The Role of Emulsifiers on the Crystallization of Cocoa Butter and Cocoa Butter-sugar Blends

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The Role of Emulsifiers on the Crystallization of Cocoa Butter and Cocoa Butter-sugar Blends

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Molecular Science

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Abstract

The crystallization of cocoa butter and 1:1 cocoa butter-sugar blends containing 2 wt% emulsifier was investigated. The emulsifiers studied were soy lecithin, polyglycerol polyricinoleate (PGPR), citric acid esters of mono- and diglycerides (CITREM) and ammonium phosphatides (YN). Lecithin, CITREM and YN enhanced nucleation and growth events substantially, however, had minimal effects on the form IV-to-V transition and enthalpy. PGPR showed a modest enhancement of crystallization kinetics but promoted the formation of form V polymorph similar to that of canola and castor oil. In the presence of sugar, emulsifiers were no longer able to influence nucleation and growth. Sugar also hindered the form IV-to-V transition in the presence of both emulsifier and oil. Overall, results showed that emulsifiers may be used to tune the isothermal crystallization of cocoa butter, though this depends on the presence of dispersed sugar.

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1.0 Introduction

Chocolate is a confectionery product produced from ground cocoa beans, sugar, emulsifier and, in the case of milk chocolate, milk solids. To produce chocolate, harvested cocoa beans are fermented, dried, ground and then refined. Grinding the beans releases cocoa butter allowing it to coat the cocoa solids. During refining, sugar is incorporated, and steel mills break the solid sugar and cocoa particles down to a uniform size, typically of 20 - 30 μm. The milling will also break up any agglomerates, and ensure all particles are coated in fat.[1] Chocolate is approximately 1/3rd cocoa butter by weight (Figure 1), the remaining 2/3rd comprising of sugar and cocoa solids. As a result, in the molten state, chocolate is very viscous, and almost paste-like.

Figure 1: A proportionally accurate representation of dark chocolate composition which contains (from left to right) cocoa nibs, sugar and cocoa butter.

The viscous nature of molten chocolate can be mitigated by the addition of more cocoa butter, increasing the quantity of continuous phase, or by introducing an emulsifier, which limits particle interactions.[2]

Tempering is a thermal process used to crystallize the fat phase of chocolate to impart desirable qualities in the finished product, such as a glossy finish, snap, and favourable melting properties

such as it melting at oral temperature.[3] Under-tempered chocolate will appear dull, crumble rather than snap, and melt more readily. As chocolate ages, it will eventually exhibit fat bloom, the whitish-grey coating that forms on the chocolate surface as a result of improper processing, temperature abuse or the presence of soft-center fillings (Figure 2).[4] While the chocolate hasn't technically spoiled, it has lost its desirable properties and, for all intents and purposes, reached the end of its shelf life. The physical properties unique to under-tempered, tempered and bloomed chocolate all arise from the crystallization behaviour of cocoa butter.

Figure 2: Examples of bloomed chocolates.

It is common in the confectionery industry to use emulsifiers to tune the flow properties of molten chocolate. However, there is the possibility that emulsifiers may also be used to adjust the crystallization and melting properties of chocolate to attain a longer shelf-life. While cocoa butter crystallization has already been studied in the presence of various additives, few ground-up mechanistic approaches have explored the structure-function relationships linking cocoa butter crystallization and the role of ingredients commonly found in chocolate, specifically the effects of sugar and emulsifier on cocoa butter crystallization rate and polymorphic transitions.

2.0 Cocoa butter crystallization in the presence of sugar and emulsifiers

2.1 Cocoa butter

Cocoa butter is a common ingredient in foods, pharmaceuticals, and cosmetics. The widespread use of cocoa butter in industry is due to its emollient properties and its melting range close to body temperature. Together, the cocoa solids and sugar suspended in cocoa butter determine the physicochemical properties of the final chocolate product.

2.1.1 Cocoa butter composition

Cocoa butter comprises a mixture of triacylglycerol (TAG) species. The major components are the monounsaturated TAGs.[5] Palmitic (P), stearic (S) and oleic (O) esters are present in the greatest quantities in cocoa butter as POP, SOS, and POS (Figure 3). Cocoa butter also contains minor amounts of other TAGs, containing linoleic (L) and arachidic (A) moieties.[6] In the major TAGs, the oleic acid moiety occupies the sn-2 position giving cocoa butter a lower melting point than if it were composed of TAGs containing oleic acid at the sn-1 or sn-3 position. Were oleic acid situated at the sn-1 or sn-3 position, there would be greater van der Waals interactions between adjacent saturated fatty acids at the remaining positions on the glycerol resulting in the higher melting temperature of the resultant fat.[7]

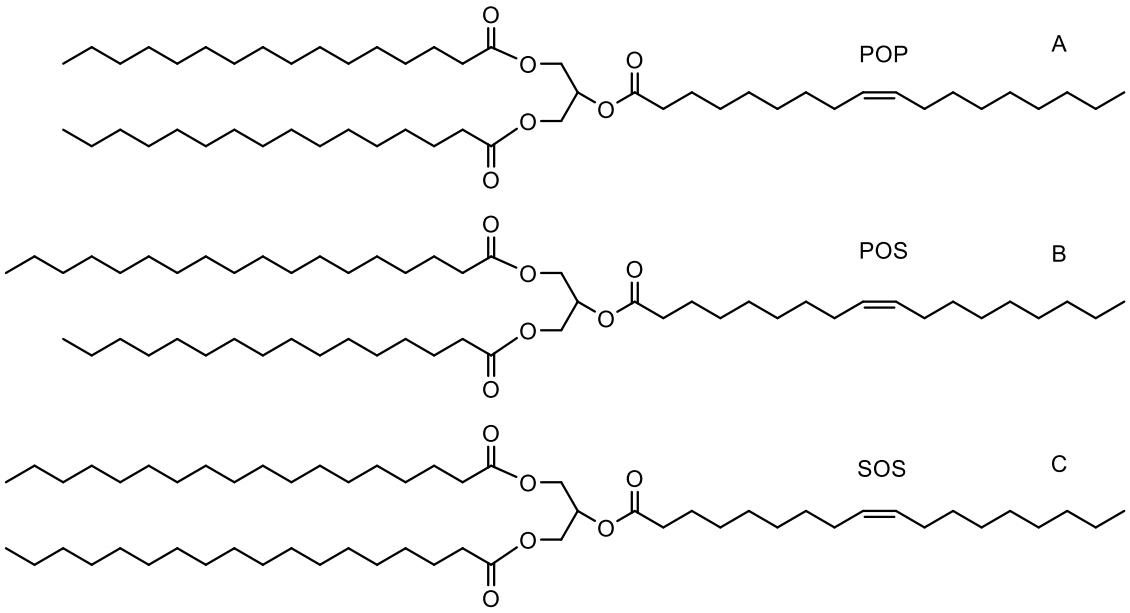

Figure 3: Major components of cocoa butter - POP (A), POS (B), and SOS (C).

This composition gives cocoa butter its desirable melting profile for use in confectionery as well as home-care products. Cocoa butter also contains higher-melting, saturated TAGs such as tristearin (SSS), as well as lower-melting, di-unsaturated TAGs such as POO and SOO, which melt below room temperature.[5,6] The presence of these minor fats gives rise to a two-step crystallization profile under isothermal conditions, where the high-melting fats will nucleate first in a primary crystallization event, followed by their growth. There is then a lag period where there are no crystallization events occurring and the solid fat content remains constant. Following this, there is a second crystallization event where the major TAGs (POP, SOS, POS) begin to crystallize. However, since there are low melting TAGs present, cocoa butter is never completely solid under most crystallization regimes, and there will always be a percentage of liquid oil present at room temperature (Figure 4). [8,9] The composition and crystallization behaviour of cocoa butter both depend on geographical origin and variety. Cocoa butters with a greater level of unsaturation will tend to be softer, whereas those containing higher levels of trisaturates will tend to be harder. [10,11]

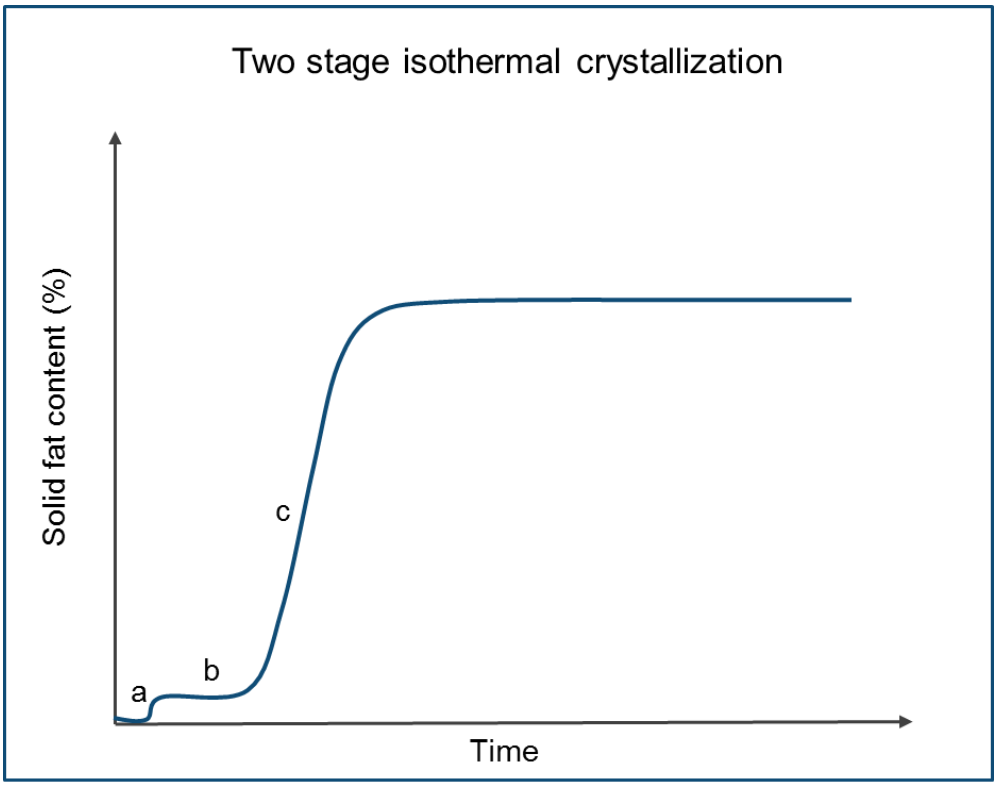

Figure 4: General two-step isothermal crystallization profile of cocoa butter. (a) Primary nucleation and growth, (b) Lag time, and (c) Secondary growth.[12]

Composition also affects the functionality of the cocoa butter in finished products. For example, filled chocolates made with a softer cocoa butters will tend to have higher rates of oil migration due to the increased liquid fraction.[13]

2.1.2 Cocoa butter crystallization

Polymorphism is a common trait among TAGs.[14] Polymorphs are distinct crystalline packing of the same material that have their own unique melting point, density, stability, and physical characteristics.[15] Cocoa butter has six identified polymorphs (I – VI), each with its own density and characteristic melting point (Figure 5).[16]

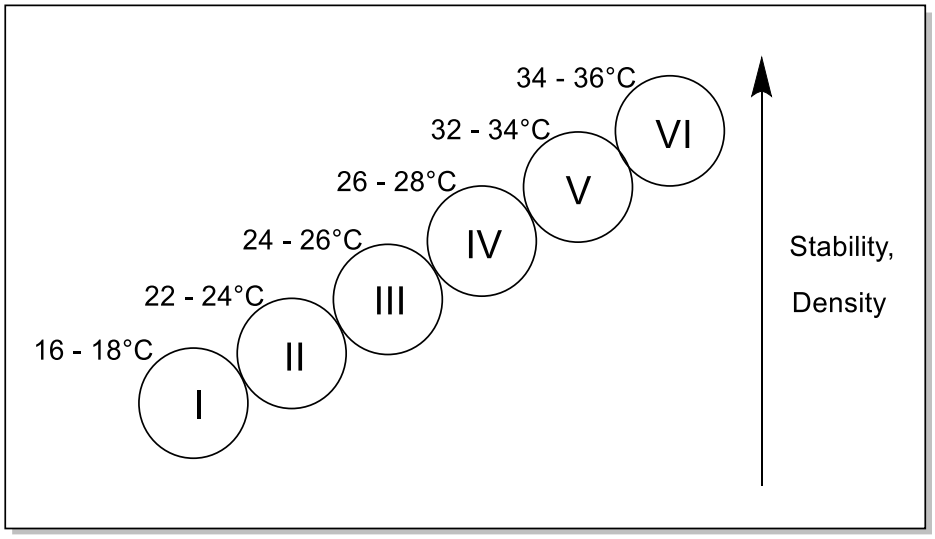

Figure 5: Schematic of cocoa butter polymorphs and associated physical attributes.[1]

The tempering of chocolate selects for the form V polymorph as it has the most desirable physical properties. In this polymorph, chocolate is solid at room temperature, melts in the mouth, has an appealing gloss and a satisfying snap, which allows flavour compounds and sugar to reach the consumer's taste buds. Form VI, while the most stable, gives chocolate a waxy texture when eaten. So-called fat bloom is closely associated with the form V-to-VI transition as the formation of the white coating on the exterior of chocolate tends to occur simultaneously with the transition.[17] Cocoa butter polymorphs below form V melt too readily and crumble rather than snap. Form V crystals will eventually transition to the form VI polymorph given its thermodynamic stability. Each polymorph has its own distinct crystal structure which allows for identification via X-ray diffraction,[16] with cocoa butter's polymorphic behaviour based heavily on its major TAG components, especially POS.[18] Wide-angle X-ray diffraction patterns have revealed that the major TAGs in cocoa butter (i.e., POP POS and SOS) tend to assume α (hexagonal, form II), β′ (orthorhombic perpendicular, forms III and IV), or β (triclinic parallel, forms V and VI) sub-cell

packing (Figure 6).[19,20] Small-angle diffraction patterns have shown that the chains may pack in a double length (α and β′ sub-cells) or triple length configuration (β sub-cell).[21]

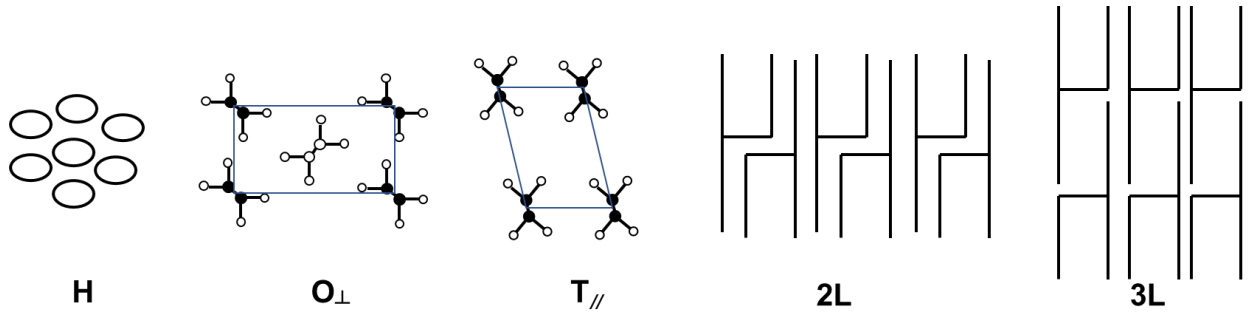

Figure 6: Sub-cell arrangements of α (hexagonal), β′ (orthorhombic perpendicular), and β (triclinic parallel)) crystals (left) and double (2L) and triple (3L) chain length structures (right).[21]

2.2 Dispersed solids

The intrinsic properties of sugar and cocoa solids give rise to chocolate's characteristic flavour and aromatic profile. In a finished chocolate, particles are milled beyond the detection limit of the tongue (< 30 μm) imparting a smooth, rather than gritty, mouthfeel. Dispersed particulates must be coated in fat to maintain to maintain a creamy texture once consumed.[22,23]

Polar particles tend to agglomerate in fat-continuous dispersions as agglomeration minimizes the contact of the polar surface with the non-polar continuous fat phase. Sugar particles immobilize fat at their surface, and agglomerates will trap more fat in the interstices between particles. In a system where the continuous phase represents ~ 30 % of the total mass and must coat the remaining ~ 70 % of dispersed phase, the presence of agglomerates will increase molten-state viscosity.[24] Sugar is the common name given to the disaccharide sucrose, which is comprised of two glucose units. It exists in a free-flowing crystalline form and typically represents ~ 50 wt% of the chocolate mass. During processing steps like refining and conching, the grinding action of the rollers creates

amorphous regions on the surface of sugar; thus, the surface has both crystalline and amorphous regions.[25,26] The presence of amorphous regions contributes to, and enhances, the tendency of sugar particles to agglomerate.[27]

One method of limiting particle interactions is by the addition of emulsifier. Emulsifiers can adsorb to the surface of particles which renders them chemically non-polar, thus limiting particle interactions.[28] Using emulsifiers to improve the flow properties of particles in suspension can also be found in other areas of materials science. Ferrofluids, for example, use emulsifiers to tune the dispersibility of particles in solvents of varying polarity.[29,30] Polar particles like titania and cellulose may also strongly bind water to their surface further enhancing interparticle interactions and increasing apparent viscosity in dispersions.[31,32] Similarly, sugar will agglomerate when dispersed within cocoa butter.

