systems of 50% CB, 50% particles and 0.5% lecithin, where cocoa powder was gradually replaced by sucrose particles (after modification) at different levels (0, 25, 50, and 75%) on a volume basis. Figures 4.49 and 4.50 show the representative pictures from stereomicroscope and camera, respectively, on the surfaces of these chocolate model systems at day 28.

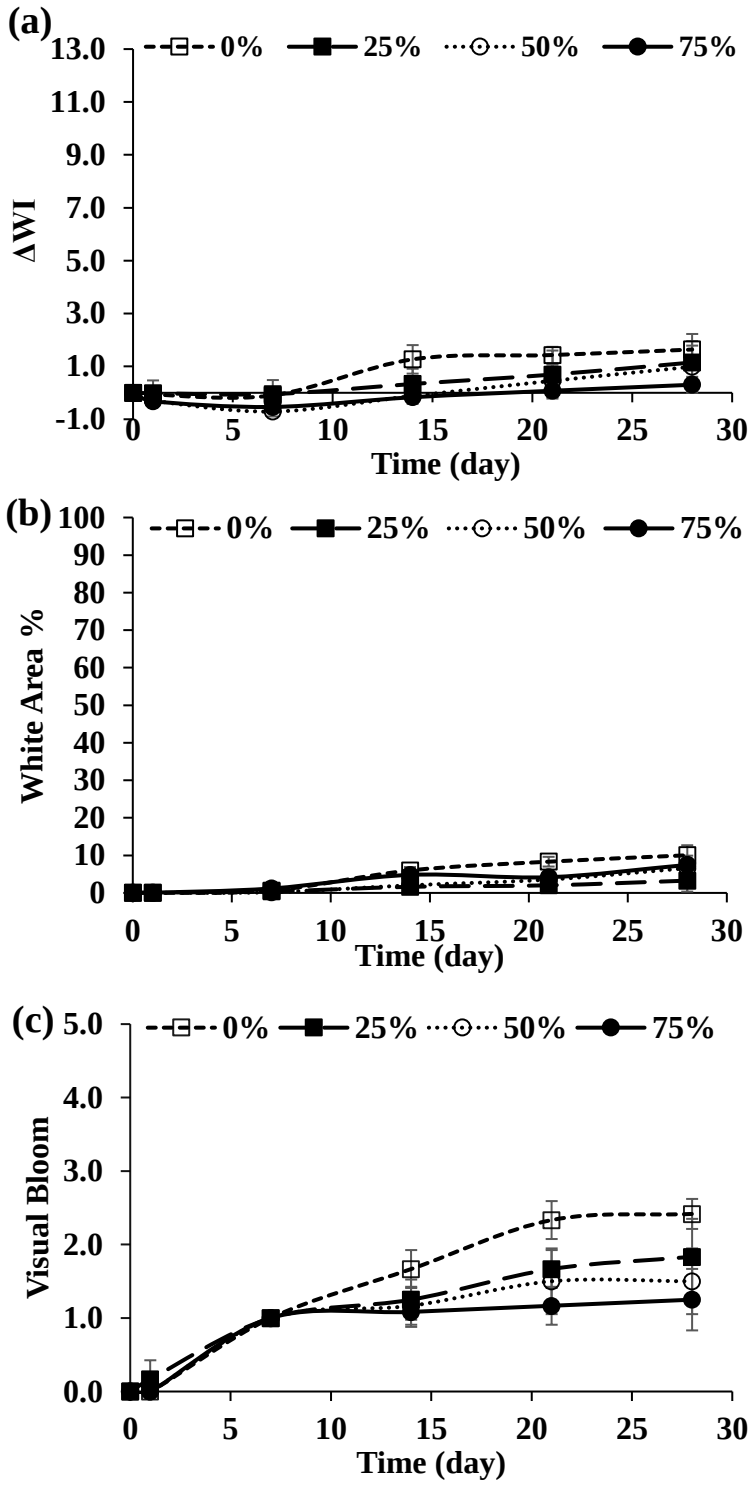


Figure 4. 48 Bloom progression represented by changes in: (a) whiteness index (ΔWI), (b) white area percentage, (c) visual bloom in chocolate model systems of 50% cocoa butter, 50% particles and 0.5% lecithin, where cocoa powder was gradually replaced by sucrose particles (after the modification method that washes the surface of sucrose with water and lecithin together with a drying process) at different levels (0, 25, 50, and 75%) on a volume basis.

