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The role of non-fat ingredients on confectionery fat crystallization

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Abstract

Confections such as chocolate and biscuit fillings are composed of a continuous fat phase that contains dispersed non-fat ingredients such as sugar and cocoa powder. Research on fat crystallization and rheology in confections often extrapolates crystallization and textural properties from bulk to mixed systems while overlooking the important role of composition or particle interactions. For example, in chocolate processing the fat phase aids dispersed phase lubrication and fluidity whereas the dispersed particles assist in fat crystallization by providing many nucleation sites. In confections with a high dispersed phase volume fraction, fat crystallization may be hindered due to reduced triacylglycerol mobility, confinement, and increased tortuosity. This is further complicated in systems with slow crystallizing fats such as palm oil whose crystallization is exceptionally sensitive to composition and processing. This review breaks down the physical chemistry of fat-based confections and discusses the impact of different non-fat ingredients towards fat crystallization and rheology. The behaviour of palm oil is further highlighted as it is becoming increasingly popular as a confectionery ingredient. Lastly,

ingredient-ingredient interactions and their role in fat crystallization are described along with force spectroscopy as a novel tool to characterize such phenomena. Force spectroscopy utilizes atomic force microscopy to measure intermolecular forces as a function of distance but remains largely unexplored in the area of food science.

Keywords

confectionery, fat, palm oil, non-fat ingredients, ingredient interactions, rheology, force spectroscopy

Abbreviations

PO palm oil

CB cocoa butter

MF milkfat

TAG triacylglycerol

DAG diacylglycerol

MAG monoacylglycerol

FA fatty acid

FFA free fatty acid

PGPR polyglycerol polyricinoleate

ϕ volume fraction of non-fat ingredients

q cooling rate

T_e cooling endpoint

SFC solid fat content

AFM atomic force microscopy

PLM polarized light microscopy

XRD x-ray diffraction

DSC differential scanning calorimetry

$\dot{\gamma}$ shear

τ_0 yield stress

η^* viscosity

1 Fat-Based Confections

1.1 Main Components

Processed foods are composed of numerous ingredients often existing in a multi-phasic environment with structures ranging from the nano- to the macro-scale (Walstra, 2003; Rao, 2007). In fat-based confections, non-fat ingredients such as sugar, salts, cocoa solids, and/or proteins are dispersed within a continuous semi-solid fat phase (Götz et al., 2005; Sokmen and Gunes, 2006; Afoakwa et al., 2009; Dahlenborg et al., 2011; Carvalho-da-Silva et al., 2013; Svanberg et al., 2013), whose fat crystal morphology, polymorphic behaviour, and interactions with non-fat ingredients determine processability, quality, and sensory properties of the final product (Figure 1). Chocolate, which is perhaps the most popular and well-studied of these confections, is solid-like at room temperature ($\leq 25\text{ }^{\circ}\text{C}$), but melts sharply once in the mouth ($33\text{--}38\text{ }^{\circ}\text{C}$) (Afoakwa et al., 2008a). The proper crystallization of cocoa butter (CB), which can account for up to 30-40 wt.% of the product, is largely responsible for the sensory behaviour that consumers have come to expect (Afoakwa et al., 2007a; Rousseau, 2007; Fernandes et al., 2013).

Often, the main ingredient of fat-based confections is sucrose (Krüger, 1999; Beckett, 2000), which provides sweetness and mouthfeel (Jamieson, 2008) and is also a source of energy ($4\text{ kcal}\cdot\text{g}^{-1}$) (Aidoo et al., 2013). Adjusting the sugar content by as little as 5 wt.% can significantly impact the perceived sweetness in some food applications (Beckett, 2008). In products such as chocolate, optimal sugar particle size is approximately $30\text{ }\mu\text{m}$ in diameter in order to maintain proper textural properties (Jeffery, 1993). Coarser particles tend to confer grittiness while finer particles impact fluidity (Beckett, 2000). A particle size distribution of 60 % coarse and 40 %

fine particles has been shown to minimize the melt viscosity of fluid chocolate (Servais et al., 2002).