Dispersed particles can act as catalytic impurities to accelerate fat crystallization. Yoshikawa and co-workers found that addition of a variety of impurities at 1 wt% increased the crystallization temperature of the trisaturated TAGs trimyristin, trilaurin and tripalmitin. The additives ranged from inorganic materials, such as carbon nanotubes or talc, to organic compounds, such as theobromine and terephthalic acid, none of which shared molecular similarities with the crystallizing TAGs.[33,34] Exploring the effects dispersed particulates have on cocoa butter crystallization is important as they comprise ~ 70 % of chocolate's total mass. While cocoa butter crystallization itself is widely studied, the effect of dispersed particulates on cocoa butter crystallization has yet to be explored in great depth.

2.3 Emulsifiers

The term ‘emulsifier’ is generally used to describe a substance that stabilizes emulsions, which are mixtures of two immiscible liquids that would otherwise separate into two distinct phases. Emulsifiers can also be used to reduce particle interactions and are added to control the rheological properties of molten chocolate.[22,35] The common theory behind their mode of action is based on the adsorption of their polar head group to the polar particle surface of sugar, with the fatty acid moieties oriented away from the surface towards the continuous fat phase.[36] This, conceptually, is similar to how certain emulsifiers can stabilize water-in-oil emulsions, where the dispersed polar aqueous phase is analogous to dispersed polar sugar or cocoa solids. Emulsifiers that adsorb to oil-water interfaces will decrease the interfacial tension between the two phases. When emulsifiers adsorb to the surface of polar particulates in chocolate, this breaks up agglomerates and releases immobilized fat, increasing the amount of continuous phase and lowering the overall viscosity as a result. Individual emulsifiers tend to impart their own unique effect on flow properties, as discussed below.

2.3.1 Lecithin

Lecithin refers to a class of emulsifiers based around a phospholipid architecture with varying acyl chains and phosphonate esters as well as varying ammonium moieties. Two of the most common varieties are derived from either soybean or sunflower feedstocks. Commercial soy lecithin contains soybean oil, phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol, as well as several other minor components such as sterols, carbohydrates and water (Figure 6).[37] The fatty acids present in the phospholipids range from saturated to polyunsaturated, and include palmitic, stearic, oleic and linoleic acids.[37] Lecithin provides a cost-effective alternative to increasing the percentage of cocoa butter in chocolate formulations as it effectively reduces molten

chocolate viscosity at low percentages.[38] Soy and sunflower lecithin seem to have different mechanisms of action in fat systems containing sugar. Soy lecithin reduces the amount of immobilized fat at the surface of sugar particles and has a more even surface coverage whereas sunflower lecithin will create localized pockets on the surface of sugar and, as a result, will not reduce the amount of immobilized fat to the same extent as soy lecithin.[39] The reduction in immobilized fat corresponds to an increase in continuous phase, thus aiding flow properties. However, at higher percentages, lecithins can have the inverse effect where the viscosity increases as more lecithin is added, which is due to a bilayer forming with polar head-groups facing outwards. The resulting increase in particle interactions will increase the viscosity accordingly.

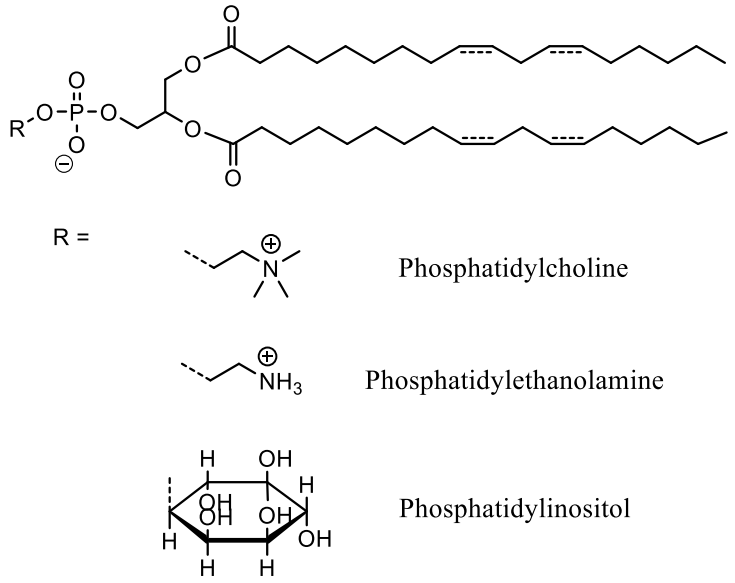

Figure 7: Major components of commercial lecithin.[40]

Though lecithin is primarily used to control the viscosity of molten chocolate, it may also affect the crystallization behaviour of cocoa butter. There is no current consensus on its prescribed effects, as in some cases, it has been shown to enhance cocoa butter crystallization rate, particularly at lower concentrations, but inhibit at higher concentrations (Figure 8).[41,42] Lecithin has also been associated with the formation of trisaturated cocoa butter TAG nuclei during primary crystallization.[9]

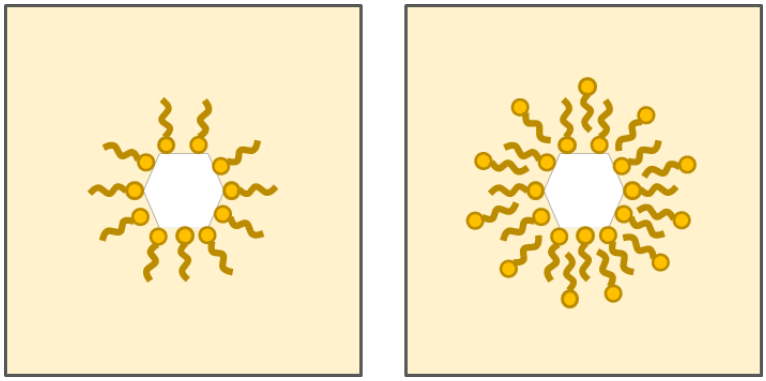

Figure 8: Schematic of a sugar crystal coated in a monolayer (left) and bilayer (right) of emulsifier molecules.

2.3.2 Polyglycerol polyricinoleate

PGPR is a synthetic, oligomeric emulsifier that has substantial interfacial tension-reducing properties.[43] PGPR contains a polyricinoleic acid chains esterified onto a polyglycerol backbone. PGPR used in commercial applications tends to contain oligomers of varying molecular weights due to its uncontrolled, high-temperature synthesis, where polyricinoleic acid and polyglycerol are synthesized independently, and then reacted together, generally *via* condensation. The repeating units in PGPR are glycerol and ricinoleic acid (Figure 9). PGPR is commercially synthesized using chemical methods but can also be produced enzymatically on a smaller scale.[44]

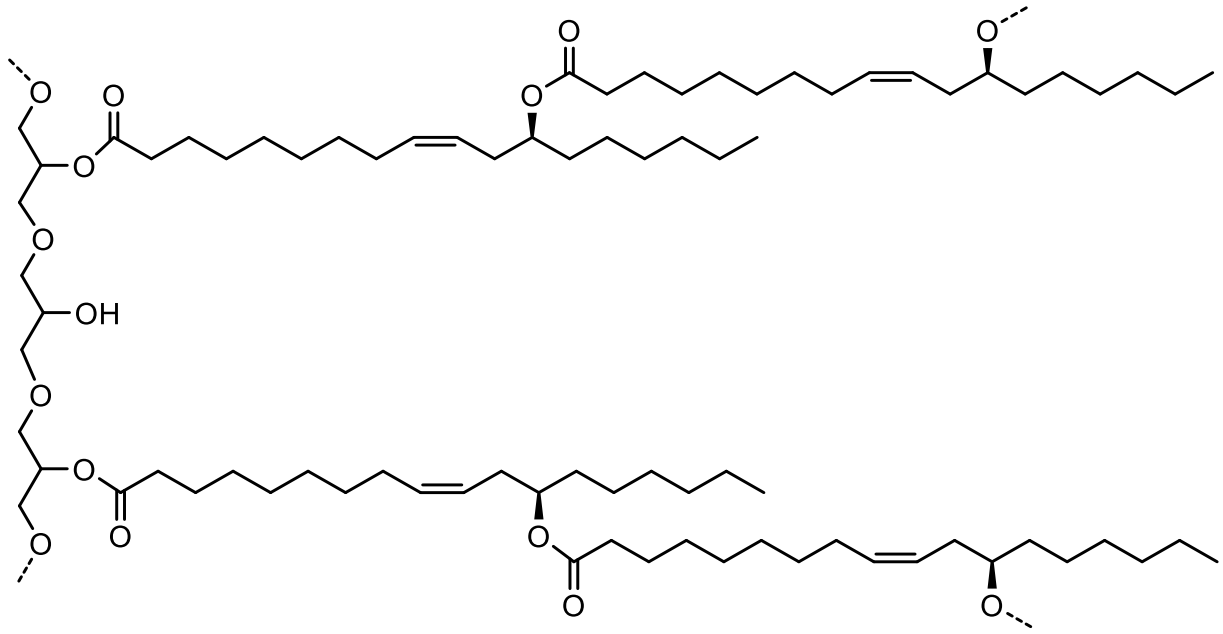

Figure 9: General chemical structure of polyglycerol polyricinoleate. [40]

PGPR reduces the yield stress in chocolate at very low concentrations (Figure 10). However, as it has a minor effect on plastic viscosity, it is commonly used in conjunction with other emulsifier such as lecithin.[1] PGPR, similar to lecithin, will coat the surface of sugar particles, increasing their lipophilicity.[28] PGPR on sugar surfaces will form "pillows" of immobilized fat which form boundary layers between particles.[45]

Figure 10: Effect of PGPR on yield stress. Chocolate containing no PGPR (left) and chocolate containing PGPR (right) have drastically different flow properties.

While some reports indicate that PGPR has no effect on the rate and thermal properties of cocoa butter crystallization, the topic lacks rigour and needs to be explored further.

2.3.3 Ammonium phosphatides

Ammonium phosphatides (YN) were developed as an alternative to lecithin, specifically for use in chocolate. The emulsifier is based around the same molecular architecture as phosphatidylcholine, except YN is the ammonium conjugate base of a phosphate, versus a zwitterionic compound. Much like PGPR, YN is produced synthetically. The fatty acid moieties of YN vary and depend on the fatty acids used in the synthesis (Figure 11).

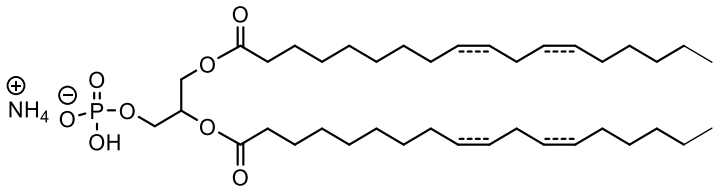

Figure 11: General chemical stucture of ammonium phosphatides.[40]

To produce YN, fatty acids are esterified onto glycerol, forming mono- and di-substituted glycerols. Phosphorus pentoxide is then used to form a phosphoric acid ester at the three-position on the glycerol. Ammonia is then used to neutralize the phosphoric acid moiety. YN performs comparably to lecithin in both their effects on molten chocolate rheology and crystallization, sometimes even outperforming lecithin.[40] Despite behaving similarly to lecithin, it is important to explore the effect of YN on cocoa butter crystallization to glean a more in-depth knowledge of its interaction with fats. YN is made from natural feedstocks but, ultimately, it is a synthetic compound, and as a result contains a greater concentration of phospholipids than lecithin, which contains soybean oil, sterols and carbohydrates. It may be considered a good analogue to observe the effect phospholipids alone will have on crystallization and polymorphic transition, as the other components in lecithin may have their own synergistic or antagonistic effects on crystallization.

2.3.4 Citric acid esters of mono- and di-glycerides (CITREM)

CITREM are another type of synthetic emulsifier where fatty acids are esterified onto a glycerol moiety with subsequent attachment of a polar citric acid group (Figure 12).

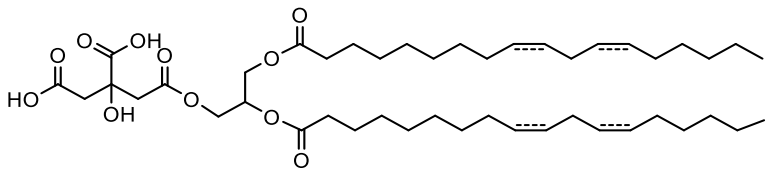

Figure 12: General chemical structure of CITREM.[40]

CITREM has shown improved rheological and textural properties of compound chocolates with a reduced fat content.[40,46] Similar to lecithin and PGPR, CITREM's viscosity-reducing capabilities arise from their adsorption to the surface of sugar, liberating immobilized TAGs.[40] Citric acid also acts as an anti-oxidant, as it can chelate metals that would otherwise catalyze the oxidation of the fat in chocolate. Since CITREM bears similar structural motifs to lecithin and YN, it may be used as a point of comparison for method of action between fatty acid chains and polar head groups.

2.4 Characterization techniques

2.4.1 Pulsed nuclear magnetic resonance spectroscopy (p-NMR)

Atomic nuclei can be considered magnets and, when placed in a magnetic field, will tend to align in the same direction as the field. When a perpendicular radio frequency is applied to the nuclei, they become excited. As the nuclei relax, a corresponding relaxation energy is released and detected. Depending on the environment of the nuclei the relaxation energy changes, thus giving insight into the magnetic environment of said nuclei. Using low-resolution pulsed nuclear magnetic resonance, such as the unit used in this study, we can observe the change in solid fat content (SFC) over time as p-NMR can detect the relaxation energy of ^{1}H nuclei in the solid state,

which relax more rapidly than ^{1}H nuclei in the liquid state.[47] This type of analysis, when run on a cocoa butter sample that is crystallizing over a defined period of time, produces the two-stage crystallization curves as described earlier. Using an Avrami model to fit the data, we can easily quantify crystallization parameters such as rates and nucleation times (Figure 13).

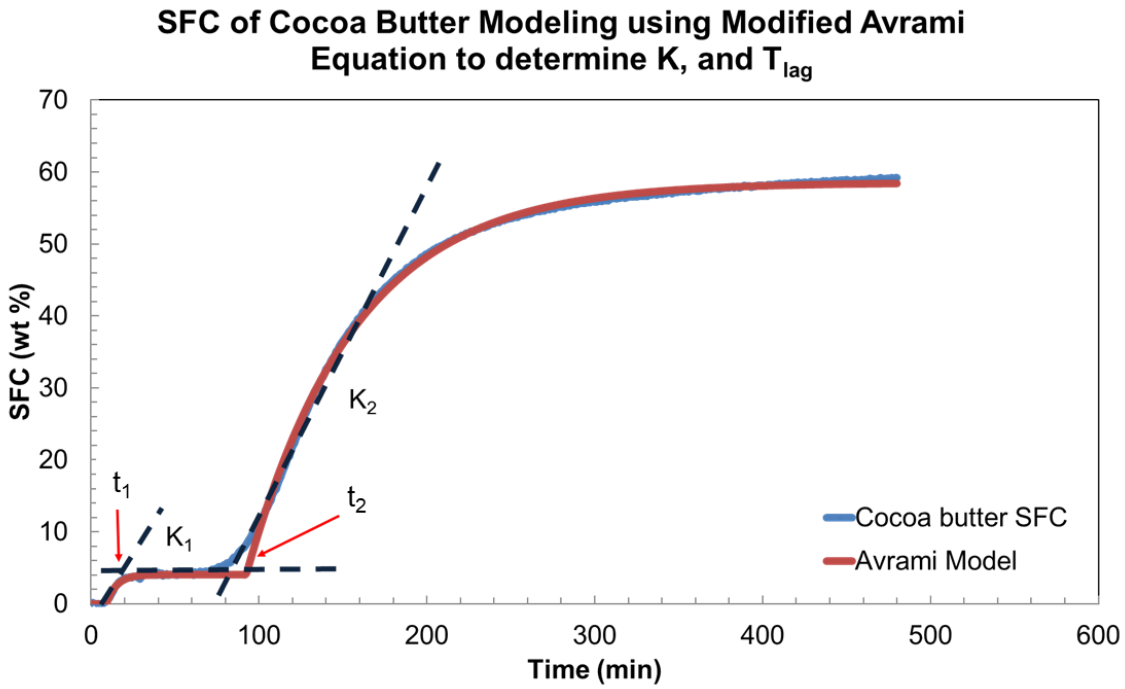

Figure 13: Avrami model fit to a cocoa butter crystallization curve.[48]

The Avrami model uses the following equation to fit two-stage crystallization curves:

$$SFC_t = \sum_1^i SFC_{\infty,i}\left(1 - e^{-K_1(t-t_{lag,i})^{n_i}}\right) \qquad (1)$$

where SFC_t is the solid fat content at time t and $SFC\infty$ is the solid fat content at equilibrium. K is a descriptor of crystal growth and nucleation rate, t_{lag} is the time indicating onset of crystallization and finally n describes the dimensionality of crystal growth. Comparison of crystallization

parameters between samples allows assignment of a numerical point of reference of the effect of the additive on crystallization.