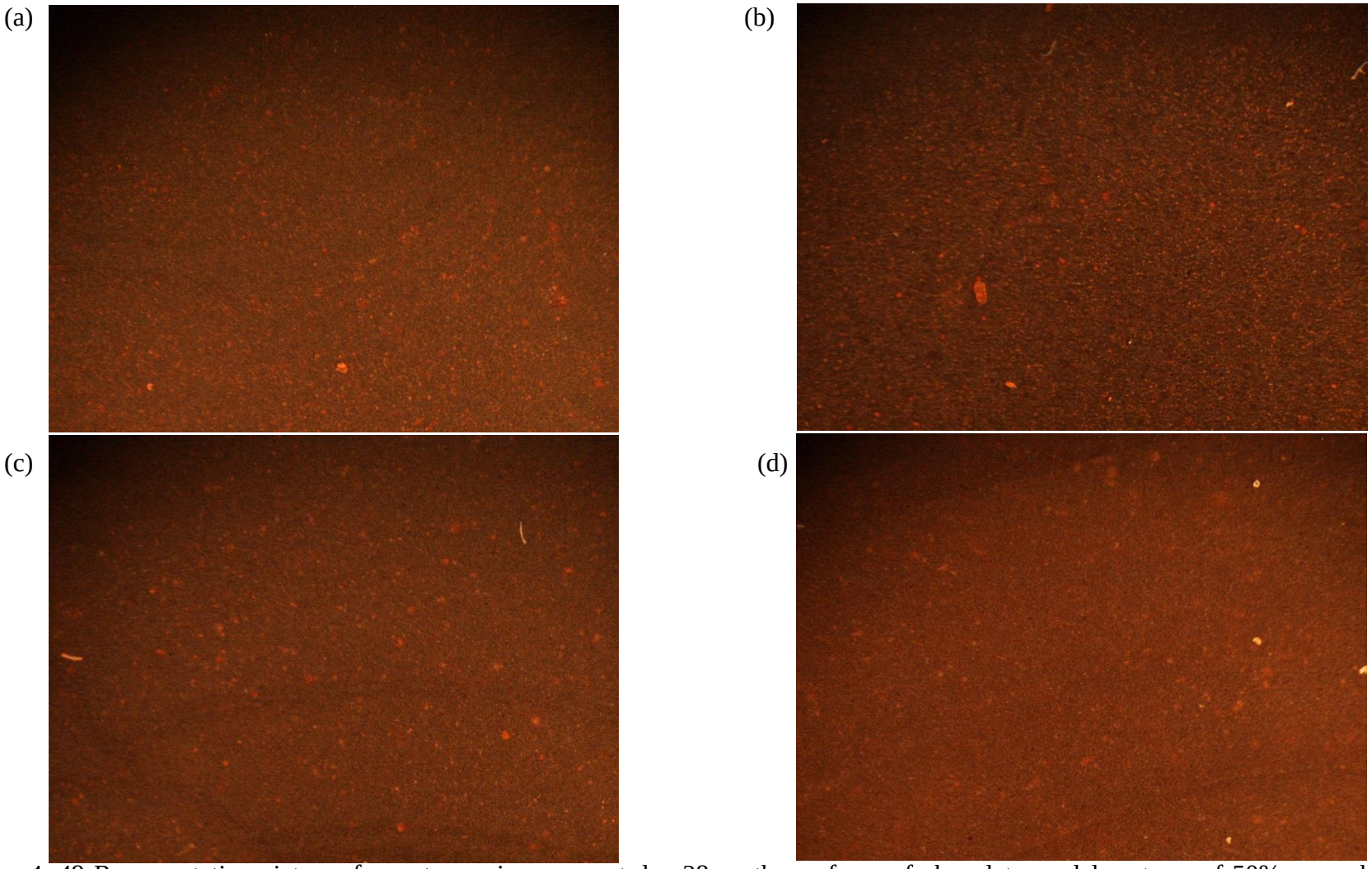


Figure 4. 49 Representative pictures from stereomicroscope at day 28 on the surfaces of chocolate model systems of 50% cocoa butter, 50% particles and 0.5% lecithin, where cocoa powder was gradually replaced by sucrose (after the modification method that washes the surface of sucrose with water and lecithin together with a drying process) at different levels on a volume basis: (a) 0%, (b) 25%, (c) 50%, (d) 75%.

(a)

(b)

(c)

(d)

Figure 4. 50 Pictures at day 28 on the surfaces of chocolate model systems of 50% cocoa butter, 50% particles and 0.5% lecithin (referenced to total mass in the control model system system), where cocoa powder was replaced by sucrose (after the modification method that washes the surface of sucrose with water and lecithin together with a drying process) at different levels on a volume basis: (a) 0%, (b) 25%, (c) 50%, (d) 75%.

In the ΔWI results, the model system with 0% (v/v) modified sucrose (referenced to the total particle volume) began to show an obvious rise in ΔWI at day 14, bloomed slowly over time and reached a plateau in ΔWI at day 28, with the highest initial bloom rate and the ΔWI values among the four systems. The model system with 25% (v/v) modified sucrose (referenced to the total particle volume) began to show an obvious rise in ΔWI at day 14 and did not reach a plateau at day 28, with a final ΔWI values lower than the 0% system. The model system with 50% (v/v) modified sucrose (referenced to the total particle volume) did not show an obvious rise in ΔWI at day 21 and did not reach a plateau at day 28, with final ΔWI values even lower than the 25% system. The model system with 75% (v/v) modified sucrose (referenced to the total particle volume) only showed obvious rise in ΔWI at day 28, with the lowest final ΔWI values among the four. In general, ΔWI values were almost all below 2, which indicates that the bloom levels were all extremely low. Model systems with higher modified sucrose concentrations started to bloom much later, with lower final ΔWI values.

The stereomicroscopy and visual analysis results showed almost the same trends. Most white area percentages were below 10%, which indicates bloom on the surface was very minor. The highest final visual levels were around 2, which means only a few white spots began to show on the surface after 4-week storage. All three methods showed that adding modified sucrose into the system actually reduced the final level of bloom. Comparing the bloom curves in Section 4.3.1.2 where bloom was promoted with unmodified sucrose samples (sharp surface), it is obvious that this washing approach reduced bloom compared to systems without the washing process.

To evaluate the effect of the modification method that washed the sucrose surface with water and lecithin together with a drying process on bloom, statistical analyses were conducted on final ΔWI, white area percentage and visual bloom values at day 28 in sucrose model systems, either unmodified or modified, with lecithin, as shown in Table 4.13. Two-way ANOVA on the final ΔWI at day 28 with 4 levels of cocoa powder replacement level and 2 levels of sucrose surface (modified or unmodified) showed that cocoa powder replacement level, the modification method, and the interaction between them all had a significant effect on the final ΔWI values ($p<0.0001$). Student t-test showed that chocolate model systems after modification of washing and drying were significantly lower in the final ΔWI values than the unmodified sucrose systems. Two-way ANOVA on white area percentage and visual bloom levels showed the same trend (all p-values were all below 0.0001, except for $p=0.0229$ in cocoa powder replacement effect on visual bloom levels).

The main difference from the modification method compared to the unmodified system was from the washing step. As shown before, surface circularity was reduced by washing. Particle with a smoother surface did not favor in fat recrystallization (Bricknell and Hartel, 1998; Hartel et al., 2016), which would potentially reduce bloom formation. On the other hand, as shown in Section 4.3.2.3, washing sucrose with water followed by a drying process would decrease the surface impurities as shown from the contact angle data. It is also possible that surface impurity of the sugar particles is the factor that reduced bloom formation.

Table 4. 13 Changes in whiteness index (ΔWI), white area percentage and visual bloom at day 28 of chocolate model systems made of 50% cocoa butter, 50% particles and 0.5% lecithin (referenced to total mass in the control model system system) with different cocoa powder replacement levels (0, 25, 50, and 75% on a volume basis) before or after the modification method that washed the sucrose surface with water and lecithin together with a drying process. Data represent means ± standard deviations.

Bloom evaluation	Surface type	Cocoa powder replacement level (%)			
		0	25	50	75
ΔWI	unmodified	1.6 ± 0.3^{d}	3.8 ± 0.3^{c}	8.0 ± 0.5^{b}	11.8 ± 0.8^{a}
	modified		1.1 ± 1.1^{de}	1.0 ± 0.8^{de}	0.3 ± 0.3^{e}
White Area	unmodified	10.0 ± 2.7^{D}	63.7 ± 6.6^{C}	85.3 ± 2.0^{B}	94.1 ± 0.4^{A}
	modified		3.3 ± 2.9^{DE}	6.6 ± 3.3^{DE}	7.5 ± 1.5^{E}
Visual Bloom	unmodified	$2.4 \pm 0.2^{\gamma\delta}$	$3.0 \pm 0.0^{\gamma}$	$3.7 \pm 0.3^{\beta}$	$4.3 \pm 0.3^{\alpha}$
	modified		$1.8 \pm 0.5^{\delta\varepsilon}$	$1.5 \pm 0.4^{\varepsilon}$	$1.2 \pm 0.4^{\varepsilon}$

$^{a,b,c,d,e;\ A,B,C,D,E;\ \alpha,\beta,\gamma,\delta,\varepsilon.}$ Means not connected within each evaluation method with the same letter are significantly different (α=0.05).