Emulsifiers are added to confections to lubricate and aid in the dispersion of sugar and other non-fat ingredients within fat-continuous environments (Beckett, 2000; Götz et al., 2005; Afoakwa et al., 2007a; Van Der Vaart et al., 2013). The most commonly used emulsifiers are soy lecithin, which contains phospholipids that contribute to its amphiphilic properties, and polyglycerol polyricinoleate (PGPR), a synthetic equivalent that shows a greater reduction in confectionery yield stress than lecithin (Vernier, 1998; Schantz et al., 2003). Other non-fat ingredients commonplace in confections includes milk solids, which are composed of casein and whey proteins, that have traditionally been added to provide a ‘creaminess’ (Haylock and Dodds, 1999), and cocoa solids, which contribute to flavour, texture, and are a source of antioxidants (Doká et al., 2013).

1.2 Rheology

This section summarizes the role of key ingredients found in confections on rheology, including the fat phase, sucrose, milk proteins, cocoa powder, and emulsifiers. The rheological properties of foods are important given their close association with processing efficiency, e.g., extrusion, enrobing, etc., and organoleptic properties (Kilcast and Clegg, 2002; Servais et al., 2002, 2004; Engelen et al., 2005; Ahmed and Ramaswamy, 2006; Denker et al., 2006; Baixauli et al., 2007; Engelen and Van Der Bilt, 2008; Ziegleder, 2009; Liu et al., 2010; Zarić et al., 2012). Oscillatory rheology is used to explore confectionery fat crystallization in the absence of shear (γ), i.e., under conditions where the material is not irreversibly deformed (Omar et al., 2005; De Graef et

al., 2006). By contrast, rotational rheology explores the influence of both torque and rotational shear on material properties such as viscosity (η^*), for example during the conching of dark chocolate (Vivar-Vera et al., 2008).

The rheological properties of confections depend on the physical properties of the continuous fat phase, e.g., solid fat content (SFC), crystal morphology, etc., the volume fraction of non-fat ingredients (ϕ), and specific ingredient interactions (Tscheuschner and Wünsche, 1979; Van Den Tempel, 1979; Jeffery, 1993; Tscheuschner, 1994; Bouzas and Brown, 1995; Kulozik et al., 2003; Liang and Hartel, 2004; Schantz and Rohm, 2005; Sokmen and Gunes, 2006; Do et al., 2007; Pérez-Martínez et al., 2007; Rao, 2007; Tang and Marangoni, 2007).

Fat-based confections are often shear-thinning, non-Newtonian pseudoplastic materials, possessing a measurable yield stress (τ_0) (Chevalley, 1999; Rector, 2000; Schantz and Rohm, 2005; Chhabra, 2006). Reduction of the continuous phase volume, as seen in fat-reduced products, results in a harder, more brittle texture (Beckett, 1999; Spearing, 2007). In fat reduced dark chocolate, viscosity has been shown to increase as a result of the reduced lubrication of sugar and other non-fat ingredients (Afoakwa et al., 2007b). In contrast, the addition of continuous phase results in a smoother, more fluid product (Aidoo et al., 2012) and plateaus at 32 wt.%, above which no effect is seen (Afoakwa et al., 2007a). The proportion of fat used in confections must also be considered during processing because of its impact on pumping, moulding, and other unit operations (Do et al., 2008). For example, chocolate may be pumped within pipelines at a flow rate of $10 \text{ t}\cdot\text{h}^{-1}$ across several dozens of metres within a production setting (Walker, 2009). Complications due to fat-reduced formulations, such as increased

hardness and difficulty to swallow (Beckett, 1999; Spearing, 2007), have been circumvented by either increasing emulsifier content or the addition of CB replacers (Wang et al., 2010), oleogels (Kaiser et al., 1998; Marangoni, 2012), or limonene (Beckett, 1999; Do et al., 2008), all of which help to maintain proper rheology.