2.4.2 Differential scanning calorimetry

During phase changes of matter, there is an associated change in energy. When liquids crystallize to form solids, energy is released as it is an exothermic process. In a similar manner, when solids melt there must be an input of energy to facilitate the endothermic phase transition. Differential scanning calorimetry (DSC) can monitor phase changes in materials. DSC tracks the energetic input or the lack thereof to maintain a substance's temperature relative to that of a reference. Therefore, we may use DSC to track changes in melting point, crystallinity and enthalpy and correlate this with polymorphic transitions in cocoa butter based on differences in melting point. For example, Figure 14 shows putative endotherms of cocoa butter crystallized isothermally. Since there are two distinct polymorphs that are present, the thermogram may then be processed *via* curve fitting techniques and integration to obtain the enthalpy (J/g), thereby providing insight into the degree of crystallinity as well as the enthalpic contributions of each given polymorph. Comparing the enthalpy of one polymorph to the enthalpy of the sample as a whole, the percentage of that polymorph, at a given time, can be obtained.

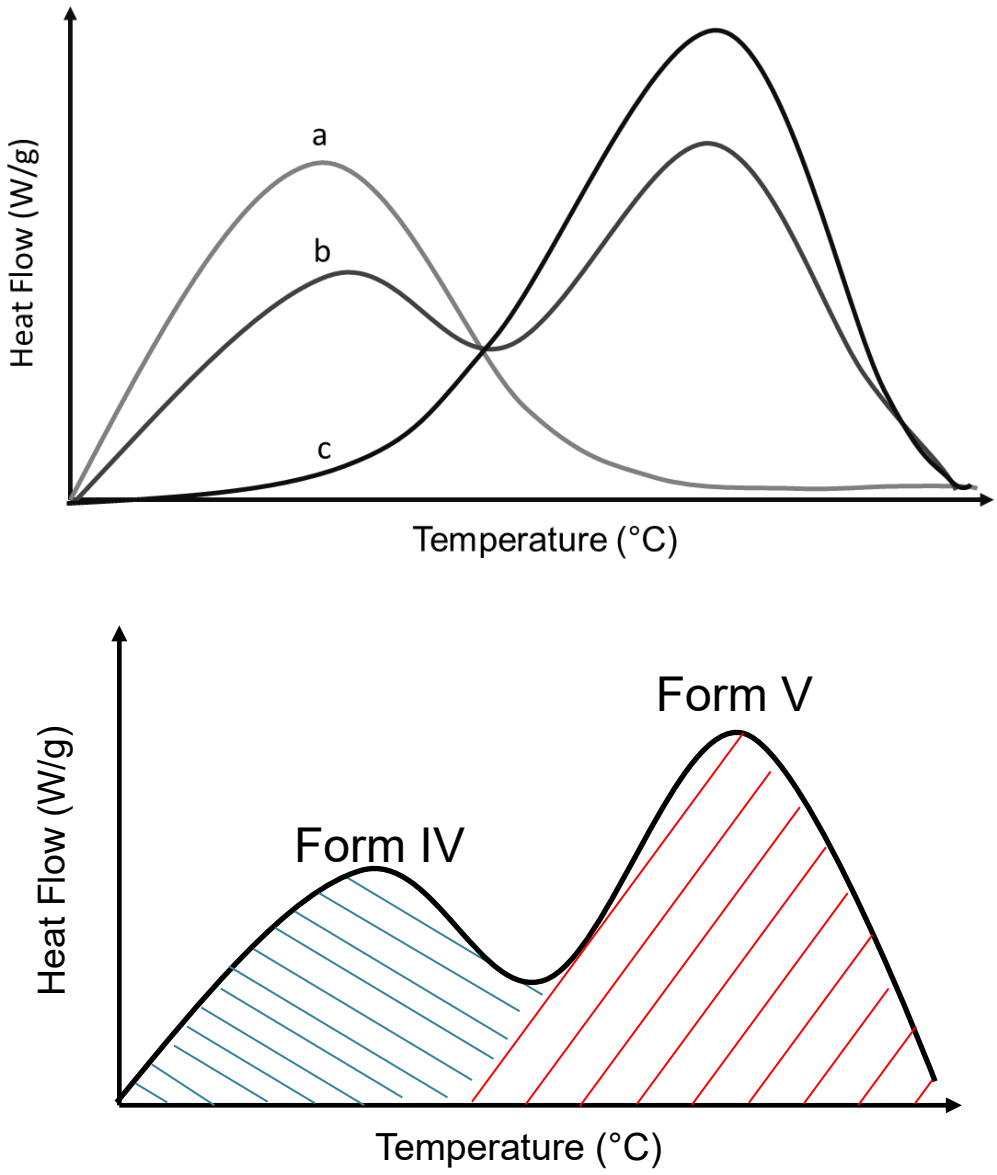

Figure 14: General arbitrary thermal profile of cocoa butter containing a lower-melting polymorph (a), a mixture of a lower and a higher-melting polymorph (b), and finally a higher-melting polymorph (c) (top). Using integration, we may estimate the relative amounts of the two polymorphs (bottom).

2.4.3 X-ray diffraction

X-ray diffraction uses the reflection of monochromatic x-rays off of the planes of a crystalline surface to describe the arrangement of molecules within a lamella and within a sub-cell. X-ray

diffraction is described using Bragg's Law (Figure 15), where n is a positive integer, λ is the wavelength, d is the interplanar spacing, and θ is the scattering angle.

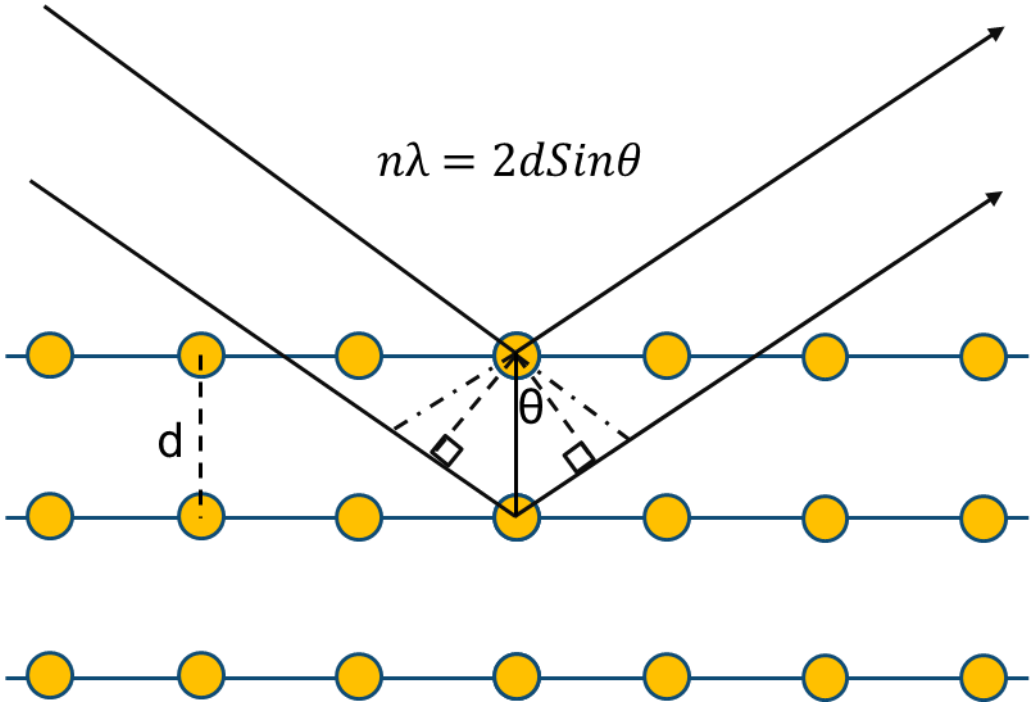

Figure 15: Schematic of Bragg's Law. [49]

X-ray diffraction has been used extensively in the literature pertaining to fat crystallization.[21,50–54] This method of analysis gives insight into molecular arrangements in a crystal lattice. Long spacings describe how TAGs are arranged within a lamella, while short spacings describe the TAG packing and orientation within a sub-cell. While DSC can give us insights into polymorphs present based on relative melting point, X-ray diffraction is a definitive indicator of the molecular packing of TAGs in a crystal structure.

2.5 Current knowledge gaps

While emulsifiers are commonly known to modify bulk fat crystallization kinetics and polymorphic transitions, there remains a lack of consensus on the mechanisms underpinning these effects. In bulk fats, the molecular structure and concentration of added emulsifier may promote or hinder crystallization events. In chocolate and other confectionery products, dispersed particles such as sugar may represent upwards of 70 % of the overall mass. Characteristics of the dispersed phase, such as surface properties, concentration and morphology, may affect the crystallization of the fat phase. While cocoa butter's crystallization behaviour in the presence of many different emulsifiers has already been established, few ground-up mechanistic efforts have elucidated the structure-function relationship linking the effects of multiple added components such as sugar and emulsifiers and their effects on cocoa butter crystallization. To this end, here, cocoa butter blends were studied with the aim of contributing to the knowledge base on the underlying mechanisms that govern cocoa butter crystallization rate and polymorphic behaviour and the effects of added sugar and emulsifiers relevant to the confectionery industry.

3.0 Objectives and hypotheses

The objectives of this thesis were to evaluate how sugar and emulsifiers affect cocoa butter crystallization, namely:

a) The ability of emulsifiers to affect cocoa butter crystallization under isothermal conditions in relation to their molecular structure.

b) The effect of a high weight percentage loading of sugar on cocoa butter crystallization under isothermal conditions.

c) The combined effect of sugar and emulsifier on cocoa butter crystallization under isothermal conditions.

The governing hypotheses were that:

1) Emulsifiers will alter cocoa butter crystallization, with their effect(s) depending on their molecular structure;
2) The presence of sugar will enhance cocoa butter crystallization, and;
3) The presence of sugar will interfere with the emulsifier's ability to impact fat crystallization.

4.0 Materials and methods

4.1 Materials

Commercial cocoa butter, canola oil and lecithin were provided by Mondelēz Canada (Toronto, ON). Cocoa butter had the following TAG composition (%): PPL (2.0), POO (3.4), PLS (3.0), POP (24.4), SOO (4.2), SSL (3.7), POS (29.3), SOS (28.8), SOA (0.8). Canola oil was purified on silica gel (60Å, 70-230 mesh, 63-200 µm) purchased from Sigma Aldrich (St. Louis, Missouri, USA) to remove any partial acylglycerols and free fatty acids. PGPR 4150, CITREM 4205 and AMP 4455 were acquired from Palsgaard (Juelsminde, Denmark) and had average molecular weights of 1500, 465 and 700 g/mol respectively. CITREM 4205 and AMP 4455 are produced using sunflower oil and have a high oleic content (75 – 90%). All emulsifiers were used as received. 10x confectioner's sugar (average particle size 60 µm) was purchased from Domino Foods (Iselin, New Jersey, USA) and used as received.

4.2 Methods

4.2.1 Preparation of stock samples

Melted cocoa butter (50 °C) was mixed thoroughly prior each to use, ensuring homogeneous mixing and minimizing error due to fractionation. Cocoa butter and emulsifier (at 2 wt%) were added to a 250 mL beaker and magnetically stirred at 40 °C for one hour. Cocoa butter and emulsifier mixtures were used to produce samples containing sugar. A 1:1 mixture of 10x confectioner's sugar and pre-mixed 2 wt% emulsifier-cocoa butter solution was magnetically stirred at 40 °C to ensure homogeneity.

In an industrial setting, ~0.6 wt% emulsifier is added to the final mass of chocolate to improve flow behaviour. However, the quantity of emulsifier is relative to the entire chocolate mass, which

considers sugar crystals, cocoa particles and milk powder, if present. Since the emulsifier is dissolved in the fat phase, the relative concentrations of fat to emulsifier are different. Here, 2 wt% emulsifier in cocoa butter was chosen relative to cocoa butter which represents ~ 30 % of the finished chocolate mass. This ensured that the relative concentrations were representative of that of a chocolate product, and with each addition, the relative concentrations remained constant.

4.2.2 Solid fat content (SFC) analysis

SFC was determined using a p-NMR (Minispec mq20, Bruker, Karlsruhe, Germany). A 2.0 mL aliquot of sample was added to an NMR tube (10 mm O.D.). Samples were then heated at 80 °C for 10 minutes to remove crystal memory. The sample was then transferred to a 20 °C water bath for 1 minute. The outside of the tube was wiped dry with a Kimwipe and immediately transferred to the NMR sample port with the sample chamber set at 20 °C. Measurements were taken every 60 seconds over 12 hours.

4.2.3 Melting temperature and enthalpy

The cocoa butter thermal behaviour was characterized using a DSC (Q2000, TA Instruments, New Castle, DE, USA). Molten samples (~ 5 mg, corrected for the presence of sugar) were placed in an aluminum DSC pan which was then hermetically sealed. Samples were heated in an 80 °C oven for 10 minutes, and subsequently aged in a 20 °C incubator. Measurements were taken after 24, 48, 72 or 96 hours. Peak fitting and integration were performed using OriginPro 2015 software (Northampton, Massachusetts, USA).

4.2.4 X-ray diffraction

Cocoa butter diffractograms were generated using a powder X-ray diffractometer (Miniflex 600, Rigaku, Tokyo, Japan), where molten samples (2.5 mL) were placed in an aluminum pan. Samples

were heated in an 80 °C oven for 10 minutes, and subsequently aged in a 20 °C incubator. Measurements were taken after 24 or 96 hours in an aluminum sample holder.

4.2.4 Statistics

All reported results are the arithmetic mean (± standard deviation) of triplicate experiments unless stated otherwise. Analyses were performed using one-way analysis of variance (ANOVA) with a *post-hoc* Tukey HSD test to compare multiple groups. A pairwise comparison was performed using the Student t-test. Differences were considered significant at $p \leq 0.05$. The ANOVA was performed using the free software [R] (www.r-project.org). The Student t-test was performed using Microsoft Excel (Redmond, Washington, USA).

5.0 Results and discussion

5.1 Crystallization kinetics

Rosen and Dahanayake postulated that emulsifiers should serve specific functions. One function is that, when added to a system, they should adsorb to an interface and change the properties of that interface. The other function is that they should aggregate in the solvent that they are dissolved in and change the properties of that solution.[55] In fat-based systems, emulsifiers potentially modify fat crystal behaviour.[56] Their ability to influence crystallization is based on the idea of molecular complementarity in the molten state. If the emulsifier structure is similar to that of the fat, there is a greater complementarity and thus an enhanced interaction between the fat and emulsifier. Conversely, if the structures are dissimilar, there is a lack of complementarity. Emulsifiers may facilitate nucleation by lowering the energetic barrier for nuclei to form, or in the case of higher-melting emulsifiers, they may nucleate first, providing a surface for fat to nucleate from. Alternatively, emulsifiers that are dissimilar may hinder nucleation by coating pre-existing nuclei,

preventing TAGs from crystallizing off of their surface. Secondary growth can also be hindered by a similar shielding effect of the emulsifier, making the crystal facets less favourable for surface nucleation and growth. Primary and secondary growth are distinct processes where the addition of an emulsifier may promote nucleation and growth in early crystallization, yet not affect, or even hinder secondary crystallization, or vice-versa.[48]

Figure 16 shows the effect of added emulsifiers on cocoa butter crystallization. In all cases, the addition of either emulsifier or oil shortened nucleation times and enhanced crystal growth rates (Table 1). The control, cocoa butter, and all emulsifier-containing samples demonstrated characteristic two-stage crystallization, with an early-stage crystallization lasting for up to ~ 2 hours, in the case of cocoa butter (t_{lag2}).

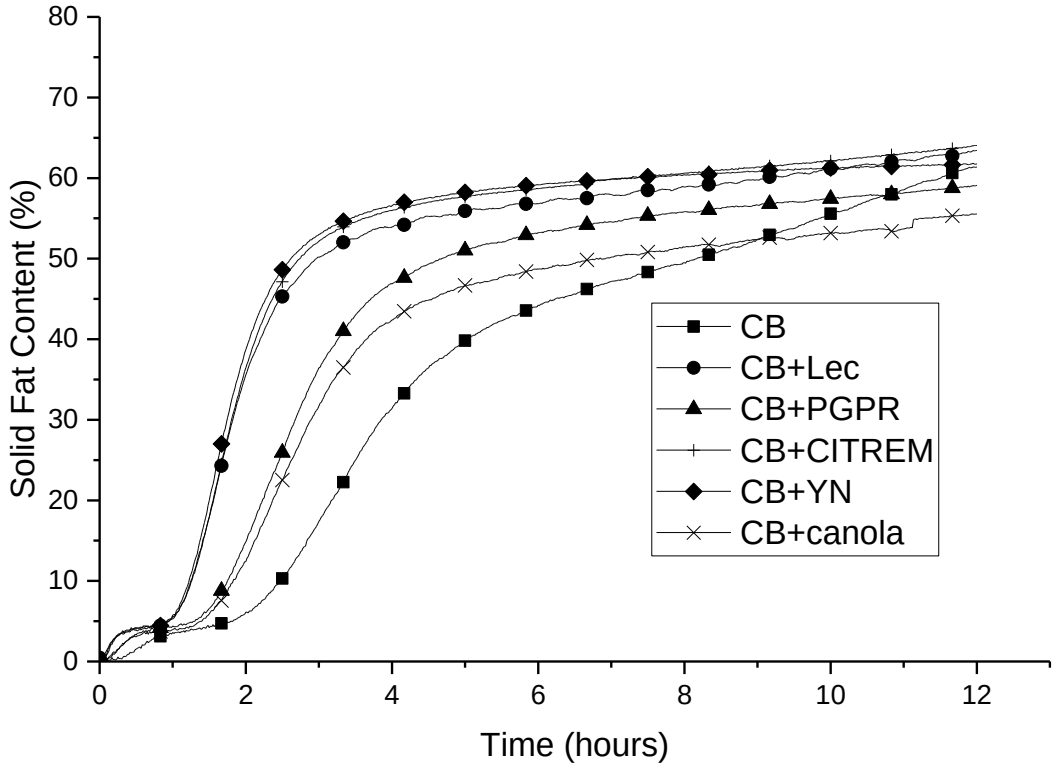

Figure 16: Isothermal crystallization of cocoa butter mixed with a series of emulsifiers (n=3).