4.4.3 "Water-free Wash"

To evaluate the effect of adding water into the white chocolate mix during the modification process, a "Water-free Wash" process, with all the same steps except for that of water addition, was conducted. Bloom was evaluated by three methods to compare to the results in systems with unmodified or water-free washed sucrose. Figure 4.51 shows the progression of bloom represented by changes in whiteness index (ΔWI), white area percentage, and visual bloom, respectively, in chocolate model systems of 50% CB, 50% particles and 0.5% lecithin, where cocoa powder was gradually replaced by sucrose (after "Water-free Wash") at different levels (0, 25, 50, and 75%) on a volume basis. Figures 4.52 and 4.53 show the representative pictures from stereomicroscope or camera, respectively, on the surfaces of these chocolate model systems at day 28.

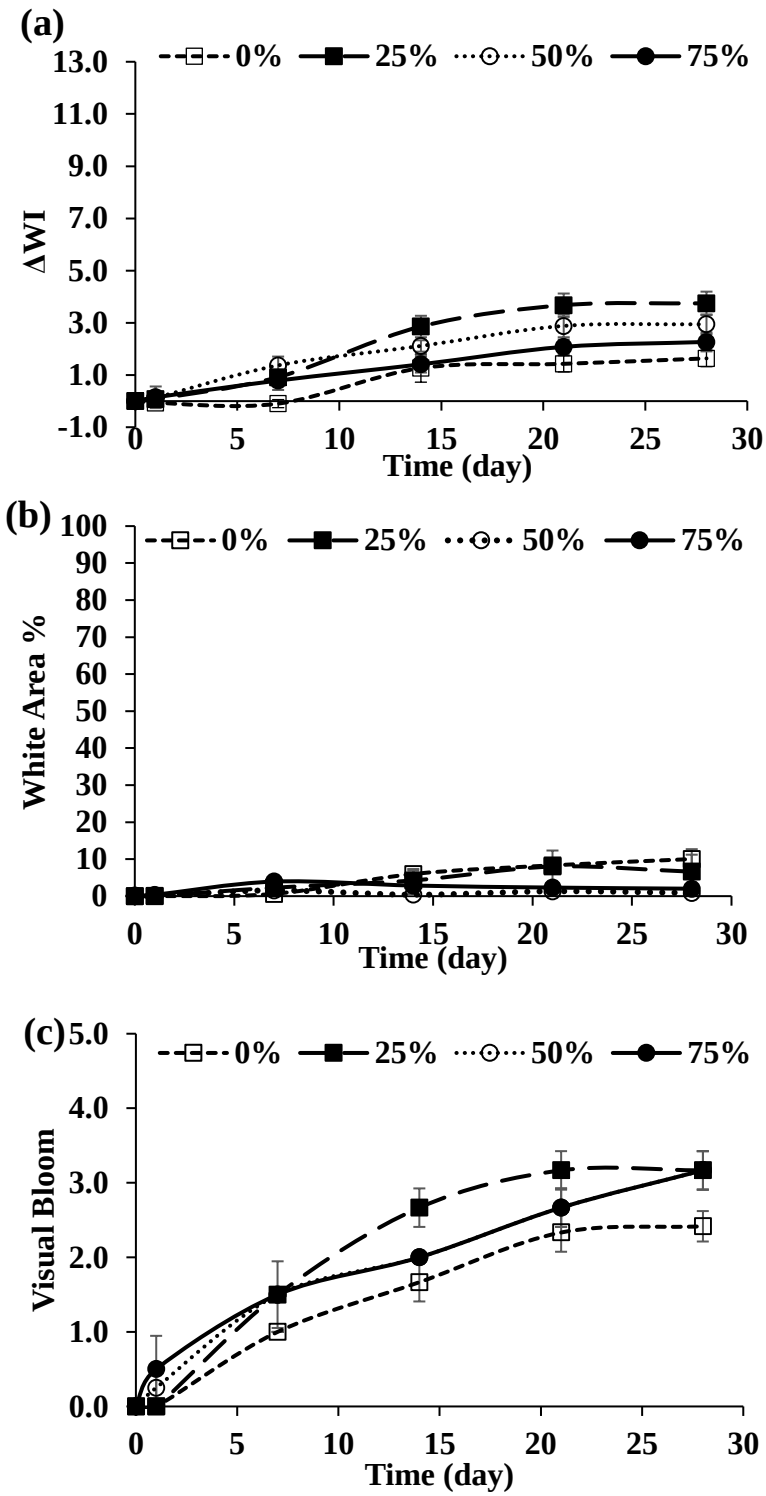


Figure 4. 51 Bloom progression represented by changes in: (a) whiteness index (ΔWI), (b) white area percentage, (c) visual bloom in chocolate model systems of 50% cocoa butter, 50% particles and 0.5% lecithin, where cocoa powder was gradually replaced by sucrose particles (after the "Water-free Wash" with all the same steps as the modification method in Section 4.4.2 except for that of water addition) at different levels (0, 25, 50, and 75%) on a volume basis.