The ϕ and their particle size distribution strongly impact rheology. Importantly, surface area and interactions with the continuous phase increase with a reduction in particulate size as this increases the energy barrier needed to overcome τ_0 and results in a much higher η^* (Beckett, 1994; Mongia and Ziegler, 2000; Ziegler et al., 2001; Walstra, 2003; De Graef et al., 2011; Fernandes et al., 2013). For the consumer, this is perceived as increased hardness and decreased spreadability (Chevalley, 1999; Beckett, 2000; Afoakwa et al., 2007b; Afoakwa et al., 2008b). Hence, control of particulate size is critical in order to maintain rheology and prevent undesirable texture attributes such as coarseness or grittiness (Jeffery, 1993; Servais et al., 2002; Afoakwa et al., 2007a).

Many commonplace ingredients will greatly contribute to the rheology of fat-based confections. Emulsifiers aid in processability and minimization of raw materials costs by improving the flow properties of chocolate and vegetable fat-based suspensions (mostly used as coatings) through lowering the friction between the particulates suspended in the liquid fat phase. As a result, τ_0 is reduced and ease of flow of these suspensions is permitted, even at low total fat content.

Using a sugar crystal as an example, to coat dispersed particulates, the hydrophobic moiety of the emulsifier will protrude into the fat phase whereas its hydrophilic moiety will adsorb onto the surface of the sugar crystal. In general terms, surface activity from an emulsifier will depend on

chemical structure, namely the balance in size between the hydrophilic headgroup and hydrophobic tailgroup. Those with larger headgroups will bind more strongly to sugar whereas others will be less strongly attached and may even be removed by the addition of different emulsifiers. Similarly, tailgroup length may affect the flow properties in different ways, implying that emulsifiers that are especially beneficial with regard to τ_0 may be poor with respect to η^* and vice versa (Beckett, 2008).

Lecithins are derivatives of *sn*-glycero-3-phosphates with one or two fatty acid residues attached to a glycerol backbone and an alcohol esterified to a phosphate group at the third glycerol position. They are commonly used to stabilize particulates in oil-continuous dispersions (e.g., sugar particles in CB), as they align into layers at the solid surface to reduce η^* stemming from particle aggregation (Johansson and Bergenstahl, 1992). For example, in chocolate manufacture, cocoa liquor (or mass) is mixed with sugar, additional CB, milk powder, and lecithin and through refining and conching the action of shear and coating of sugar with fat and lecithin improves chocolate flow properties. The addition of soy lecithin decreases τ_0 and η^* in chocolate until a maximum concentration of $4 \text{ g}\cdot\text{kg}^{-1}$. Beyond this point, continued addition begins to increase τ_0 (Table 1) (Chevalley, 1999; Schantz and Rohm, 2005). This is attributed to the micelles produced from lecithin phospholipids that disrupt particulate flow (Vernier, 1998).

PGPR is a synthetic food-grade emulsifier consisting of polyglycerol as the hydrophilic group and interesterified ricinoleic fatty acids as the hydrophobic group (molar mass between $1200\text{--}2000 \text{ g}\cdot\text{mol}^{-1}$) (Middendorf et al., 2015). It is increasingly being sought as a replacement for lecithin given its η^* -reducing effects that are generally similar to lecithin but at lower wt.%

values. For example, Ghorbel et al. (2011) found PGPR decreased τ_0 more significantly than either lecithin or total fat content in molten milk chocolate. Both emulsifiers had similarly influenced η^* . The interaction between emulsifier and fat on the one hand—and those between the emulsifier and solid particles on the other hand—play an important role not only in determining chocolate flow properties but also texture of the final product. Rousset et al. (2002) stated that PGPR enhanced the lipophilicity of sugar particles thereby decreasing sugar-sugar interactions and increasing the fluidity of the fat-based suspension. Finally, Middendorf et al. (2015) concluded that PGPR interacts with CB immobilized on the surface of sugar in order to form pillow-like deposits, which act as spacers to reduce interaction between sugar particles. In addition to providing creaminess, milk solids also impact rheology where casein, which possesses both polar and non-polar sites, behaves similar to an emulsifier (Haylock and Dodds, 1999).