Table 1: Avrami model results of the effect of 2 wt% emulsifiers on the isothermal crystallization of cocoa butter.

	t_{lag1} (min)	t_{lag2} (min)	$K_1 \cdot 10^{-4}$	$K_2 \cdot 10^{-6}$	n_1	n_2	SFC_{Eq1}	SFC_{Eq2}
CB	25 ± 1^{a}	111 ± 3^{a}	9.1 ± 0.0^{a}	2.2 ± 0.7^{a}	1.0 ± 0.0^{a}	1.5 ± 0.0^{a}	4.1 ± 0.0^{a}	45.2 ± 0.7^{a}
Lecithin	4 ± 2^{b}	69 ± 0^{b}	10.9 ± 1.8^{a}	221 ± 87.5^{b}	1.0 ± 0.0^{a}	1.0 ± 0.0^{c}	4.5 ± 0.0^{b}	53.0 ± 0.3^{bc}
PGPR	11 ± 4^{b}	86 ± 5^{c}	9.1 ± 0.0^{a}	15.6 ± 12.7^{ac}	1.0 ± 0.0^{a}	1.3 ± 0.0^{b}	4.4 ± 0.0^{d}	50.3 ± 1.6^{b}
Canola oil	13 ± 3^{b}	87 ± 2^{c}	9.1 ± 0.0^{a}	8.4 ± 4.8^{ac}	1.0 ± 0.0^{a}	1.4 ± 0.1^{ab}	4.2 ± 0.0^{a}	46.1 ± 1.8^{a}
CITREM	3 ± 2^{b}	70 ± 0^{bc}	9.4 ± 0.4^{a}	144 ± 5.0^{b}	1.1 ± 0.1^{a}	1.1 ± 0.0^{c}	4.5 ± 0.0^{bc}	56.7 ± 2.4^{c}
YN	3 ± 1^{b}	67 ± 0^{bc}	11.3 ± 1.4^{a}	140 ± 29.7^{bc}	1.1 ± 0.0^{a}	1.1 ± 0.0^{c}	4.4 ± 0.0^{cd}	54.9 ± 0.2^{bc}

Avrami constants for primary (t_{lag1}) and secondary (t_{lag2}) nucleation, primary (K_1) and secondary growth (K_2), post-primary (SFC_{Eq1}) and secondary (SFC_{Eq2}) SFCs and the dimensionality of crystal growth during primary (n_1) and secondary (n_2) crystallization ($r^2 \geq 0.9989$). Data represents the mean (n=3) ± standard deviation; ANOVA-designated significant differences within columns are shown as superscript letters.

The addition of 2 wt% emulsifier enhanced most aspects of cocoa butter crystallization. The presence of emulsifier had little effect on the primary nucleation rate ($p \geq 0.05$). Lecithin shortened the onset of nucleation from 25 minutes to 4 minutes. Secondary growth occurred ~ 40 minutes earlier and the rate increased from 2.2×10^{-6} s^{-1} to 2.2×10^{-4} s^{-1}, an increase of 100 ×. Lecithin has been known to accelerate cocoa butter crystallization.[41,34] In the high-melting fraction of cocoa butter that crystallizes during primary nucleation, there tends to be a greater fraction of phospholipids that are native to the cocoa butter, indicating that they facilitate nuclei formation.[9] Lecithin has a very polar head group, and in a hydrophobic solvent like cocoa butter, there may be more of a tendency to aggregate and form structured regions off which a high melting fraction may nucleate. Svanberg et al. also saw that the addition of 0.5 % lecithin increased the crystallization rate of cocoa butter.[57] The onset times that Svanberg and coworkers observed for cocoa butter as well as a 0.5 wt% lecithin + cocoa butter blend were similar to the present work. In our system

containing 2 wt% lecithin, the resulting concentration of phospholipids was far greater than in native cocoa butter, perhaps leading to more numerous regions of order which in turn could provide a greater number of sites to enhance primary nucleation and growth.[9,58] Generally, liquid-state emulsifiers do not enhance primary stage nucleation while solid-state emulsifiers may enhance early-stage nucleation via a templating effect, where the emulsifier will nucleate first and act as surfaces for ensuing TAG crystallization.[59,60] The ability of lecithin to enhance secondary growth was also observed, though this may have been a by-product of a greater number of high-melting nuclei present after primary growth resulting in a greater surface area for secondary growth to occur, however, non-phospholipid compounds in lecithin may have also caused this enhancement. Finally, lecithin and other charged emulsifiers have previously been used in synthetic/materials chemistry to template crystallization, suggesting that lecithin's ability to accelerate cocoa butter crystallization is expected.[61, 62]

Both YN and CITREM decreased the onset of nucleation from 25 minutes in cocoa butter alone to 3 minutes and decreased the onset of secondary growth by ~ 40 minutes, similar to lecithin. This may be the result of CITREM and YN containing greater amounts of emulsifier when compared to lecithin, given the presence of non-phospholipid constituents in the soy lecithin. Both YN and phosphatidylcholine share structural similarities, which suggests that they share similar mechanisms regarding the formation of ordered regions that promote TAG nucleation, and it is indeed the charged phospholipids in lecithin that accelerate crystallization events versus the non-phospholipid compounds present in commercial lecithin.[63] As all three emulsifiers are liquid at room temperature, there were no high-melting components to act as solid-state nuclei for heterogeneous nucleation. As noted, CITREM shares similar fatty acid moieties with YN as they are made from the same high-oleic acid oil feedstock. It is more likely that the polarity of the head

group was responsible for the increase in nucleation times as other liquid-state emulsifiers with oleic acid moieties, such as sorbitan monooleate and sorbitan trioleate, have minimal effects on cocoa butter nucleation despite bearing the same fatty acids.[48]

PGPR also accelerated cocoa butter crystallization, but to a lesser extent than lecithin, YN and CITREM. This was likely due to the fact that PGPR is oligomeric in nature, and unlike the other small-molecule emulsifiers, it cannot integrate itself into fat crystal nuclei. In the presence of PGPR, the onset of crystallization occurred earlier, after ~ 11 minutes, rather than at ~ 25 minutes in cocoa butter alone. Secondary growth also occurred earlier, after ~ 86 minutes instead of ~ 111 minutes. Rates also increased to a far smaller extent in the presence of PGPR than any other emulsifier, further demonstrating its muted effects on cocoa butter crystallization (Table 1). Given its structural dissimilarity to TAGs, all changes in cocoa butter crystallization were attributed to a dilution effect. For comparison, the effects of 2 wt% canola oil on cocoa butter crystallization closely resembled those of PGPR. Canola oil is primarily comprised of triolein, with a *cis* configuration at the olefin. Due to its very kinked structure, it is also unlikely that canola oil would integrate itself into the crystal nuclei. As a result, canola oil and PGPR only served to increase the liquid fraction of cocoa butter, mimicking softer cocoa butters with a high oil content, which also have faster nucleation rates.[6]

In contrast to our results, PGPR was previously shown to have no tangible effect on cocoa butter crystallization rate, as analyzed by Miyasaki et al..[41] The discrepancy in results may arise from the difference in concentration, where the greatest quantity of PGPR used in their compositions was 0.8% whereas here our concentration was 2 wt%, more than double, thus our systems had a greater level of dilution. Another culprit may be the degree of undercooling. Our samples were isothermally crystallized at 20 °C whereas in the aforementioned work, crystallization occurred

isothermally at 15 °C. With a greater degree of undercooling, the thermodynamic driving force for crystallization to occur is greater which would potentially diminish the effect of an additive.[56]

The Avrami constant n describes the dimensions that crystal growth occurs in. Lower values of n indicate growth in fewer directions, i.e., rod-like crystal growth, whereas higher values of n indicate disc-like or spherulitic growth patterns.[64] The addition of emulsifier and oil did not have a significant effect on primary growth mode ($p \geq 0.05$) where values remained constant at ~ 1, indicating rod-like growth from instantaneous nuclei. Secondary growth was hindered by lecithin, YN and CITREM showing values ~ 1.0 whereas cocoa butter alone and in the presence of PGPR and canola oil tended towards disc like growth ~ 1.4 or towards a higher dimensionality. The value of n should be an integer, however previous works has shown values of n as fractions.[48,65] Without corresponding microscopy it is difficult to confirm growth modes as they relate to added emulsifier or oil.

Early stage SFC_{Eq} was modestly enhanced in the presence of emulsifiers, increasing slightly from ~ 4.1 to ~ 4.5 %, where no increase was seen with the addition of canola oil. Second stage SFC_{eq} showed similar trends, as canola oil showed no increase in SFC, and the emulsifiers enhanced equilibrium SFC. Since the emulsifiers contributed no crystallizable fat, the increase in SFC was due to TAGs present in the cocoa butter. Speculatively this may be due to the emulsifier causing a decrease in solubility of cocoa butter's high melting TAGs at 20 °C, resulting in a slight increase in SFC.

The governing hypothesis of this thesis was that the addition of dispersed sugar would restrict the ability of all emulsifiers to modify the crystallization kinetics of cocoa butter. It was expected that the presence of sugar would limit the ability of emulsifier molecules to associate with each other

and with the fat, owing to their preferential interaction with the sugar crystal surface. The effect of dispersed sugar on cocoa butter isothermal crystallization (Figure 17) along with the associated nucleation parameters (Table 2) confirmed these significant effects.

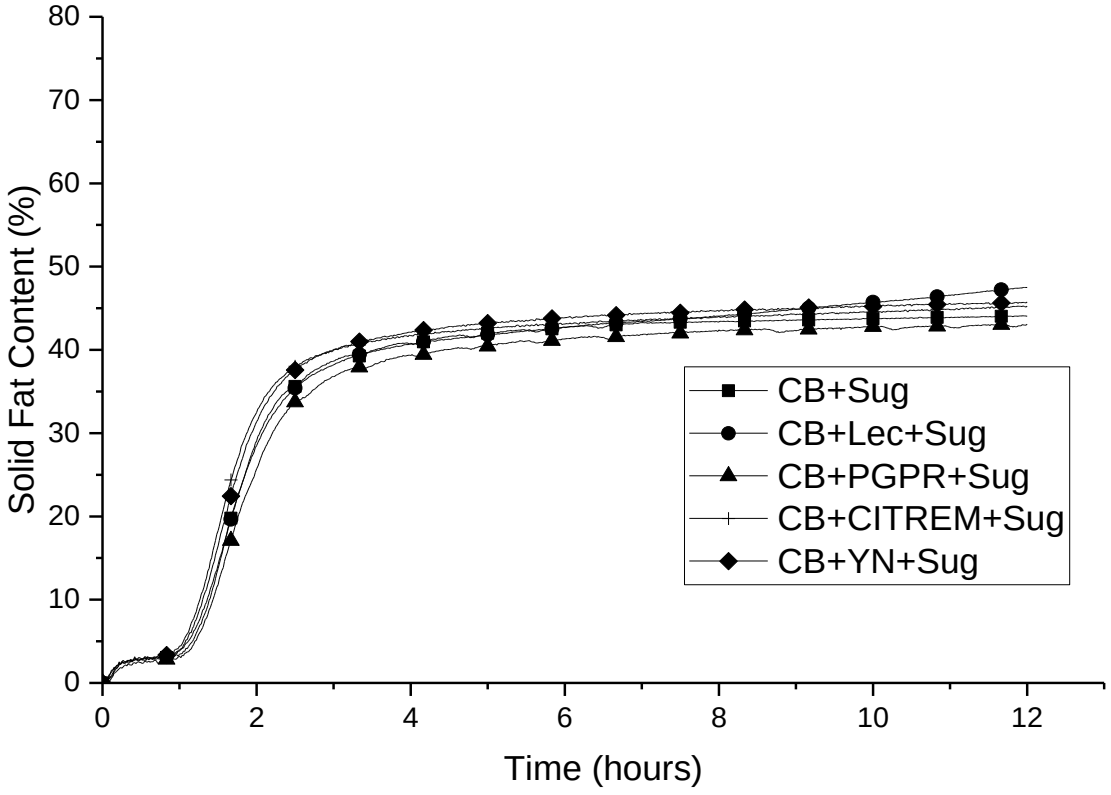

Figure 17: Isothermal crystallization of cocoa butter mixed with a series of emulsifiers in the presence of sugar (n=3).

Table 2: Avrami model results of the effect of 2 wt% emulsifiers on the isothermal crystallization of cocoa butter – sugar blends.

	t_{lag1} (min)	t_{lag2} (min)	$K_1 \cdot 10^{-4}$	$K_2 \cdot 10^{-4}$	n_1	n_2	SFC_{Eq1}	SFC_{Eq2}
CB	3 ± 3[a]	77 ± 8[a]	9.1 ± 0.0[a]	6.3 ± 0.9[a]	1.0 ± 0.0[a]	0.9 ± 0.0[a]	4.1 ± 0.0[a]	38.6 ± 1.8[a]
Lecithin	3 ± 2[a]	75 ± 3[a]	9.1 ± 0.0[a]	9.5 ± 2.5[a]	1.0 ± 0.0[a]	0.9 ± 0.1[a]	4.4 ± 0.0[a]	38.6 ± 0.5[a]
PGPR	8 ± 1[a]	81 ± 10[a]	9.1 ± 0.0[a]	7.9 ± 2.9[a]	1.0 ± 0.0[a]	0.9 ± 0.0[a]	4.2 ± 0.0[a]	37.2 ± 0.9[a]
CITREM*	3	69	9.1	3.5	1.1	1.1	4.4	38.67
YN*	3	72	9.1	4.5	0.9	1.0	4.4	39.5

Avrami constants for primary (t_{lag1}) and secondary (t_{lag2}) nucleation, primary (K_1) and secondary growth (K_2), post-primary (SFC_{Eq1}) and secondary (SFC_{Eq2}) SFC and the dimensionality of crystal growth during primary (n_1) and secondary (n_2) crystallization as affected by added emulsifier. Data represents the mean (n=3) ± standard deviation, * = samples represent single experiments and thus could not be analyzed using ANOVA; ANOVA-designated significant differences within columns are shown as superscript letters.

The NMR could not differentiate signal contributions from sugar crystals and fat crystals, which resulted in a falsely high recorded SFC values. In the presence of sugar at t = 0 where an SFC of ~ 0 % was expected, there was instead an SFC of ~ 30 %. To evaluate only the crystallizing cocoa butter, the t = 0 SFC was subtracted from all values which allowed for the generation of Avrami constants from the resulting curve. Across all emulsifiers tested, sugar muted any effect added emulsifier had on crystallization, resulting in similar Avrami constants regardless of the emulsifier added ($p \geq 0.05$). The onset of primary and secondary growth across all samples occurs within the 3 - 8 minutes and 69 - 81 minutes respectively, with primary and secondary growth also showing very similar rates across samples, i.e., 9×10^{-4} s^{-1} for primary growth and ~ $4 – 10 \times 10^{-4}$ s^{-1} for secondary growth. This suggested that, at a 1:1 ratio of cocoa butter + 2 wt% emulsifier combined with sugar, the surface effects of sugar dominated crystallization, and the ability of any emulsifier to affect crystallization was diminished either through immobilization at the surface of sugar or simply through the sheer volume of sugar present. Similar to our observations, Dhonsi and Stapley

as well as Svanberg and coworkers saw an increase in the rate of cocoa butter crystallization when sugar was added, citing heterogeneous nucleation arguments.[34,66] However, the addition of lecithin to a cocoa butter-sugar blend showed conflicting results. Svanberg et al. saw that addition of lecithin enhanced the rate of crystallization whereas Dhonsi and Stapley saw a decrease in the crystallization rate of a cocoa butter-sugar blend in the presence of lecithin. Contrary to both publications, our results suggested that lecithin in a cocoa butter-sugar blend did not enhance the rate of crystallization. The discrepancy between studies may arise from sample preparation as Dhonsi and Stapley sheared their samples during crystallization whereas, similar to our work, Svanberg crystallized their samples statically. Compositional differences may have also caused divergent crystallization behaviour. Dhonsi and Stapley used a cocoa butter to sugar ratio (44:56) similar to our composition (1:1) whereas Svanberg used a greater quantity of cocoa butter to sugar (~ 2:1). The amount of lecithin present also varied across studies. Here, 2 wt% lecithin relative to the fat phase was used whereas Dhonsi and Stapley used 0.2 wt% and Svanberg et al. used 0.5 wt%, both on a sample mass basis. On this basis, the present concentration would be 1% emulsifier. Dhonsi and Stapley used viscosity to monitor onset of crystallization whereas Svanberg et al. used confocal microscopy to quantify the amount of crystalline material and determine a rate. In the present work, p-NMR was used to monitor the solid fat content. Finally, lecithin origin may have also come into play given that lecithin may act both a crystal inhibitor and promoter depending on concentration and composition, which may have also biased the results obtained.[67]

Sugar did not change calculated mode of growth in the primary stage (n_1) where, similar to samples containing no sugar, the value suggested rod-like growth from instantaneous nuclei, i.e., n ~ 1.0. The presence of sugar reduced the dimensionality of secondary growth, where rather than the disc-like crystals that formed with cocoa butter alone, rod-like growth was observed.