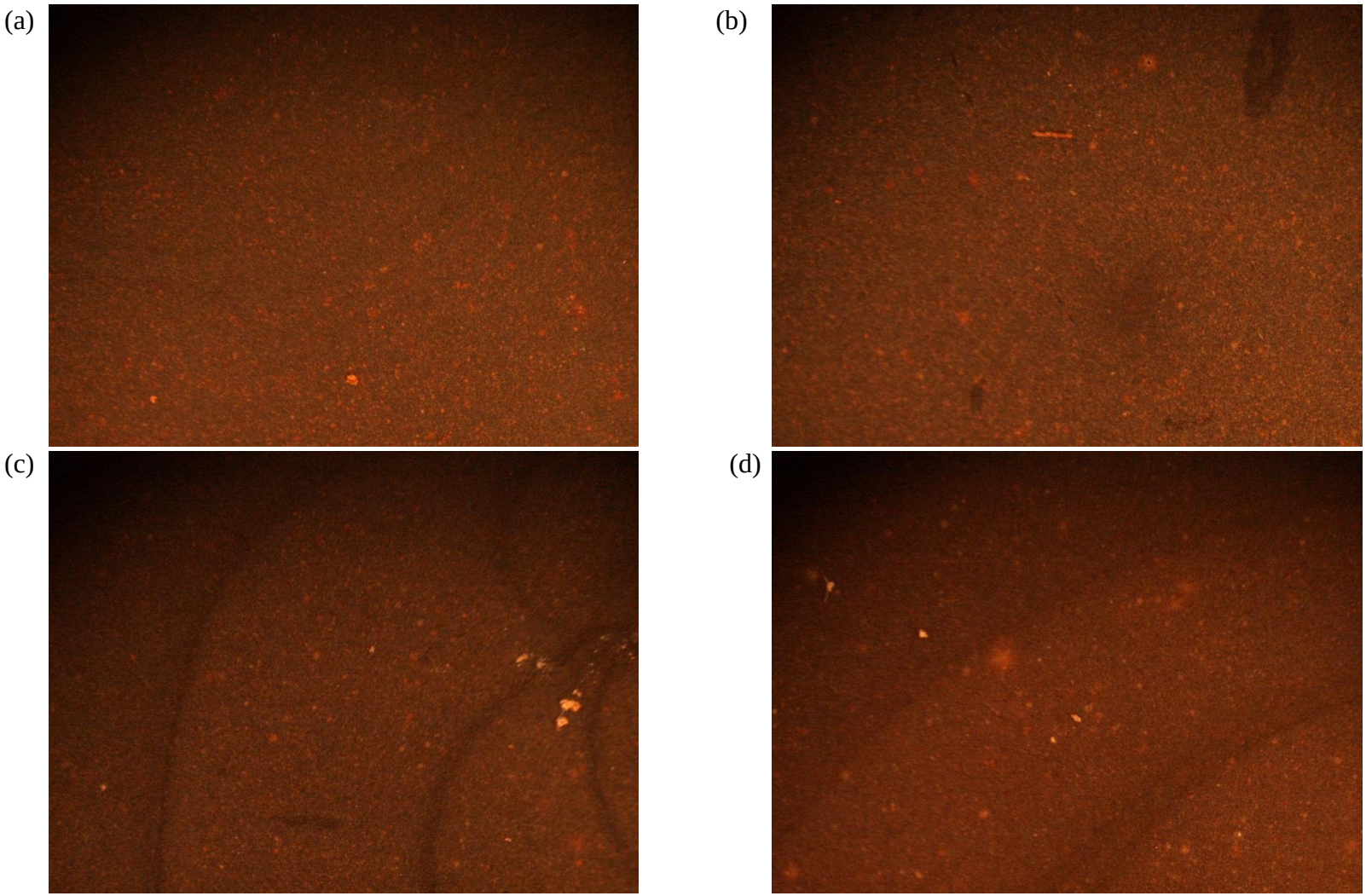


Figure 4. 52 Representative pictures from stereomicroscope at day 28 on the surfaces of chocolate model systems of 50% cocoa butter, 50% particles and 0.5% lecithin, where cocoa powder was gradually replaced by sucrose (after the "Water-free Wash" with all the same steps as the modification method in Section 4.4.2 except for that of water addition) at different levels on a volume basis: (a) 0%, (b) 25%, (c) 50%, (d) 75%.

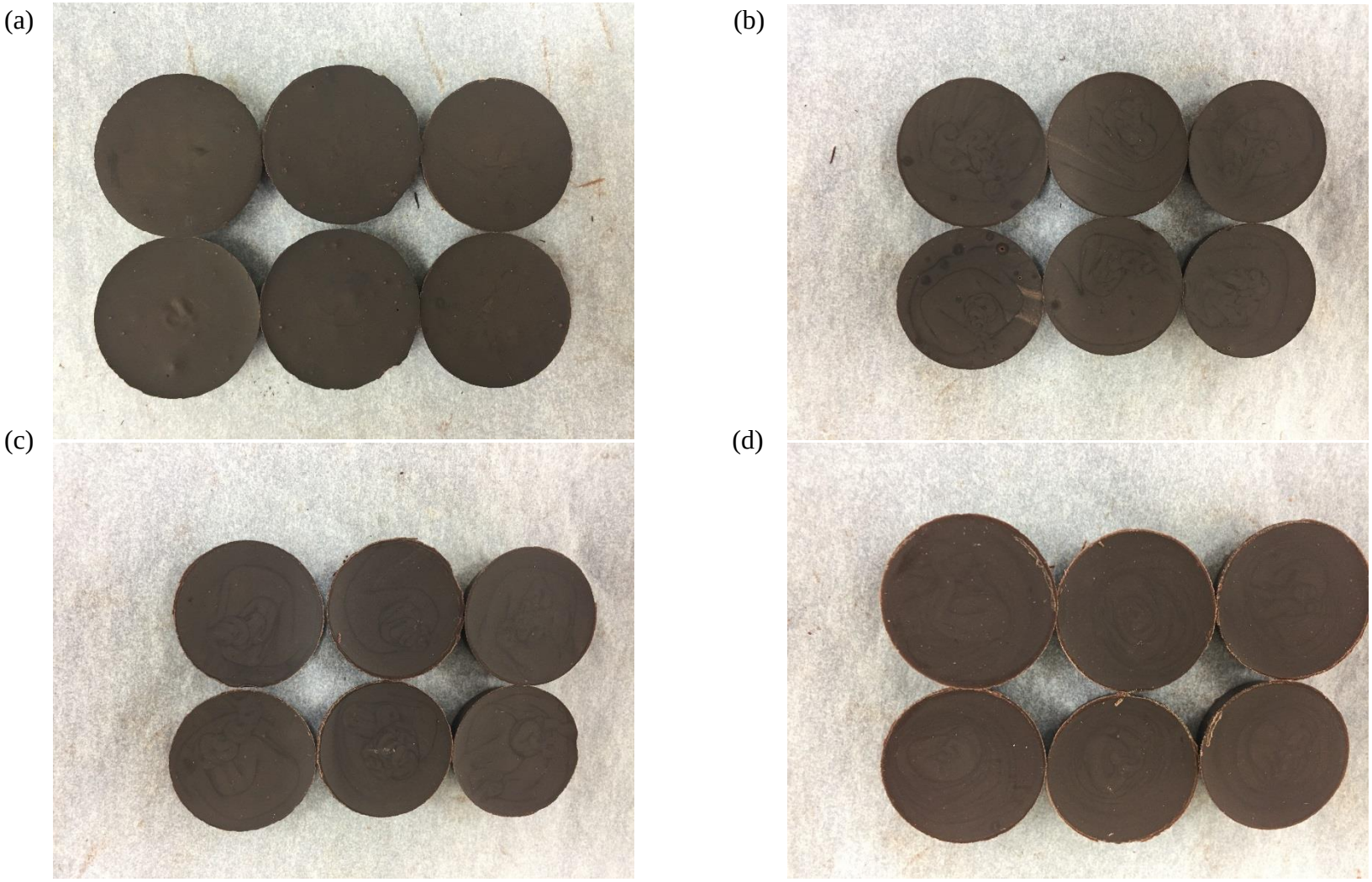


Figure 4. 53 Pictures at day 28 of chocolate model systems of 50% cocoa butter, 50% particles and 0.5% lecithin (referenced to total mass in the control model system system), where cocoa powder was replaced by sucrose (after the “Water-free Wash” with all the same steps as the modification method in Section 4.4.2 except for that of water addition) at different levels on a volume basis: (a) 0%, (b) 25%, (c) 50%, (d) 75%.