An increase in demand of low-calorie confections has resulted in many sugar-free products (Zumbé and Brinkworth, 1993; Sokmen and Gunes, 2006). Sugar substitutes, which include bulk sweeteners such as sugar alcohols, e.g., sorbitol and xylitol, and saccharide alternatives, e.g., trehalose and isomaltulose (Olinger, 1994; Kroger et al., 2006; Aidoo et al., 2013), have a negative impact on rheology (Clayton and Conn, 2005). For example, sugar alcohols were found to reduce the quality of chocolate and result in poorer rheology (Zumbé and Brinkworth, 1993). A further study showed that maltitol produced a η^* most similar to sucrose whereas isomalt significantly increased it (Sokmen and Gunes, 2006). In another study, the substitution of sucrose with steviol glycosides, which are natural sweet-tasting compounds isolated from the plant *Stevia*

rebaudiana, in combination with inulin and dextrose as bulking agents was found to not significantly alter hardness of chocolate (Shah et al., 2010).

Moisture often has a dire effect on fat-based confections. It causes dispersed particulates to cluster through attractive polar forces and capillary action, resulting in poor mixing and increased particle heterogeneity as well as deleterious effects on η^* (Nováková et al., 2002). This is in large part due to the low affinity sugar has for the continuous phase. The effect of moisture has also restricted the usage of hygroscopic low-calorie ingredients such as glucose or fructose (Afoakwa et al., 2007a). To counteract this effect, confections are formulated with a higher fat content, though this is clearly not cost-effective. For example, CB is adjusted by a three-fold addition for every one-fold addition of unwanted moisture in order to correct its rheology (Beckett, 2000; Aidoo et al., 2012). Approaches such as high intensity conching (Nováková et al., 2002) and ultrasound (Yucel and Coupland, 2011) may also remove these aggregates.

A nascent area is that of the role of micro-viscosity and rheology on particle networks. In particular, characterizing the micro-rheological properties of liquid-state oil may improve our understanding of the role of confinement and non-fat ingredient interactions on fat crystallization. Therefore, it is important to isolate this behaviour from the aforementioned bulk rheology as much as possible. It was previously reported that the micro-viscosity of localized oil was influenced by the fractal dimension of fat-oil mixtures and not their SFC. Relying on the twisted intramolecular charge transfer properties of the molecular rotor citrus red, micro-viscosity was determined to increase as oil confinement and fractal dimension increased (Du et al., 2014). While determination of oil micro-viscosity may possibly give insight into its

migration and impact on crystallization in systems containing non-fat ingredients, any existing study remains to be published.

Large deformation experiments, e.g., cone penetrometry and two-plate compression, are used to determine the textural properties of foods, e.g., mechanical firmness and adhesiveness, and are often used to corroborate sensory tests (Hayakawa and DeMan, 1982; Rousseau and Marangoni, 1999; Liang and Hartel, 2004; Omar et al., 2005). These experiments may also be used in conjunction with rheology (Beckett, 2000; Bourne, 2002). The main limitation with texture analysis is the inability to reproduce chewing and the absence of saliva, which can invert phases within fat-based confections, changing the way in which they are perceived (Lee and Pangborn, 1986; Carvalho-da-Silva et al., 2013). Tribology, which studies the interaction between food and oral epithelia, has been developed to minimize this challenge. The tribological properties of some fat-based confections have been studied, though much work remains to be done in this area (Carvalho-da-Silva et al., 2011; Lee et al., 2002).