Much like the other crystallization parameters, sugar mitigated the effect of emulsifier on SFC_{eq} in both early and late-stage crystallization.

In the absence of emulsifier, sugar enhanced cocoa butter crystallization when based on the primary nucleation time decreasing from ~ 25 to ~ 9 minutes and the onset of secondary nucleation decreasing from ~ 111 to ~ 64 minutes. In the presence of sugar, secondary growth rate increased over 280 ×, demonstrating that sugar greatly enhanced the crystallization kinetics of cocoa butter, presumably via heterogeneous nucleation.[34,66]

5.2 Thermal behaviour

Emulsifiers may affect polymorphic transitions through mechanisms similar to how they affect nucleation. Emulsifiers that share structural similarities to the solidifying fat may integrate themselves into the fat crystal lattice, allowing them to modify the properties of the crystal rather than that of the oil phase. Conversely, dissimilar emulsifiers will not be able to change the properties of fat crystals directly and may instead alter crystallization by increasing liquid fraction and molecular motion *via* dilution, or by changing the polarity of the mother liquor.[68] Transitions mediated by the liquid phase, through a partial melt and recrystallization process, can be inhibited by the addition of emulsifier, though this effect is dependent on cooling rate.[69] Melting point determination can be used as a technique to determine how emulsifiers integrate themselves into fat crystals. With a greater degree of molecular complementarity, a mixed melting profile can be observed.[70] Table 2 shows that addition of emulsifier had no tangible effect on the melting points of the form IV and V polymorphs.

Table 3: Melting point of cocoa butter-emulsifier blends.

Composition	*m.p. form IV (°C)*	*m.p. form V (°C)*
CB	26.8 ± 0.1	30.7 ± 0.3
CB + Lecithin	26.8 ± 0.1	30.8 ± 0.1
CB + PGPR	27.2 ± 0.4	31.4 ± 0.4
CB + CITREM	26.6 ± 0.1	31.1 ± 0.3
CB + YN	26.4 ± 0.1	31.1 ± 0.2
CB + Canola oil	a	30.9 ±0.2
CB + Castor oil	a	31.0 ± 0.1

a = Samples did not have a measurable amount of form IV after 24 hours of isothermal aging at 20°C and a melting point could not be determined. ($n \geq 3$).

The melting points for all samples ranged from 26.4 – 27.2 °C for form IV and from 30.7 – 31.4 °C for form V, with narrow standard deviations for all samples. The lack of integration of these additives into the cocoa butter crystals was expected as they were of different molecular weights and contained varying degrees of unsaturation, which suggested that they were likely too large or structurally dissimilar to integrate themselves into the crystal lattice.[71] Previously, it has been seen that simply altering the number of carbons in fatty acid chains can determine whether an emulsifier can affect the melting point of fat crystals. Ueno and coworkers showed that palm mid-fraction (predominantly POP) in the presence of 5% sorbitan tripalmitate, a sucrose fatty ester with emulsifying capability sharing the same fatty acid moieties found in palm mid fraction, showed mixed melting behaviour with the emergence of a melting peak distinct from palm mid-fraction and sorbitan tripalmitate alone. The addition of sorbitan tristearate and sorbitan tribehenate, with similar molecular architecture with only varying chain length, showed no influence on the melting of palm mid-fraction, as its melting profile remained unchanged in both instances.

Under isothermal cooling to 20 °C, cocoa butter will preferentially crystallize in the form II polymorph and transition rapidly to the form IV polymorph as it is the more kinetically stable at that temperature.[12] Over time, form IV will transition to form V *via* liquid phase-mediated recrystallization or through a solid-state transition, depending on thermal treatment.[68] Crystallization is not an equilibrium process, i.e., TAGs do not remove themselves from the crystal lattice and re-integrate based on lability. Instead, the liquid phase-mediated recrystallization consists of the partial melting of β′ crystals and reformation into the β polymorph.[67,72] Figure 18 shows the effect of emulsifiers on the transition from form IV to form V.

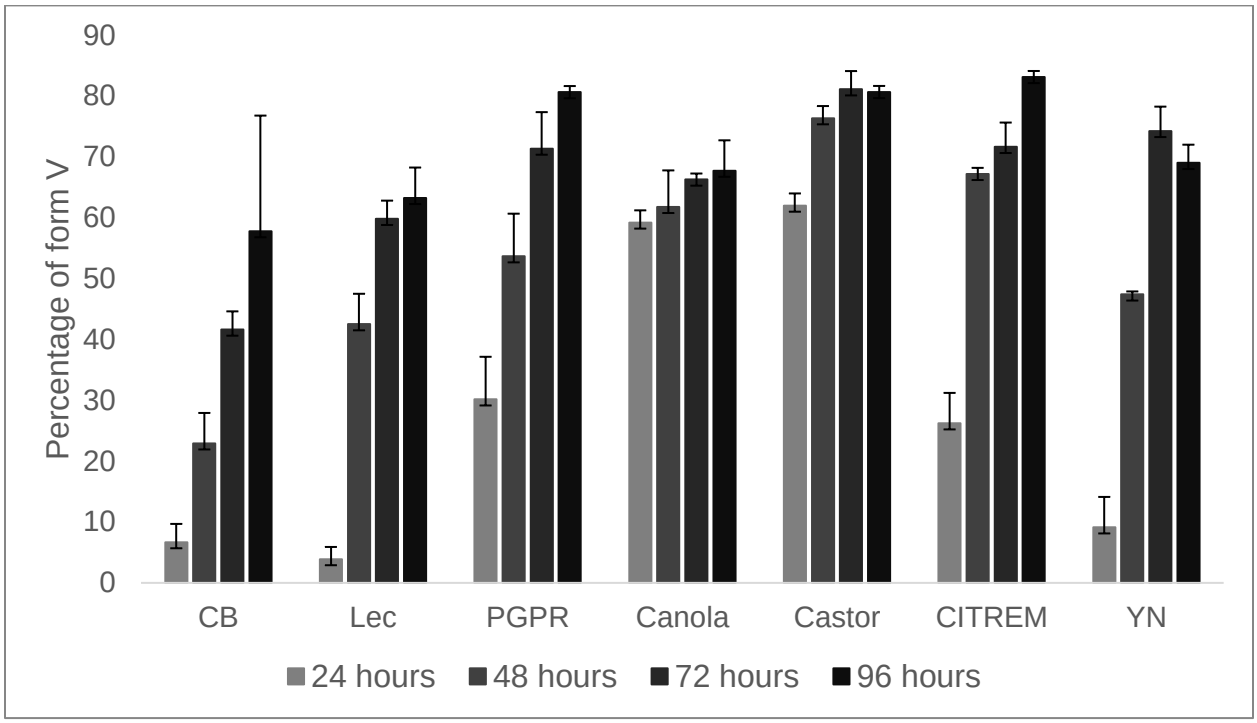

Figure 18: Evolution in the percentage of cocoa butter transitioned from form IV to V during isothermal storage at 20 °C as affected by emulsifier type (n=3).

Small-molecule emulsifiers such as lecithin, CITREM and YN did not affect the form IV – V transition. Cocoa butter alone showed a slow increase in percentage of form V present over a period of 96 hours, increasing from 7 % in the first 24 to 57 % after 96 hours. Lecithin followed a

similar trend, increasing from 4 % to 63 %. After 96 hours, CITREM and YN, following the same trend, evolved from 26 % to 83 %, and 9 % to 69 % form V, respectively. The addition of PGPR did slightly accelerate the transformation, however, supporting the concept that it is free in the liquid oil and acts as a diluent. The presence of PGPR resulted in 30 % form V after 24 hours and 81 % after 96 hours. Canola oil and castor oil also promoted the form VI to V transition likely through a similar mechanism, with only slight changes in form V content over 96 hours. The slow crystallization of fats from a solvent will generally lead to the formation of more stable polymorphs, namely the β polymorph.[73] In a similar manner, PGPR and the added oils promoted the formation of form V crystals within the first 24 hours. Despite some emulsifiers accelerating polymorphic transition, the additives did not seem to substantially effect the total enthalpy of form IV and form V crystals (Figure 19).

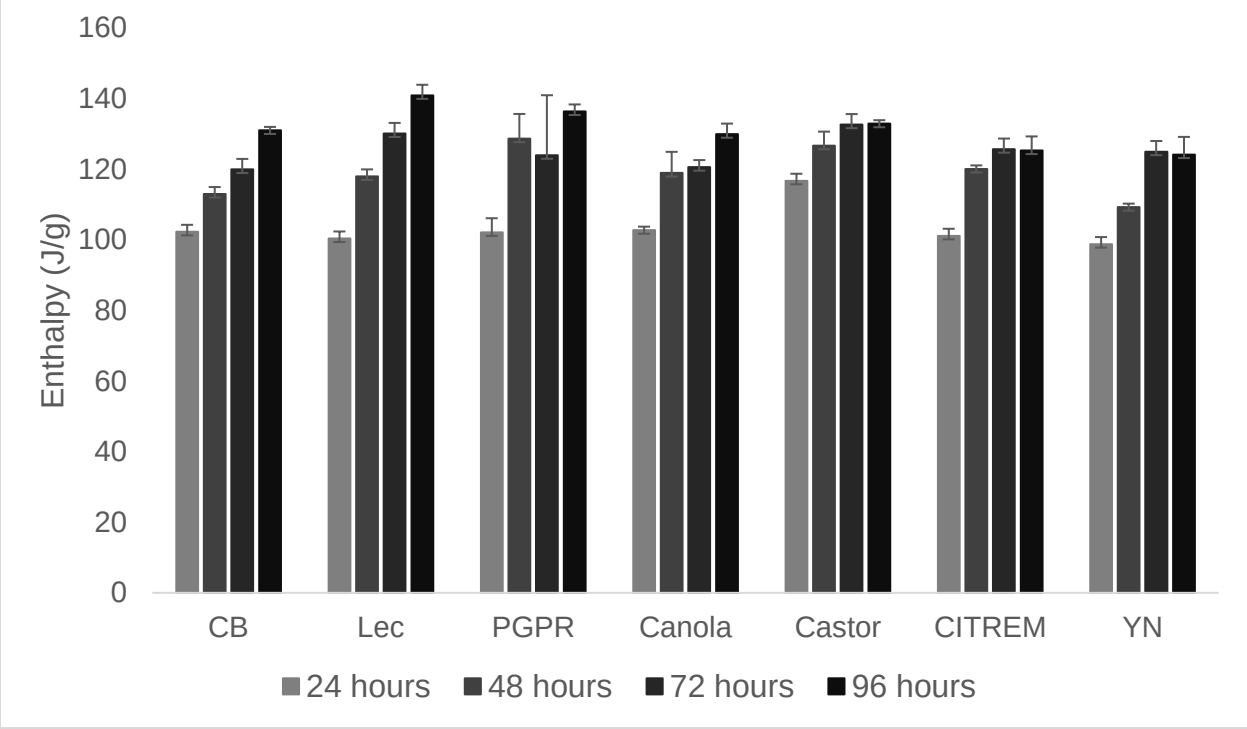

Figure 19: Evolution in the total enthalpy of melting or cocoa butter during isothermal storage at 20 °C as affected by emulsifier type (n=3).

Neither the addition of emulsifier nor the addition of oil affected the total enthalpy of cocoa butter ($p \geq 0.05$). Cocoa butter alone showed enthalpies ranging from 102 – 131 J/g over 96 hours, with the addition of lecithin resulting in similar enthalpies (100 – 140 J/g). In a similar fashion, presence of CITREM and YN resulted in melting enthalpies ranging from 101 – 125 J/g and 99 – 124 J/g, respectively. The presence of PGPR and the oils also produced similar enthalpies. Overall, this mirrored the results seen in the melting points where, due to a lack of molecular complementarity, there was no tangible effect on melting enthalpy across compositions, as there was no presumed emulsifier incorporation into the TAG crystalline lattices (Table 3).

Table 4: Melting point of cocoa butter-emulsifier blends in the presence of sugar after isothermal ageing for 96 hours (n ≥ 3).

Composition	***m.p. form IV (°C)***	***m.p. form V (°C)***
CB + Sugar	27.0 ± 0.0	30.3 ± 0.3
CB + Lecithin + Sugar	26.8 ± 0.0	30.6 ± 0.3
CB + PGPR + Sugar	27.0 ± 0.2	30.6 ± 0.3
CB + CITREM +Sugar	26.8 ± 0.0	31.0 ± 0.3
CB + Canola oil + Sugar	27.1 ± 0.2	30.7 ± 0.3
CB + Castor oil + Sugar	27.2 ± 0.2	30.9 ± 0.3

Similarly, the presence of sugar had no effect on the melting point of the form IV or V polymorphs, either alone or in the presence of emulsifier, as melting points ranged from 26.8 – 27.2 °C for form IV and from 30.3 – 31.0 °C for form V ($p \geq 0.05$).

The addition of sugar significantly hindered the form IV to V polymorphic transition (Figure 20).[74] As seen in the nucleation and growth studies, the presence of sugar minimized the effect of added

emulsifier, presumably due to an association of emulsifier and the sugar crystal surface. Unexpectedly, the same effect was seen with both canola and castor oil. This result was rather perplexing, as it was expected that canola oil would remain free in the fat phase as it is not surface-active. In regards to castor oil, given that it is tangibly more polar than canola oil, it may have shown some interaction with the surface of the sugar crystals. A potential explanation might be the presence of a micro-viscous environment at the surface of the sugar crystals, which would reduce the increased molecular motion effect that the oils and PGPR had in the absence of sugar.[75,76,74] Though there likely were interactions between the sugar and emulsifier that hindered the ability of emulsifier to affect cocoa butter polymorphic evolution, this was not explored further.

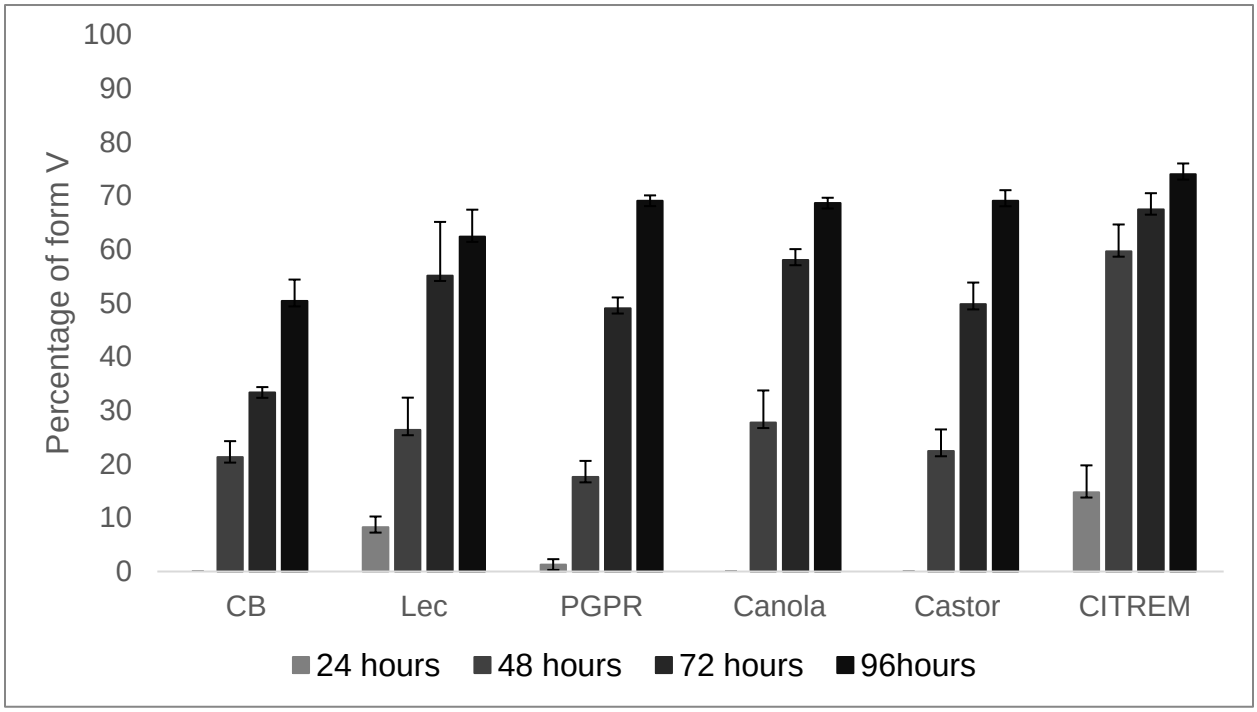

Figure 20: Evolution in the percentage of cocoa butter transitioned from IV to V during isothermal storage at 20 °C as affected by presence of sugar and emulsifier (n=3).