The ΔWI results showed that, the model system with 25% (v/v) "Water-free Washed" sucrose (referenced to the total particle volume) began to an show obvious rise in ΔWI at day 7, bloomed slowly over time and reached a plateau in ΔWI at day 21. The model system with 50% (v/v) "Water-free Washed" sucrose (referenced to the total particle volume) began to show an obvious rise in ΔWI at day 7 and reached a plateau in ΔWI between day 21 and 28. The model system with 75% (v/v) "Water-free Washed" sucrose (referenced to the total particle volume) began to show an obvious rise in ΔWI at day 7 and slowly reached a plateau in ΔWI at day 28. The 0% (v/v) "Water-free Washed" sucrose model system behaved exactly the same as the 0% (v/v) modified sucrose system. In general, however, systems with "Water-free Washed" sucrose had higher ΔWI than the control (0% sucrose system). As shown from Section 4.4.2, systems with modified sucrose with regular washing and drying process all showed lower ΔWI than the control system, opposite to the case of "Water-free Wash" system. The only difference between "Water-free Wash" and the regular wash is the moisture addition step. This implies that the moisture addition step might be an important step during the sugar surface modification process to give a bloom prevention effect. On the other hand, even though both gave a bloom prevention effect, compared to systems with unmodified sucrose with the same sugar concentration, the ΔWIs in the "Water-free Wash" system were lower. Thus, the effect of "Water-free Washed" sucrose on bloom promotion was less than modified sucrose with the regular wash and drying process.

Similar trends were shown in the stereomicroscopy and visual analysis. All white area percentages were below 10% indicating that bloom levels on the surface were still low. The stereomicroscopy results confirmed that adding "Water-free Washed" sucrose into systems

increased bloom extents compared to the control system (0% sucrose system). The final visual bloom levels were between 2 and 3, which means a few spots or some area on the chocolate surface turned white after 4-week storage.

Statistical analyses were conducted on the final ΔWI, white area percentage and visual bloom values at day 28 in "Water-free Washed" sucrose model systems as shown in Table 4.14. One-way ANOVA on the final ΔWI at day 28 with 4 levels of cocoa powder replacement level showed that cocoa powder replacement level had a significant effect on the final ΔWI values ($p<0.0001$). Systems with 25, 50, and 75% cocoa powder replacement level were all significantly higher than the 0% cocoa powder replacement system. One-way ANOVA on visual rates showed similar result ($p<0.0001$ for both tests). Unexpectedly, for tests on white area percentage, 0 and 25% cocoa powder replacement systems were significantly higher than 50 and 75% replacement systems, even though p-value of one-way ANOVA was still below 0.0001. This is because the bloom levels were too low (below 10%), which makes bloom evaluation within this range unreliable, as described in Section 4.3.1.4. Comparing to Table 4.13, where systems with sucrose after the modification process with washing and drying decreased or did not change bloom extents, systems with sucrose after the "Water-free Wash" process increased bloom extents or did not show differences. Thus, the effect of the "Water-free Wash" process was the opposite of the modification process with washing and drying.

Table 4. 14 Changes in whiteness index (ΔWI), white area percentage and visual bloom levels of chocolate model systems made of 50% cocoa butter, 50% sucrose (modified by "Water-free Wash" process with all the same steps as the modification method in Section 4.4.2 except for that of water addition) and 0.5% lecithin (referenced to total mass in the control model system system) with different cocoa powder replacement levels (0, 25, 50, and 75% on a volume basis) at day 28. Data represent means ± standard deviations.

Bloom evaluation	Cocoa powder replacement level (%)			
	0	25	50	75
ΔWI	1.6 ± 0.3^{d}	3.7 ± 0.4^{a}	2.9 ± 0.4^{b}	2.3 ± 0.4^{c}
White Area (%)	10.0 ± 2.7^{A}	6.6 ± 4.6^{A}	0.9 ± 0.1^{B}	2.0 ± 0.6^{B}
Visual Bloom	$2.4 \pm 0.2^{\beta}$	$3.2 \pm 0.3^{\alpha}$	$3.2 \pm 0.3^{\alpha}$	$3.2 \pm 0.3^{\alpha}$

$^{a,b,c,d; A,B; \alpha,\beta.}$ Means not connected within each evaluation method with the same letter are significantly different (α=0.05).

In order to explain the results in the "Water-free Wash" samples, particle shape and circularity were checked. Optical microscopy showed that particles in the white chocolate mix after the "Water-free Wash" process still had sharp edges, as shown in Figure 4.54, which is expected since there was no water added to dissolve the jagged edges on the sucrose surface. Circularity of the particles before or after "Water-free Wash" was evaluated. The average surface circularity before "Water-free Wash" was 0.74 (standard deviation = 0.07), compared to 0.75 (standard deviation = 0.07) after "Water-free Wash". One-way ANOVA conducted on the circularity with two levels of particle type showed that "Water-free Wash" did not have significant effect on particle circularity (p=0.2347), as indicated by the microscope pictures. Thus, the shape of the particle was not a factor in the bloom results.

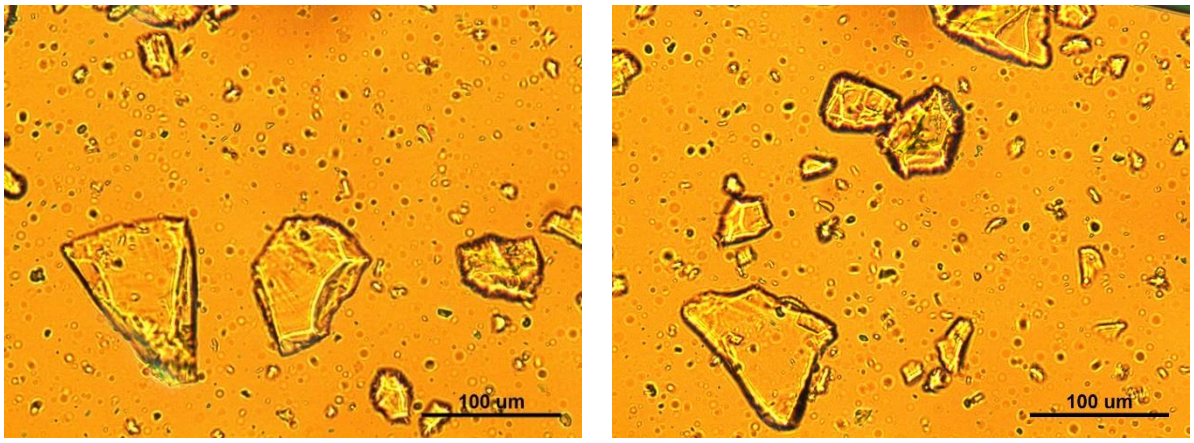


Figure 4. 54 Two representative pictures from optical microscope of sugar particles after the "Water-free Wash" process with all the same steps as the modification method in Section 4.4.2 except for that of water addition).