2 Fats and Oils

2.1 Molecular Structure

Like all food fats, confectionery fats are mixtures of solid- and liquid-state triacylglycerols (TAGs) where fatty acids (FAs) are esterified to a glycerol skeleton (Figure 2) (Timms, 1984). These molecules conform to an asymmetric tuning fork or chair geometry due to steric hindrance at the *sn*-2 position (Larsson et al., 2006). Hindrance is exacerbated with unsaturation at this position, e.g., oleic acid in CB TAGs, which is partly responsible for its sharp melting behaviour (Smith and Dahlman, 2005; Afoakwa et al., 2007a).

Mixtures of fats may be simple, consisting of just a few key TAGs, e.g., CB (Sato et al., 1989; Loisel et al., 1998; Beckett, 2000; Smith and Dahlman, 2005), or complex with several hundreds, e.g., milkfat (MF) (Gresti et al., 1993). As composition becomes more complex, the thermal profile of the fat becomes less defined and broader, e.g., MF melts between -40 °C and 40 °C (Larsson, 1994). Both origin and environmental factors, e.g., temperature and rainfall, can alter the composition of the source, influencing physical properties of the fat (Table 2) (Timms, 2003; Marty and Marangoni, 2009).

Common minor components include partial acylglycerols, e.g., monoacylglycerols (MAGs) and diacylglycerols (DAGs), free fatty acids (FFAs), and other components, e.g., carotene, tocopherol, etc. (Gee, 2007; Gibon et al., 2007; Kellens et al., 2007; Smith et al., 2011). DAGs have higher slip points than TAGs, which can negatively impact the intended application (Ab Latip et al., 2013). For example, in one study the slip points of commercial and DAG-enriched margarines were determined to be 30.5 ± 0.2 °C and 35.5 ± 0.1 °C, respectively (Saber et al., 2012). With the consideration of oral temperature, a DAG-enriched margarine would have a longer, waxier thermal profile. DAGs are also believed to slow fat crystallization, although this mechanism is not fully understood (Siew and Ng, 1999; Tshiamala, 2013). Though emulsifiers are a mainstay of the fat and oil industry, there remains a dearth of information on their structure-function relationship and the mechanisms by which they influence TAG nucleation and growth. In this regard, they may significantly impact fat nucleation, crystal growth or both, depending on their composition, added amount and mechanism of action (Smith et al., 2011).

2.2 Polymorphism and Morphology

The key polymorphs identified in most fats and oils, in order of increasing stability, are the α – hexagonal, β' – orthorhombic, and β – triclinic forms (Figure 3) (Talbot, 1999; Bresson et al., 2011; Tshiamala, 2013). These different packing configurations describe the lateral packing of molecules and provide the fingerprint short spacings. While polymorph subtypes have been reported in pure TAG systems, e.g., sub- α , pseudo- β' , sub- β , etc., they are not universally accepted (D'Souza et al., 1990).

X-ray diffraction (XRD) is used to identify fat crystal polymorphs (Figure 4) (D'Souza et al., 1990; Schenk and Peschar, 2004; Da Silva et al., 2009). With wide-angle XRD, the simplest definition is based on short spacings where one strong peak at 4.15 Å is observed for α crystals, peaks at 3.8 Å and 4.2 Å for β' crystals, and one strong peak at 4.6 Å and two lesser peaks at 3.7 Å and 3.9 Å for β crystals (Larsson, 1966; Hernqvist, 1988a; DeMan, 1992; Verstringe et al., 2012). These forms are driven by van der Waals forces to further pack into bilayer or trilayer lamellae two to three alkyl chains thick, respectively, interpreted as the long spacing of the crystals (Talbot, 1999; Larsson et al., 2006).

Other than XRD, many techniques can be used to characterize the crystallization behaviour of fats (Van Malssen et al., 1994, 1999; Loisel et al., 1998; Van Langevelde et al., 2001a, 2001b), including differential scanning calorimetry (DSC) (Foubert et al., 2008; De Cock, 2011; Tshiamala, 2013), Raman spectroscopy (Kobayashi, 1988; Bresson et al., 2005, 2006, 2011; Hu et al., 2005; Schoukens and De Clerck, 2005; Da Silva et al., 2009), and dilatometry (Wille and Lutton, 1966) although this final technique is rarely used nowadays.