The effect of sugar and emulsifier on the total enthalpy of melting of cocoa butter crystals was also determined (Figure 21). Lecithin, PGPR and CITREM had no appreciable effect on melting

enthalpy in the presence of sugar ($p \geq 0.05$). Cocoa butter alone showed melting enthalpies of 102 - 131 J/g over a period of four days. Lecithin and CITREM had showed similar enthalpies ranging from 90 – 120 J/g, and 101 – 139 J/g, respectively. Canola and castor oil did reduce the overall enthalpy slightly, but this was not surprising as they increased the liquid fraction in the samples, thereby reducing the amount of fat that could crystallize.

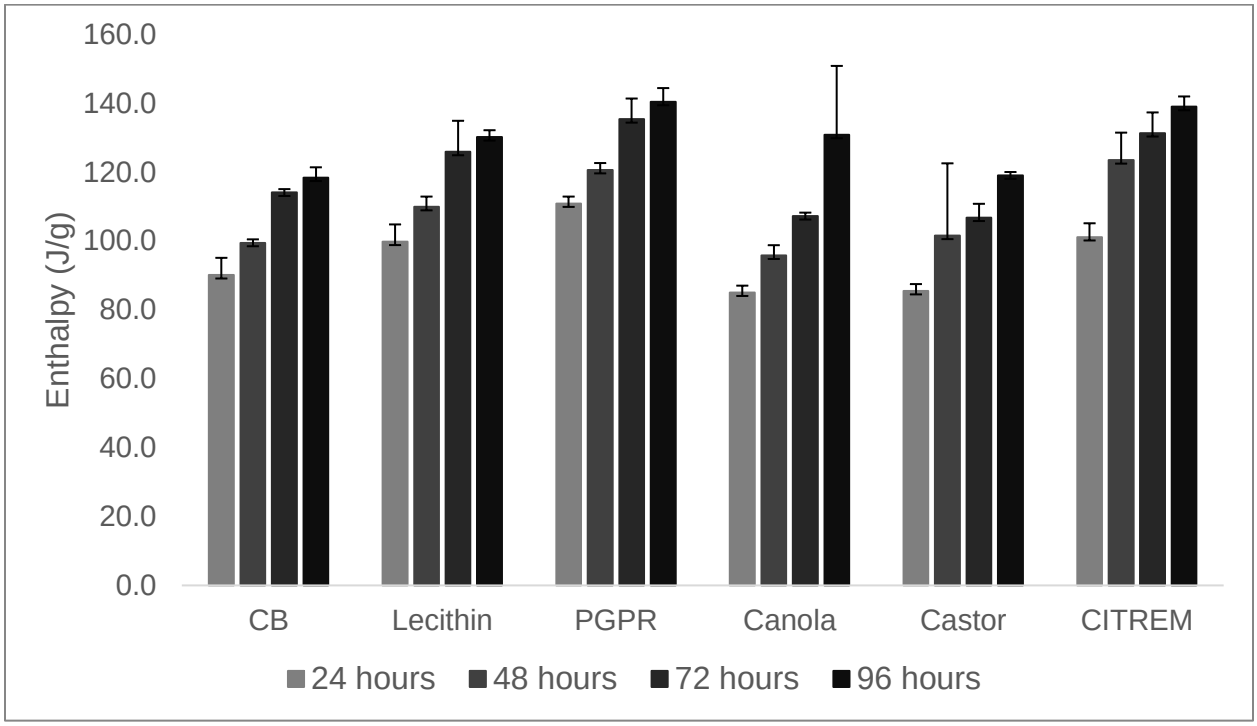

Figure 21: Evolution in the total enthalpy of melting of cocoa butter-sugar blends during isothermal storage at 20 °C as affected by emulsifier type (n=3). YN results removed at the request of industrial partner.

A visual representation of how the emulsifiers and sugar affected the form IV to V transition was made by comparing the melting enthalpies of samples containing only cocoa butter and emulsifier as the x-axis and those containing both sugar and emulsifier as the y-axis (Figure 22). This approach permitted the direct comparison of the effect of sugar on the proportion of forms IV and V in the cocoa butter + emulsifier blends.

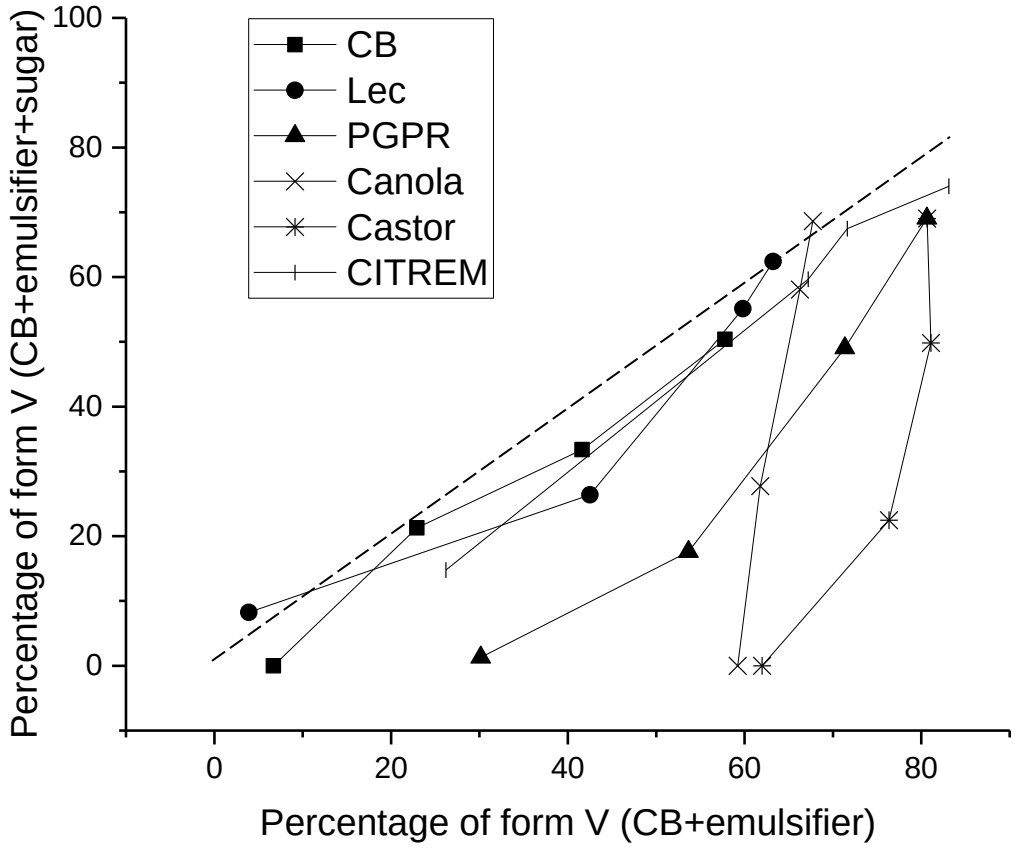

Figure 22: Effect of the presence of sugar versus emulsifier on the form IV to form V transition.

In using y = x (dashed line) as a median, values above the line would suggest that sugar had a more positive effect on the form IV to V transition whereas, below the line, emulsifier promoted the form IV to V transition to a greater extent. This graphical representation suggested that emulsifiers had a greater effect alone than in their counterpart also containing sugar, and that certain types of emulsifiers showed similar effects. For example, lecithin and CITREM showed similar trends which suggested a limited effect of the sugar on the proportion of forms IV and V. PGPR, canola oil and castor oil also showed similar trends whereby the initial amounts of form V present after

24 hours were greater than the other additives. However, their steep slopes suggested little effect at later storage times.

5.3 Crystal polymorphic identity

The effect of added emulsifier on the form IV and V cocoa butter polymorphs is shown in Figure 23. It was not possible to carry out experiments in sugar-containing samples given the strong diffraction pattern associated with sugar crystals.[77]

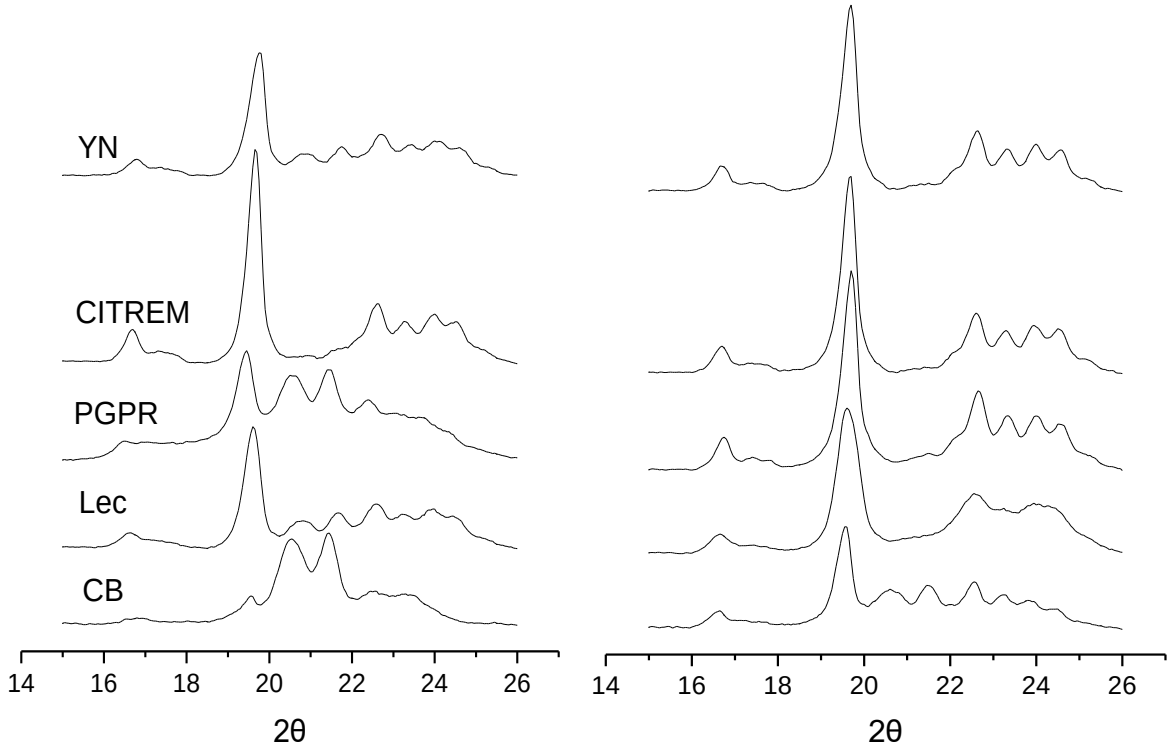

Figure 23: Wide-angle X-ray diffraction of isothermally aged cocoa butter at 20 °C after 24 hours (left) and 96 hours (right) of storage.

Table 5: The d-spacings (Å) of isothermally aged cocoa butter at 20 °C after 24 hours (left) and 96 hours (right) of storage.

Composition	*Short spacings 24 hrs (Å)*	*Short spacings 96 hrs (Å)*
CB	4.32(s) 4.13(s)	4.53(vs) 4.30(ms) 4.13(ms) 3.93(ms) 3.81(ms)
CB + Lecithin	4.53(vs) 4.23(ms) 4.10(ms) 3.93(ms) 3.70(ms)	4.53 (vs) 3.93(ms)
CB + PGPR	4.44 (s) 4.22(s) 4.05(s)	4.50(vs) 3.92(ms) 3.70(ms)
CB + CITREM	4.51(vs) 3.92(ms) 3.70(ms)	4.50(vs) 3.93(ms) 3.81(ms) 3.70(ms)
CB + YN	4.49(vs) 4.25(ms) 4.08(ms) 3.92(ms) 3.70(ms)	4.50(vs) 3.92(ms) 3.81(ms) 3.70(ms)

Cocoa butter after 24 hours showed a majority of form IV with d-spacings of 4.32 and 4.13 Å which corresponded well to the results of Chapman.[77] After 96 hours, cocoa butter had begun to transition from form IV to form V, with the appearance of a sharp peak with a very strong intensity at 4.53 Å. The form V polymorph is generally associated with an intense peak around 4.5 Å accompanied by a moderately strong signal at 3.9 Å, however, variability of these values has been reported.[78] After 24 hours of crystallization, lecithin and YN had begun to transition, with both showing a strong, sharp peak at ~ 4.5 Å. Yet, a portion of the cocoa butter remained in form IV

based on the signals at ~ 4.2 and ~ 4.1 Å. PGPR also showed spacings at ~ 4.2 and ~ 4.1 Å, indicating the presence of form IV. However, there was an anomalous peak at 4.44 Å that corresponded to a short-spacing indicative of form III.[78] The intensity of this peak suggested that it did not represent form III as this peak generally appears as a shoulder rather than a stronger signal.[79] CITREM completely transitioned to form V after 24 hours of storage, given the presence of peaks at ~ 4.5 and ~ 3.9 Å. After 96 hours, all samples containing emulsifier had transitioned to form V. Cocoa butter alone, however, still contained a measurable amount of form IV. The data collected was not representative of the DSC results, due to possible sedimentation, or differences in heat transfer. What the data did indicate was that after 96 hours, there was indeed the presence of the form V polymorph in the systems containing emulsifier. Overall, the diffractograms suggested that these liquid-state emulsifiers did not influence TAG packing.

5.4 Mechanistic considerations

The mechanisms governing the ability of emulsifiers to impact fat crystallization in bulk and dispersed systems have yet to be fully elucidated. Some mechanistic considerations have been presented to describe the possible interactions between emulsifiers and crystallizing fat.[56] However, few mechanisms describing the interactions between emulsifier and sugar on fat crystallization exist. Figure 24 depicts some mechanistic considerations relevant to the systems studied.

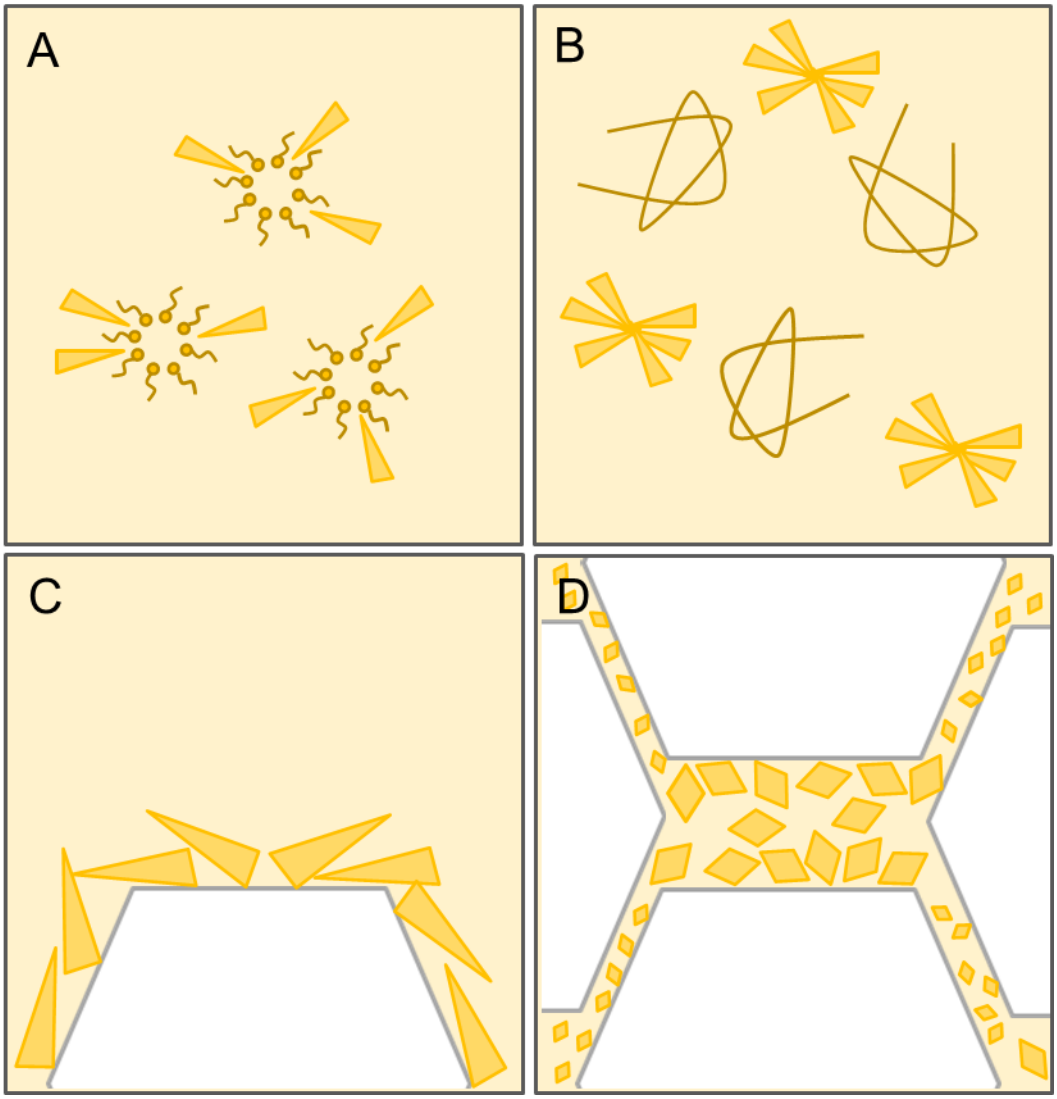

Figure 24: Possible mechanisms influencing cocoa butter crystallization through templating (A), dilution (B), heterogeneous nucleation (C) or viscosity (D) effects.