Moisture might be another factor since it was shown in Section 4.3.2.1 that CSS, with a much higher moisture content than sucrose, had higher Casson viscosity than sucrose, indicating higher particle interaction. Water bridges enhance adhesion and change particle arrangement in the dispersion system. Thus, systems with higher moisture content may have higher particle interaction resulting in higher bloom extent (Johansson and Bergenstahl, 1992a; Barbin et al., 2005). To make system with "Water-free Washed" sucrose, every step before tempering was the same as the system with unmodified sucrose except that the "Water-free Wash" procedure had an extra two-day mixing at 60ºC, which might potentially dry out some moisture from the system. In chocolate, lecithin may be present as reverse micelles (Wolf, 2017). Kanamaru and Einaga (2002) reported that in a lecithin-water-organic solvent system, reverse micelles were formed by lecithin in organic solvent and their size increased with the addition of water. Thus, it is possible that water is trapped within the lecithin reverse micelles and the drying process reduced their size, which might potentially increase the tortuosity for fat migration during storage and inhibit bloom formation (Hartel et al., 2016).

Dispersion time of lecithin during chocolate making is another potential factor. In the "Water-free Wash" process, there was an extra two-day mixing for the white chocolate mix and lecithin was added into the system in two steps. It would take time for lecithin to be well dispersed and go to the interface between particles and liquid fat. Prawira and Barringer (2009) reported that longer conching time resulted in smoother chocolate, which potentially supported that dispersion time of lecithin might be an important factor. Thus, it is possible that the "Water-free Wash" process favors lecithin dispersion that enhances lecithin's effect on bloom reduction.

4.4.4 Particle surface circularity

Optical microscope and Image J were used to quantify the shape of the different sugar particles. Figure 4.55 shows representative pictures from optical microscope of sugar particles including sucrose, maltitol, CSS and PD. By visual evaluation, sucrose had a relatively sharp surface and CSS had a very round surface. PD and maltitol were midway among them. Circularity of these sugar surfaces was calculated by Image J, as shown in Table 4.15. One-way ANOVA showed that the sugar type had a significant effect on the surface circularity of sugar particles ($p<0.0001$). Tukey HSD showed that circularity was highest in CSS and lowest in sucrose. For PD and maltitol, there was no significant difference between them. This agreed with the visual evaluation.

Table 4. 15 Circularity calculated by Image J of different sugars (sucrose, maltitol, corn syrup solids and polydextrose). Data represent means ± standard deviations.

Modification	Sucrose	Maltitol	Corn syrup solids	Polydextrose
Circularity	0.74 ± 0.07^{c}	0.84 ± 0.07^{b}	0.90 ± 0.07^{a}	0.83 ± 0.06^{b}

[a,b,c.] Means not connected with the same letter are significantly different.

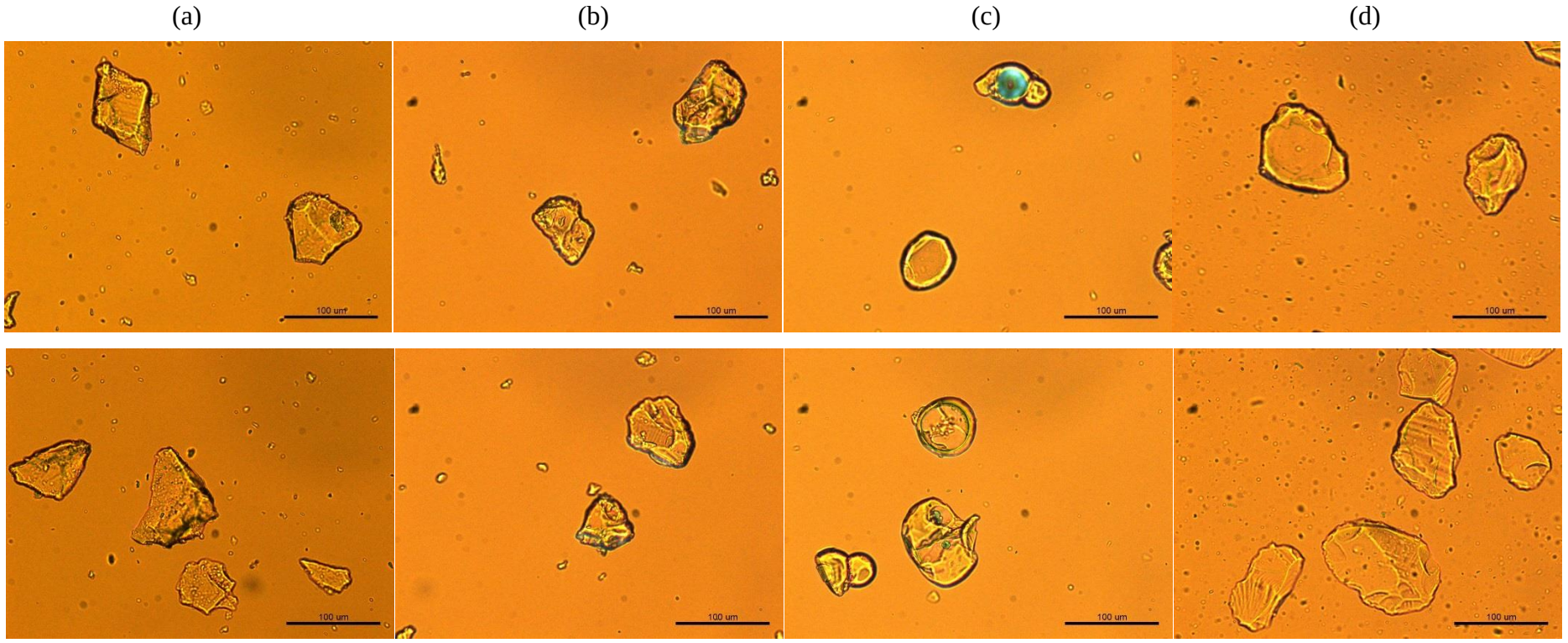


Figure 4. 55 Representative pictures (two for each sugar type) from optical microscope of sugar particles: (a) sucrose, (b) maltitol, (c) corn syrup solids, (d) polydextrose.