Polymorphs are associated with specific fat crystal morphologies under static crystallization. For example, α crystals are least dense and form platelets $\sim 5 \mu\text{m}$ in length (Rousseau et al., 2005). Both β' and β crystals are acicular where crystal growth is more energetically favoured along its length. These needle-like crystals are typically $1\text{--}2 \mu\text{m}$ and $50 \mu\text{m}$ in length for β' and β , respectively. Morphology may affect textural properties of bulk fats and oils. Rounded crystals, such as those shaped by shear, will flow past each other more easily to produce a smoother texture whereas sharper, more angular crystals will fracture and become brittle. For example, the ideal textural properties for palm-based margarines, including proper smoothness and spreadability, are obtained if β' crystals are formed. In more complex foods, the effect of crystal morphology on texture is not fully understood because of factors such as multiple phases, ingredient interactions, and compartmentalization (Hartel, 2001).

As in other fats, polymorphism in confectionery fats is monotropic evolving from less to more stable polymorphs. CB polymorphism has been studied extensively because of its close association to organoleptic properties and shelf life. A previous XRD study (Wille and Lutton, 1966), later to be confirmed by DSC (Huyghebaert and Hendrickx, 1971), showed a total of six polymorphs to exist in CB, i.e., γ , α , β'_2 , β'_1 , β_2 , and β_1 , melting over a range of 17 to 36°C (Table 3), with β_2 crystals exhibiting optimal macroscopic properties (Smith and Dahlman, 2005). The triple chainlength packing of higher order polymorphs further allows for higher thermodynamic stability and lower occupied volume compared to double chainlength packing (Talbot, 1999). With proper tempering, β_2 crystals initially form at $1\text{--}3 \text{ vol.}\%$, establishing a template for further crystallization of the remaining melt (Svanberg et al., 2013). More contemporary methods use pre-made β_2 crystals to seed the melt and facilitate proper

crystallization of the chocolate mass (Windhab, 1999; Zeng, 2000). Svanberg et al. (2011a) showed that seeded samples of molten chocolate formed multiple nucleation sites and a large degree of homogeneity was not observed in the absence of seeding. Instead, large spherical crystals were observed in some areas, but absent in others.

Importantly, β_2 crystals contribute to a glossy finish, clean fracture, i.e., snap, quick melting at oral temperature, flavour release, and contraction for demoulding of shelled confections (Timms, 1984, 2003; Seguine, 1991; Stapley et al., 1999; Talbot, 1999; Beckett, 2000, 2008; Khan and Rousseau, 2006; James and Smith, 2009). Shelf life is also prolonged because of the ability for β_2 crystals to restrict liquid oil from migrating to the surface and promote fat bloom, characterized by the onset of large β_1 crystals, i.e., $> 5 \mu\text{m}$, that have the ability to diffuse light, giving a dull, grey appearance of the surface (Cebula and Ziegleder, 1993; Lohman and Hartel, 1994; Loisel et al., 1997; Ziegleder, 1997; Hartel, 1999; Sato, 2001; Timms, 2003; Dibildox-Alvarado et al., 2004; Lonchampt and Hartel, 2004; Smith et al., 2007; Nopens et al., 2008; Dahlenborg et al., 2011; Glicerina et al., 2013). Furthermore, with β_1 crystals, melting point is increased and the product adopts a gritty texture (Afoakwa et al., 2009). Internally, β_1 crystals may also form but are typically smaller and more irregularly shaped (Jewell, 1972; Hartel, 1999; Lonchampt and Hartel, 2004; James and Smith, 2009; Dahlenborg et al., 2011; Dahlenborg, 2014). With proper storage, it may take years for properly-tempered chocolate to produce enough bloom-inducing β_1 crystals (Bomba, 1993; Subramaniam, 2000; Rousseau et al., 2010). With under-tempering, this process occurs in just days (Vaeck, 1960; Campbell, 1967; Hernqvist, 1988b; Rousseau, 2007; Bresson et al., 2011), as β'_1 crystals rapidly form due to the

lack of a proper template and undergo a $\beta'_1 \rightarrow \beta_2$ transition on the surface of chocolate (Schlichter-Aronhime and Garti, 1988; Seguiné, 1991; Hodge and Rousseau, 2002).