Emulsifier clustering in solution forming templates for crystallization to occur has previously been reported for phospholipids in cocoa butter by Davis and Dimick.[9] Rizzo, Norton and Norton also described the formation of mono- and diglyceride micelles in a coconut and sunflower oil blend, which enhanced crystallization.[80] The ability for lecithin, CITREM and YN to enhance cocoa butter crystallization can be attributed to this clustering and templating phenomenon. However, PGPR and the oils are unlikely to participate in this behaviour due to their structure. Norton and

coworkers described the exclusion of triolein during the crystallization of tristearin and tripalmitin, with the presence of triolein also promoting the formation of higher melting polymorphs, similar to the present observed effect of PGPR and the oils.[71] Sonwai and coworkers saw similar enhancement of cocoa butter crystallization and the promotion of higher melting polymorphs in the presence of canola oil, and attributed the result to a dilution effect.[48]

The presence of sugar introduced a surface off which fat may crystallize *via* heterogeneous nucleation, similar to the effects Yoshikawa and coworkers saw with the introduction of 1 % solid particles such as talc and graphite on trisaturates.[81] Beyond their propensity to enhance nucleation, sugar surfaces also provided sites for emulsifiers to adsorb onto, which rendered the now-coated particles lipophilic.[28]

The crystallization kinetics of cocoa butter in the presence of sugar, with or without emulsifier, were similar to, or greater than that of cocoa butter and emulsifier alone. Thus, it is difficult to determine if the impact of emulsifier was affected by the presence of sugar. West and Rousseau saw similar trends in palm oil where sugar enhanced crystallization and hindered polymorphic evolution.[82] Acevedo, Block and Marangoni observed that increased viscosity decreased molecular mobility during fat crystallization and promoted the formation of less stable polymorphs.[74] Thus since the presence of sugar hindered polymorphic evolution even in the presence of non-surface-active oils, its effects were likely due to an increase in viscosity and a decrease in molecular motion.

6.0 Conclusion

Through techniques such as p-NMR, DSC, and X-ray diffraction, we have observed the effects of 2 wt% emulsifier on cocoa butter nucleation, crystal growth and polymorphism both in the presence and absence of sugar. We demonstrated that small-molecule emulsifiers promoted crystallization of bulk cocoa butter during the early stages of nucleation and growth. We observed the ability of PGPR to modestly enhance nucleation and growth and have compared its effect to that of canola oil and suggested a dilution mechanism rather than an integration into the cocoa butter crystal lattice. The small-molecule emulsifiers demonstrated minimal effect on the form IV to V transition, given their liquid state and lack of molecular complementarity whereas PGPR behaved similarly to added oil, accelerating the transition from form IV to V. The presence of sugar accelerated cocoa butter nucleation and growth but muted the effect of emulsifiers which was surmised as resulting from their adsorption to the sugar crystal surface. The sugar retarded the form IV to V transition both in the presence and absence of emulsifiers, potentially through a form IV stabilization mechanism. Lastly, the ability of emulsifiers to impact the sub-cell packing of the polymorphic forms of cocoa butter showed that, despite the variety of emulsifiers explored, there was no significant impact on the sub-cell packing due to the likely inability of an emulsifier to become integrated within the crystal lattices of the cocoa butter crystals.

7.0 Future work

Future work should include the effects of emulsifiers on the microstructure of cocoa butter and cocoa butter-sugar blends, since in many fat-based systems, microstructure determines physical properties such as texture and oil migration. Determining the effect of emulsifier concentration and varying the concentration of sugar in our blends may also provide important mechanistic insights into how these components interact.

On a larger scale, it would be worthwhile to develop a library of emulsifiers and to catalog their effects on the nucleation, growth and polymorphic transitions of cocoa butter and cocoa butter-sugar blends. It is commonly known that molecular structure determines the function of emulsifiers, and functionality could be distilled down to steric arguments, electronics arguments or a combination of the two. For a complete library, emulsifiers containing headgroups of varying composition and charge and acyl chains of varying length, unsaturation and reactivity should be tested. By comparing the structural identity of emulsifiers, more accurate mechanisms for emulsifiers as crystal modifiers could be developed and contribute to creating a toolbox where a desired effect on cocoa butter crystallization from the addition of a specific structural motif with an emulsifier may be achieved. This would benefit the food and nutrition industries with the design of higher functionality foods, and even the pharmaceutical industry for the possible design of novel drug delivery vehicles.

Appendix 1: Supplementary

Figures 25 and 26 show the residual plots of the Avrami model fit to the isothermal crystallization data for samples containing emulsifier and emulsifier and sugar respectively. Since the values are relatively close to zero, we can suggest that the model fits fairly well.

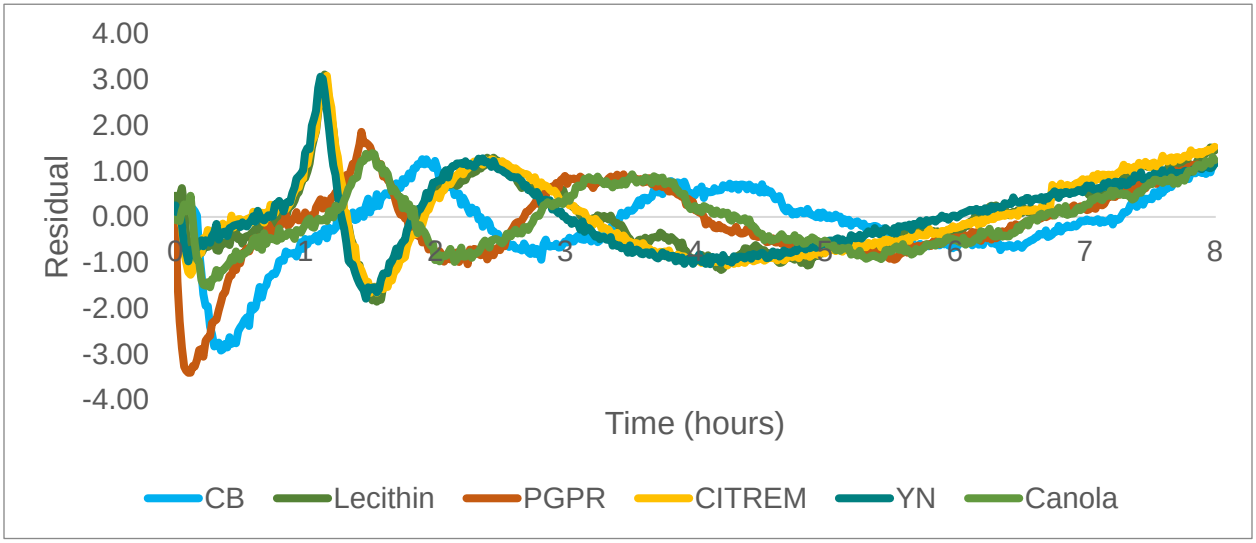

Figure 25: Residual plot of the Avrami model fitted to crystallization curves of cocoa butter containing emulsifier.

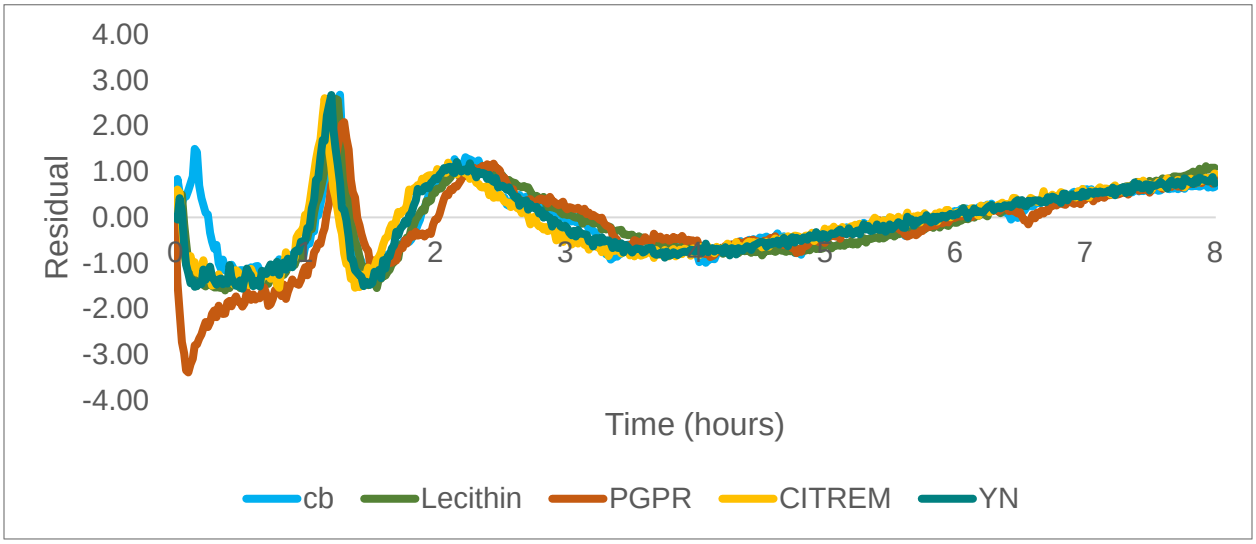

Figure 26: Residual plot of the Avrami model fitted to crystallization curves of cocoa butter containing emulsifier and sugar.

Figures 27 to 39 show the residual plots for the curve fitting to the DSC thermograms. The low values indicate a good fit.

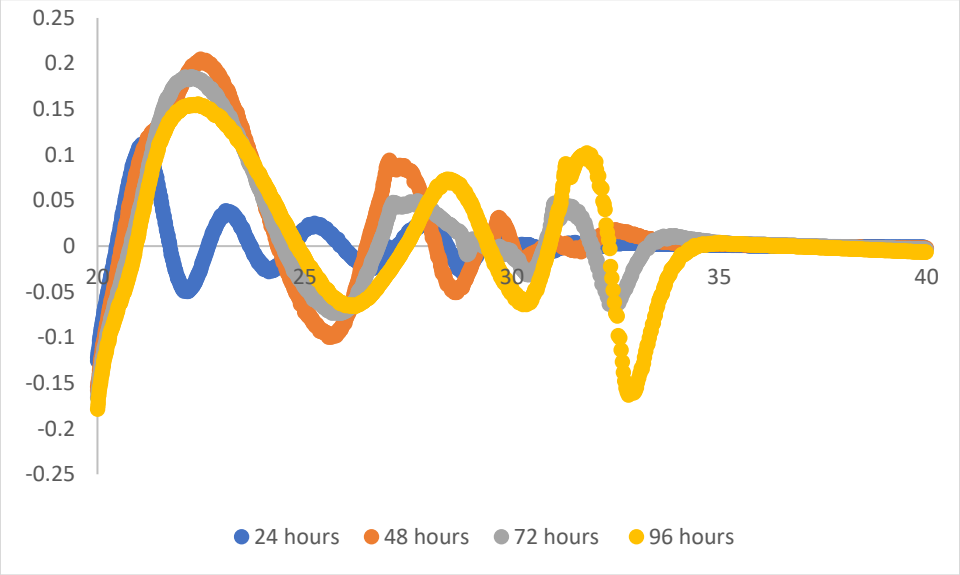

Figure 27: Residual plot of the curve fitting on thermal curves of cocoa butter.

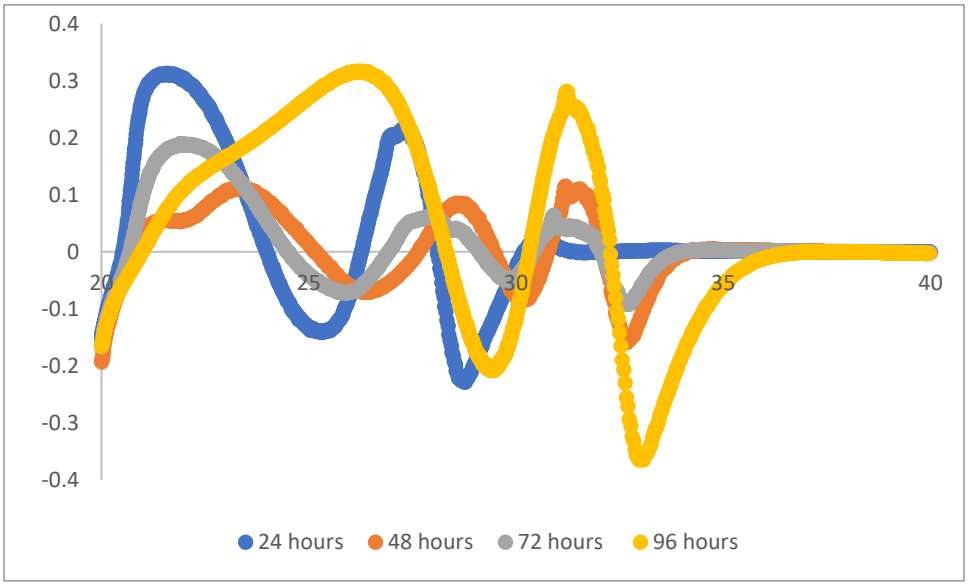

Figure 28: Residual plot of the curve fitting on thermal curves of cocoa butter containing lecithin.

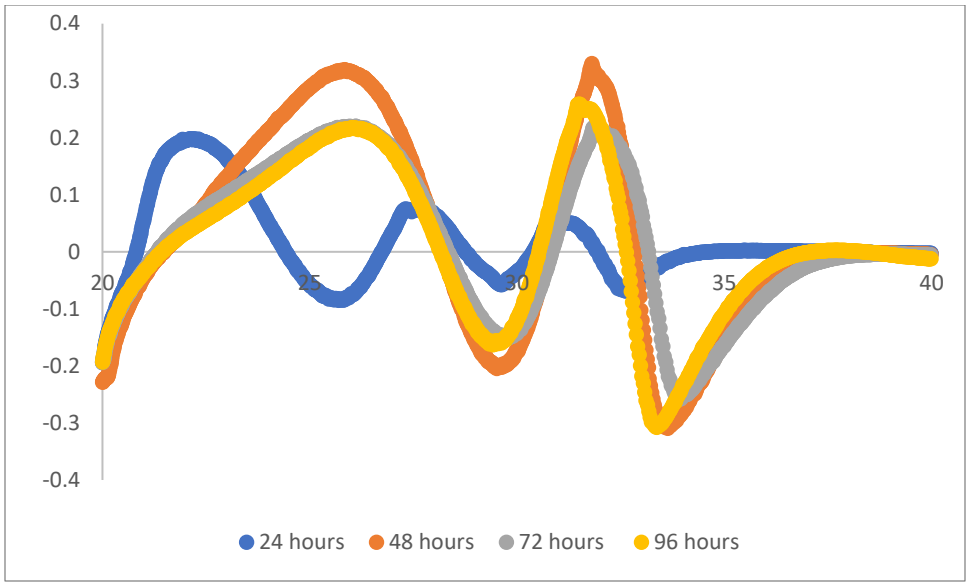

Figure 29: Residual plot of the curve fitting on thermal curves of cocoa butter containing PGPR.

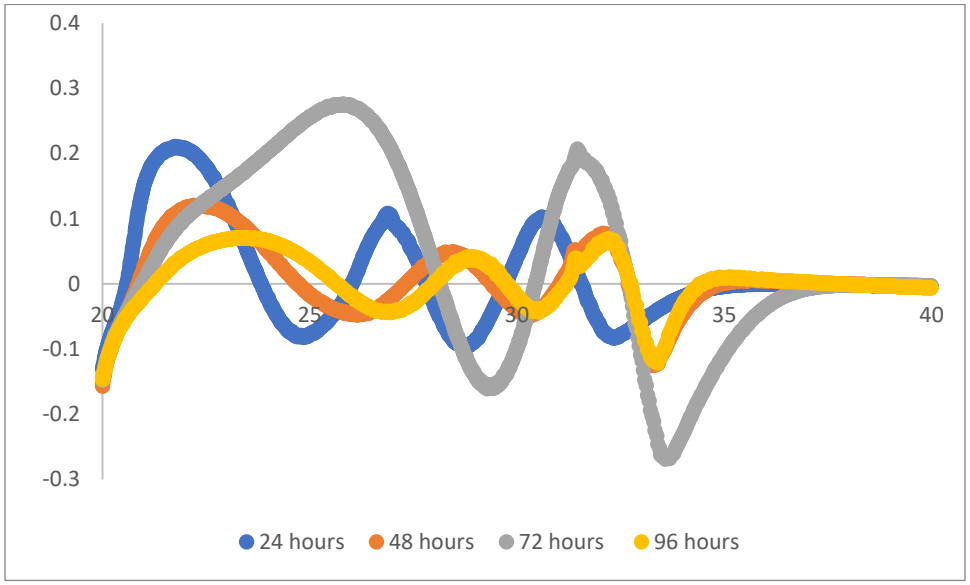

Figure 30: Residual plot of the curve fitting on thermal curves of cocoa butter containing CITREM.