In order to evaluate the correlation between particle circularity and bloom, the final ΔWIs in the model systems with unmodified sucrose, sucrose after the regular washing and drying, maltitol, CSS and PD at a concentration of 50% concentration (referenced to the total particle volume in the dispersed phase) were plotted against circularity in Figure 4.56. In general, particles with lower circularity resulted in higher final ΔWI. This might be expected since particles with lower circularity usually have sharper edges that potentially provide nucleation sites for recrystallization. Also, particles with a smoother surface do not favor recrystallization and the round shape favors greater particle packing that might block the migration pathway (Bricknell and Hartel, 1998; Hartel et al., 2016). However, not all particles followed this trend. For instance, even though the circularity of CSS was significantly higher than maltitol, its final ΔWI was higher than maltitol. This might be due to the high moisture content in CSS. As stated in Section 4.3.2.1, with moisture content not controlled, bloom extents with those particles cannot be precisely compared.

In sum, there was a negative trend between circularity and bloom in some systems, but not all sugar particles followed this trend. Unmodified sucrose had the lowest circularity (sharpest surfaces), which favors recrystallization for bloom, thus having the highest bloom level when added into chocolate system. Systems with maltitol and sucrose after regular washing and drying had smoother particle surfaces that might not favor fat recrystallization. Unexpectedly, CSS also had a relatively higher bloom level compared to maltitol, even though its surface was the smoothest, which might be due to the high moisture content. Circularity correlates with bloom results in some systems, but there was no strong evidence that circularity is a significant factor that can completely explain the occurrence of bloom.

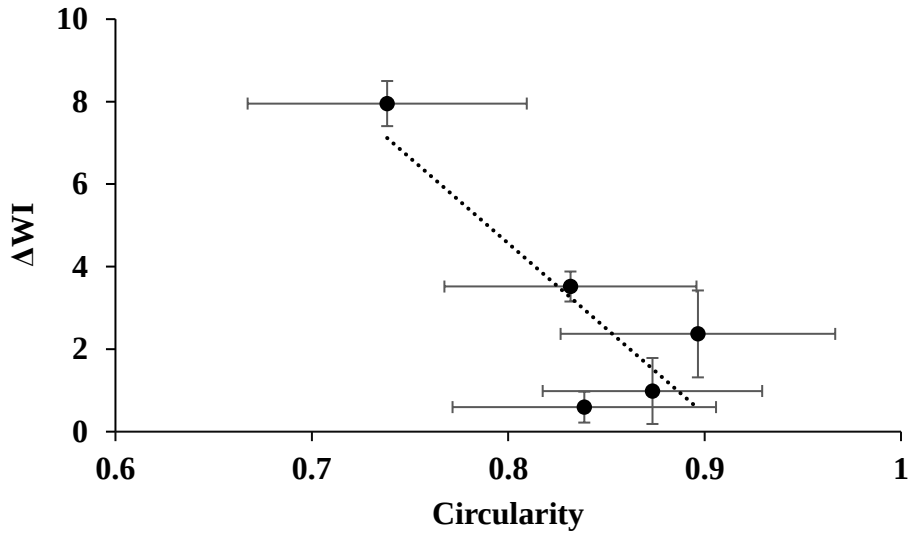


Figure 4. 56 Plots of changes in whiteness index (ΔWI) in chocolate model systems (50% cocoa butter and 50% particles) after 28 days, where cocoa powder was replaced by unmodified or modified sucrose (with regular washing and drying), maltitol, CSS or PD at 50% level (v/v) with 0.5% lecithin (referenced to total mass in the control system) against circularity of these particles.

5 Summary, conclusions and recommendations

5.1 Summary

5.1.1 Effects of fat phase on fat bloom in chocolate model systems

Isothermal diagrams of mixtures of cocoa butter stearin (CB-S) and liquid oils (peanut oil, cottonseed oil, canola oil and sunflower oil) were constructed to find formulations to make chocolate with solid fat content (SFC) of 45, 55 and 65%. The 20 ºC isothermal diagrams between CB-S and all liquid oils were similar and linear indicating little interactions between CB-S and liquid oils. Chocolate model systems were formulated by mixing 50% cocoa powder and 50% fats with formulations obtained before. Chocolates were stored under fluctuating temperatures and bloom was tracked by differential scanning calorimetry (DSC) and Whiteness Index (WI) measurement. DSC confirmed that polymorphic transition of cocoa butter from βV to βVI still occurred in the chocolate model system. WI curves over time indicated that decreasing SFC significantly increased initial bloom rate resulting in higher final bloom extent. This agreed with the literature (Ramsom-Painter et al., 1997; Ali et al., 2001) and supported the fat dissolution, migration and recrystallization theory of bloom since lower SFC provides more liquid oils with more dissolved high-melting fats to migrate to the chocolate surface and reduces viscosity to provide less resistance for migration. Liquid oil type had no significant effect on final WI values thus being a minor factor for bloom.

5.1.2 Effects of temperature fluctuation frequency on fat bloom during storage

Chocolate model systems with canola oil with SFC of 45, 55 and 65% as well as commercial chocolate were stored under different storage temperature fluctuation frequencies (3-hour, 7-hour, and 11-hour). WI curves over time showed that increasing SFC of cocoa butter tremendously enhanced chocolate bloom resistance. Temperature fluctuation frequency was also a significant factor but the effect was more complicated. In the chocolate model system, which had no sugar, with lower SFC, a slower temperature fluctuation promoted more severe bloom, whereas in commercial chocolate, which had large amount of sugar, with higher SFC, a slower temperature fluctuation reduced fat bloom. This discrepancy in the WI trend between commercial chocolate was likely due to the changes in the microstructure from different SFCs, particulate levels and lecithin levels were different. This might have a great difference on the particle network in the chocolate system (Johansson and Bergenstahl, 1992a) and change the migration pathway, kinetics and even the nature of migration during bloom. Further, the absence of sucrose in the model system might change the nature of recrystallization during bloom. In sum, the changes in the migration and recrystallization steps due to the differences in the microstructure between the two systems might be potential explanations for the discrepancy in bloom results.

5.1.3 Effects of different nonfat particles on fat bloom during storage

Chocolate model systems where cocoa powder was replaced by sugar particles (sucrose, maltitol, corn syrup solids (CSS) and polydextrose (PD)) by 25, 50 or 75% on a volume basis were stored and evaluated for bloom extents by change in whiteness index (ΔWI), white area

percentage and visual bloom. All three bloom evaluation methods showed similar trends. Increasing sucrose level significantly promoted bloom, either with or without 0.5% lecithin. Bloom extents in maltitol, CSS and PD-based systems were not as severe as in the sucrose system. Only 75% CSS, 50 and 75% PD systems showed significant bloom compared to the control. Adding 0.5% lecithin significantly reduced bloom extents in all model systems except for the 50% CSS system.