2.3 Crystallization

Fat crystallization may occur from either melts or solutions (Figure 5) (Himawan et al., 2006; Kellens et al., 2007). This process is driven by the reduction of activation free energy as the system is shifted from equilibrium and either the melt is cooled below its melting point or the solution has reached saturation. For melts, the chemical affinity (Φ), which is the driving force promoting TAGs to associate, is expressed by:

$$\Phi = \Delta H_f \frac{(T_f - T)}{T_f} \quad (1)$$

where ΔH_f is the latent heat of fusion, T_f is the melting point, and T is the temperature of the system (Hartel, 2001). For solutions, this is expressed by:

$$\Phi = RT \ln \left(\frac{\gamma X}{\gamma_s X_s} \right) \approx RT \ln \left(\frac{C}{C_s} \right) \quad (2)$$

where R is the gas constant, γ is the activity coefficient, γ_s is the activity coefficient at equilibrium, X is the mole fraction, X_s is the mole fraction at equilibrium, C is the concentration, and C_s is the saturation concentration (Hartel, 2001). Obviously, as T is reduced in either melts or solutions, so is Φ . In systems of great TAG diversity, crystallization proceeds from both melt and solution. This is explained by the presence of lower-melting TAGs serving as solvent for fat crystals composed of higher melting TAGs. Butter is just one example of a mixed system where crystallization from both melt and solution occurs (Hartel, 2001).

The first step in crystallization is primary nucleation where molecules either combine from the molten state, i.e., homogeneous, or form on the surfaces of impurities, i.e., heterogeneous (Metin and Hartel, 2005; Basso et al., 2010). Liquid-state TAGs assemble through local energy fluctuations into embryos under homogeneous nucleation (Walton, 1969; Zettlemoyer, 1969; Mullin, 1993). This process is reversible but the rate of association eventually exceeds that of dissociation as the system continues to shift away from equilibrium. These embryos must form prior to the formation of stable nuclei of a critical size. For melts, this critical size (r_c) is estimated by:

$$r_c = \frac{2\sigma_s v T_f}{\Delta H_f (T_f - T)} \quad (3)$$

where σ_s is the interfacial tension and v is the molecular volume (Hartel, 2001). The rate of homogeneous nucleation (J_{hom}) from the molten state is:

$$J_{hom} = A \exp \left\{ \frac{16\pi\sigma_s^3 v^2 T_f^2}{3kT\Delta H_f^2 (T_f - T)^2} \right\} = A \exp \left\{ -\frac{\Delta G_c}{kT} + \frac{\Delta G'_v}{kT} \right\} \quad (4)$$

where k is Boltzmann's constant, ΔG_c is the critical free energy for a stable nucleus, and $\Delta G'_v$ is the free energy for molecular diffusion. Nucleation is further influenced by polymorphism where the rate increases with lower order (Hartel, 2001). A reduced σ_s in α crystals is one suggestion behind their tendency to nucleate before either β' or β crystals (Sato, 1988), where their greater chain mobility may significantly reduce interface energy.