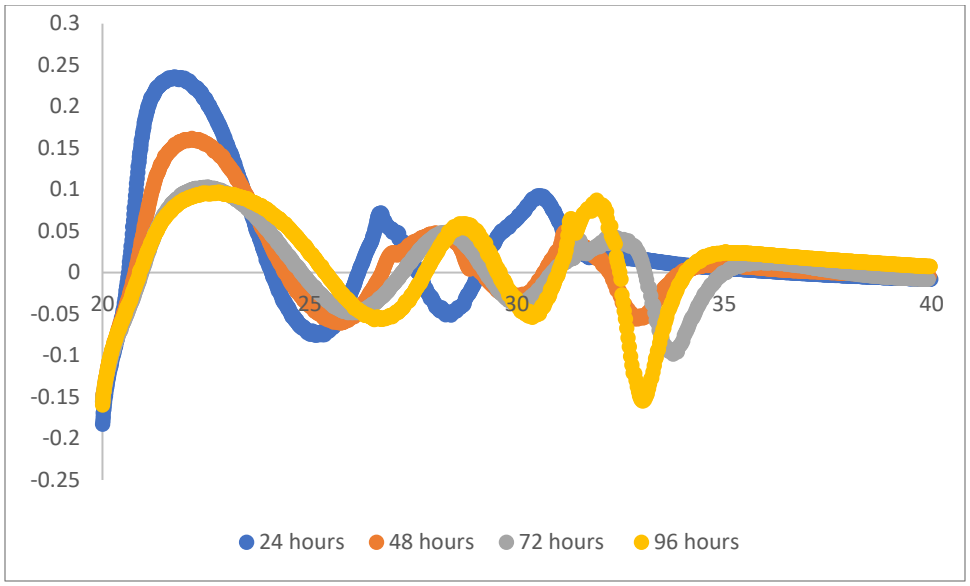

Figure 31: Residual plot of the curve fitting on thermal curves of cocoa butter containing YN.

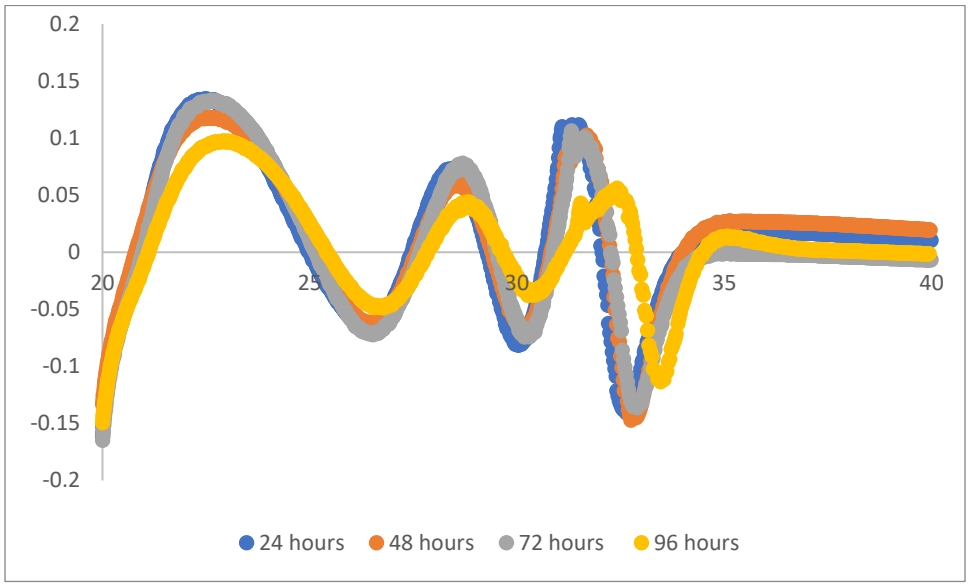

Figure 32: Residual plot of the curve fitting on thermal curves of cocoa butter containing canola oil.

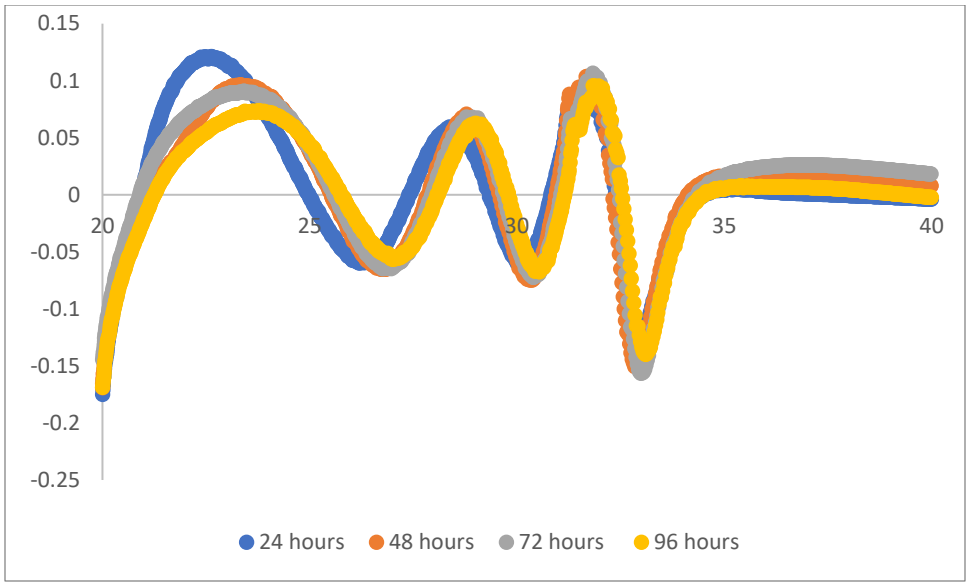

Figure 33: Residual plot of the curve fitting on thermal curves of cocoa butter containing castor oil.

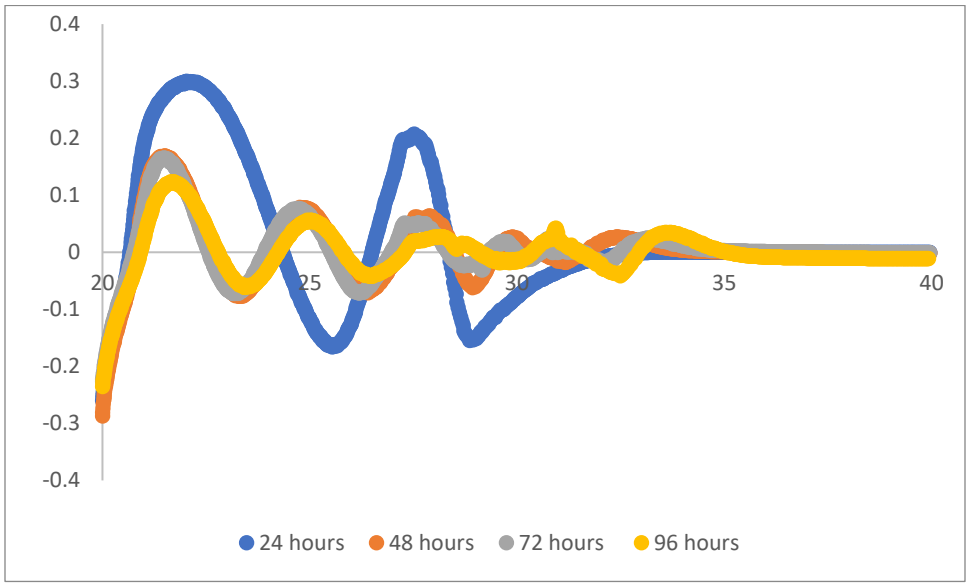

Figure 34: Residual plot of the curve fitting on thermal curves of cocoa butter containing sugar.

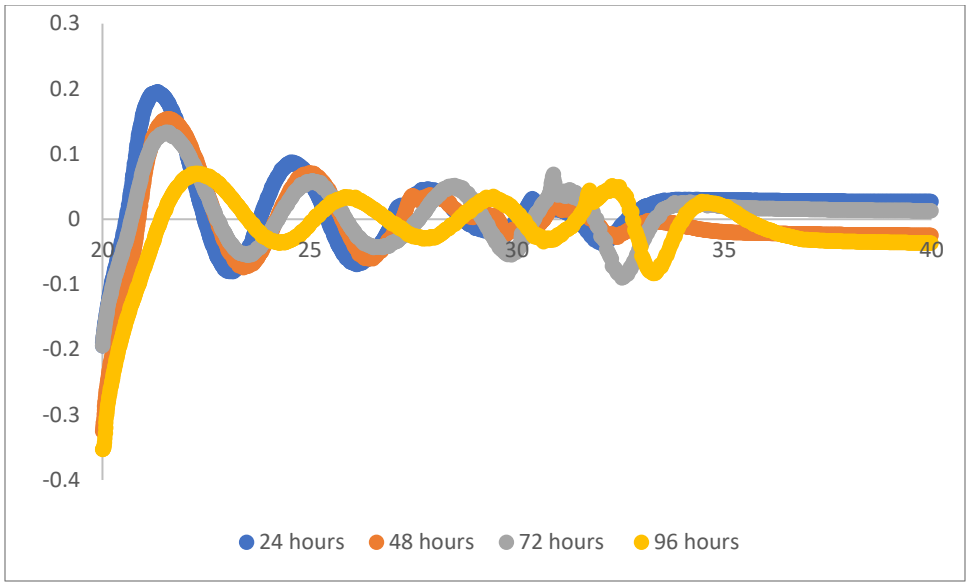

Figure 35: Residual plot of the curve fitting on thermal curves of cocoa butter containing sugar and lecithin.

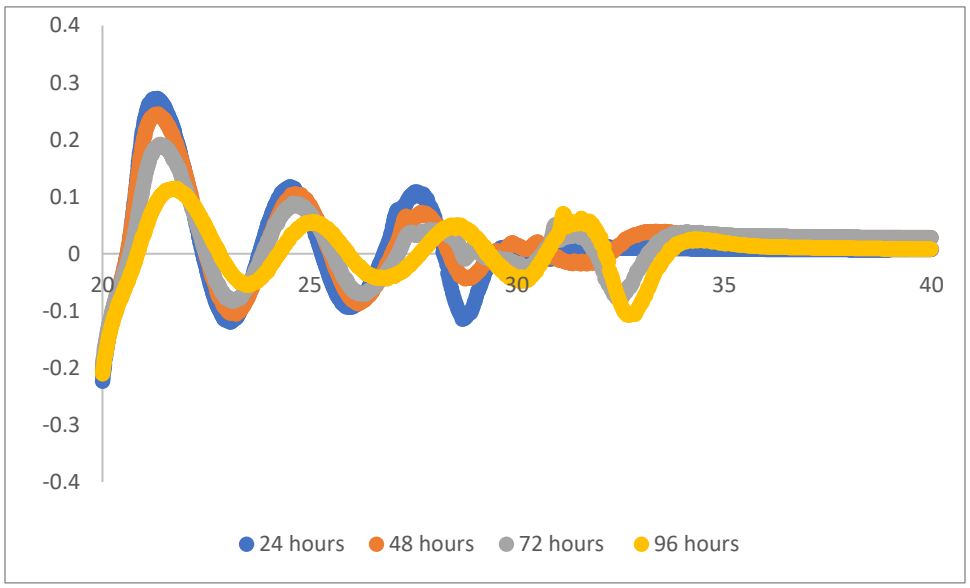

Figure 36: Residual plot of the curve fitting on thermal curves of cocoa butter containing sugar and PGPR.

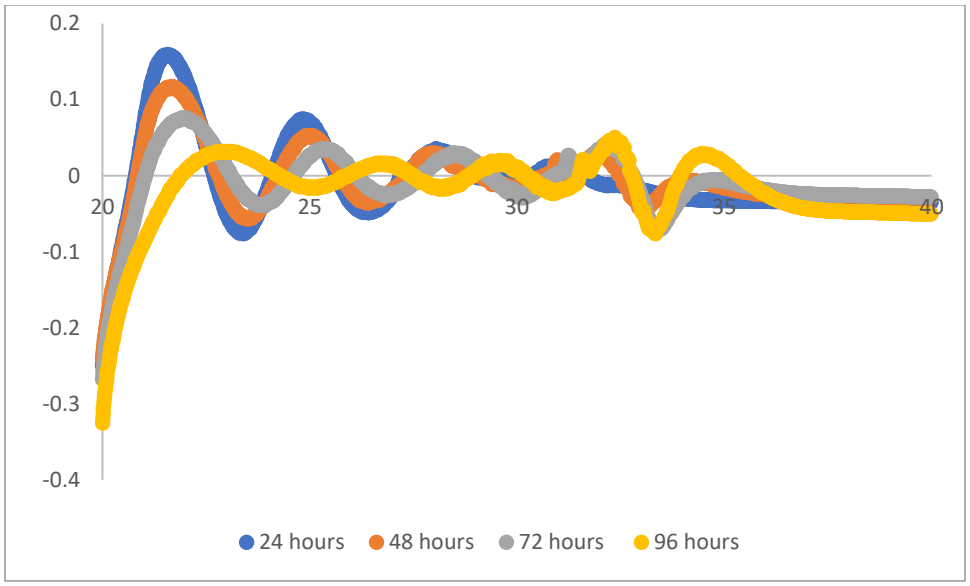

Figure 37: Residual plot of the curve fitting on thermal curves of cocoa butter containing sugar and CITREM.

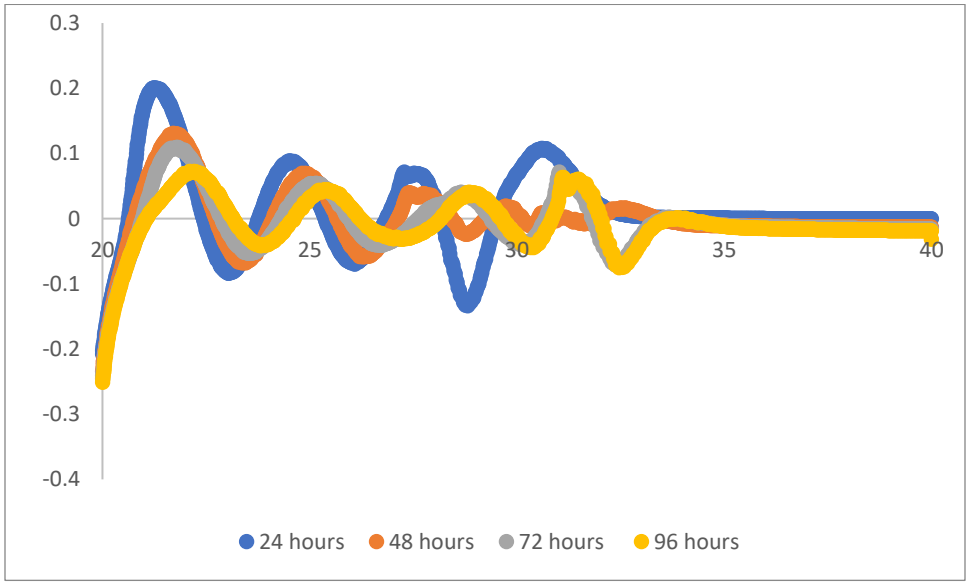

Figure 38: Residual plot of the curve fitting on thermal curves of cocoa butter containing sugar and canola oil.

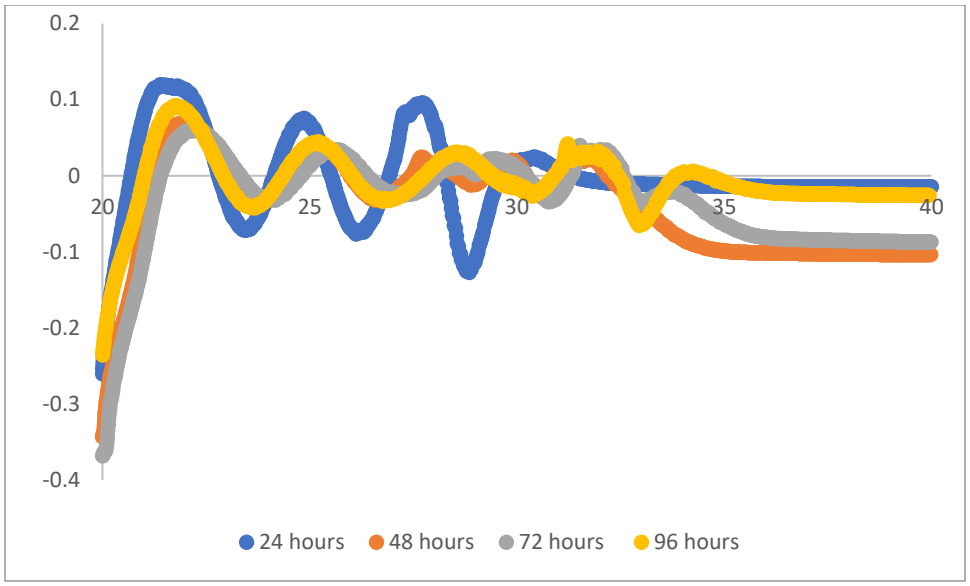

Figure 39: Residual plot of the curve fitting on thermal curves of cocoa butter containing sugar and castor oil.

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