Particle interactions in sugar-in-fat dispersions where four sugars were dispersed in melted cocoa butter were evaluated by Casson viscosity, sedimentation volume and contact angle. CSS had the highest Casson viscosity, maltitol had the lowest, and sucrose and PD were in the midway. The high viscosity of CSS and PD systems might be due to their high hygroscopicity. When lecithin was added, Casson viscosity was only significantly reduced in the sucrose system, showing no changes in the other three. The sucrose system had a significantly higher sedimentation volume than the other three systems and the reduction effect of lecithin was only shown in the sucrose system. Contact angles among the four sugars were very close. Only sucrose had a significantly higher contact angle than CSS. Surface impurity might influence contact angle, which may be an explanation for the difference between CSS and sucrose system, as shown from the difference in contact angle among compressed sugar, rock candy and washed rock candy. Only the sucrose system showed strong correlation between particle interaction and bloom. The correlations were weak in the other three due to the high hygroscopicity of CSS and less reduction effect of lecithin on particle interaction in maltitol, CSS and PD systems.

5.1.4 Effects of particle surface properties on fat bloom during storage

The surface of sucrose particles in the chocolate model system was modified by a washing and drying process and bloom in the model system after modification during storage was evaluated by the same methods as the previous phase. After modification, the surface of sucrose was significantly smoother and rounder as shown from the rheology and optical microscopy. Bloom was significantly reduced as expected. This might be due to the reduced surface circularity and impurity after washing. However, a control "Water-free Wash", another washing process that followed every step in the previous modification process except for that of water addition resulted in bloom extents that were higher than that of regular washing. This might due to the extra drying and mixing process in the "Water-free Wash". The extra drying process might dry out more moisture that was trapped in lecithin reverse micelles thus reducing the micelle size (Kanamaru and Einaga, 2002) to increase migration tortuosity and reduce bloom. The extra mixing step might favor lecithin dispersion to enhance its reduction effect on bloom. Comparing among different sugar particles, there was a negative correlation between surface circularity and bloom extents, but not all of them followed the same trend. There is still no sufficient evidence that surface circularity has a significant effect on bloom.

5.2 Conclusions

It was hypothesized in this study that fat bloom during storage is formed with three steps: (1) dissolution of high-melting fats in low-melting fats in chocolate, (2) migration of liquid fats onto the surface of chocolate by diffusion or capillary force, and (3) recrystallization of high-melting fats on the chocolate surface (Hartel, 1999; Matsuda et al., 2001; Hartel and Lonchampt,

2004; Dahlenborg, 2014). It is the particles in the chocolate matrix that changes the microstructure of chocolate and particle interactions thus changing the bloom steps. Several results in this study are consistent with this hypothesis. For the fat phase, reducing SFC significantly promoted bloom since reducing SFC increased the amount of liquid fats together with more dissolved high-melting fats to migrate and recrystallize. Of the sugar particles, sucrose, a crystalline sugar that favors cocoa butter recrystallization for visual bloom, had the highest bloom extent compared to maltitol, CSS and PD. On the other hand, when modified by a washing and drying process, bloom was significantly reduced in the sucrose model system since the particle surface was smoother and rounder, which did not favor cocoa butter recrystallization and migration (higher tortuosity). For lecithin, adding 0.5% lecithin significantly reduced bloom in most model systems, potentially because of its effect on changing particle networks.

However, there are several results in this study that are not clearly explained. Comparing the bloom results in commercial chocolate and model system, the trend in the effect of temperature fluctuation frequency was in the opposite way: slower temperature fluctuation promoted bloom in the chocolate model system while reducing bloom in commercial chocolate. It is hypothesized that the difference in the microstructure due to different SFCs, particulate levels and lecithin levels might be a potential explanation as they all influenced the migration and recrystallization steps. When analyzing the correlation between particle interaction and bloom, the correlation was only strong in the sucrose system. It is suggested that moisture content, a factor that was not fully controlled, might greatly influence bloom and particle interaction measurements, especially in the highly hygroscopic CSS and PD systems.

Furthermore, comparing bloom extents in the “Water-free Washed” sucrose system where sucrose was modified using the same steps but with no water added and the untreated sucrose system, bloom was significantly reduced in the “Water-free Washed” sucrose system. It is suggested that it might be possible that the moisture content and lecithin dispersion time was not controlled in the two systems. It is concluded from these findings that besides particles in chocolate, moisture content might be another important factor that greatly influences particle interactions and bloom.

5.3 Recommendations

The discrepancy in the bloom trend between model system and commercial chocolate was not fully understood. Both of the bloom behaviors, either increasing temperature fluctuation frequency to promote bloom in commercial chocolate or reducing temperature fluctuation frequency to inhibit bloom in the chocolate model system, were shown in literature (Quevedo et al., 2005; Rothkopf et al., 2017), but there was no systematic study that controls particulate level, SFC, lecithin, and crystallization procedures to explain the discrepancy. Future work could be done to develop a chocolate model system that is more similar to the commercial chocolate to evaluate the effects of these parameters.

More sugar particles could be evaluated for bloom. Based on the hypothesis that interactions between particles may influence change the microstructure of chocolate to influence the migration and recrystallization steps during bloom, future work could focus on adding amorphous sucrose, fructose, lactose, erythritol and inulin to better understand the correlation between particle interaction and bloom.

Moisture content is the factor that was not successfully controlled especially in phase three and four of this study. High hygroscopicity of PD and CSS significantly increased their moisture contents. Thus, it is unclear whether the high bloom levels in 75% CSS and 50 and 75% PD systems were due to the particles themselves or just the moisture. It also influenced rheology of CSS and PD dispersions, which greatly influenced the correlation between bloom and particle interaction results. In future works, it would be good if specific drying procedures as well as humidity control system could be applied to control the moisture contents in the model systems. It would even be interesting to evaluate the effect of different moisture content levels on fat bloom.

Further, lecithin plays an important role in bloom. One possible mechanism is that it interacts with water in chocolate to influence bloom. This might explain the difference in bloom results between model system after regular washing and after "Water-free Wash". Specific microscope technologies such as confocal microscopy and atomic force microscopy, as well as rheological technologies could be used to better understand the role of moisture and lecithin in chocolate during bloom formation.

Lastly but not least, even though this study tried three methods to quantify particle interactions in the model systems, there was still no direct and effective method to measure particle interactions. Storage modulus and yield stress might be good indicators of particle interactions; thus, strain sweep in the sugar-in-fat dispersion systems would be worth trying. Adsorption of emulsifiers to dispersed fat and sugar crystals in oils measured by high-performance thin-layer chromatography and zeta potential measurements in the dispersion system could also be potential methods.

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