The theory behind heterogeneous nucleation (J_{het}) is a modification of homogeneous nucleation, such that:

$$J_{het} = A \exp \left\{ -\frac{\Delta G'_c}{kT} \right\} \quad (5)$$

where $\Delta G'_c$ is the critical free energy for heterogeneous nucleation (Hartel, 2001). $\Delta G'_c$ in this case is the combination of tensions at the melt-foreign surface, crystal-foreign surface, and melt-crystal interfaces (Garside, 1987). $\Delta G'_c$ may be explained by:

$$\Delta G'_c = \Delta G_c \left\{ \frac{1}{4} (2 + \cos\theta)(1 - \cos^2\theta) \right\} \quad (6)$$

where θ represents the angle of wetting between crystal and nucleating solid. This angle may be calculated by:

$$\cos\theta = \frac{\sigma_{sl} - \sigma_{cs}}{\sigma_{cl}} \quad (7)$$

where interfacial tensions for melt-foreign surface, crystal-foreign surface, and melt-crystal are σ_{sl} , σ_{cs} , and σ_{cl} , respectively. The presence of a foreign surface, such as a sugar particle, should lower θ , which in turn requires fewer liquid-state TAGs to assemble a nucleus of critical size. In return, $\Delta G'_c$ is lowered and nucleation occurs more easily (Hartel, 2001). Furthermore, a greater degree of lattice matching, i.e., physical or chemical complementarity, between the foreign surface and fat crystal impacts nucleation of the system (Metin and Hartel, 2005).

Foreign surfaces that facilitate nucleation may be inherent to the system, e.g., dust, dirt, etc., or they may be intentionally added. For example, heterogeneous nucleation of fat crystals off the surface of sugar particles was observed in non-seeded CB-sugar systems (Svanberg et al., 2011a). It has been theorized that one catalytic impurity per cubic millimetre results in a nucleation large enough to ensure rapid phase transition at a given supersaturation (Walstra,

2003). Homogeneous nucleation rarely proceeds in food systems because of this (Land et al., 1999; Cacciuto et al., 2004; Eremina et al., 2005; Rak et al., 2005).

The deviation from equilibrium required for nucleation is reduced in the presence of foreign surfaces because they provide active sites that lower the energy barrier by facilitating proper orientation of liquid-state TAGs (Bowser, 2006; Rousseau and Sonwai, 2008; Svanberg et al., 2011a, 2011b; Fernandes et al., 2013; Hartel, 2013). For example, crystallization induction time is reduced in the presence of sugar for palm oil (PO) (Figure 6), cocoa solids for CB (Svanberg et al., 2011a), common minor components of fats and oils (Savage and Dimick, 1995), and solvents such as acetone (Hartel, 2001). Furthermore, local shear rates near non-fat ingredient surfaces increase nearly two-fold, providing enough energy to promote transition to higher ordered polymorphs (Mazzanti et al., 2003; Dhonsi and Stapley, 2006). The effect of various lipophilic molecules on nucleation has been previously reviewed (Smith et al., 2011; Yoshikawa et al., 2014).

The effect of inorganic additives on trilaurin (LLL) crystallization was recently studied by Yoshikawa et al. (2014). These additives appeared to enhance crystallization and the $\beta' \rightarrow \beta$ transition. Furthermore, there existed an interaction between type of additive and TAG orientation on the surface of LLL crystals. XRD studies revealed greater peak intensities for long spacing over short spacing in the presence of talc, suggesting orientation where lamellar planes were parallel to additive surface. In contrast, these planes grew normal to the surface of graphite (Figure 7) (Yoshikawa et al., 2014). Crystallization suppression may sometimes be observed in

the presence of an additive (Cacciuto et al., 2004). For example, lecithin has suppressed fat crystallization of CB-sugar models (Dhonsi and Stapley, 2006).

Crystals are fewer but larger in size if nucleation is slow, hindering their ability to flow across each other with minimal friction and causing products to become grainy or coarse in texture. For example, the textural quality of margarines is significantly reduced if crystals exceed 10 μm (Walstra, 2003). In contrast, high nucleation rates result in many smaller crystals and a pasty consistency (Hartel, 2001). The rate of nucleation may be quantified by refractive index, turbidimetry, or light scattering although this has been met with challenges (James, 1982).

Secondary nucleation depends on the presence of existing fat crystals and can occur in parallel to primary nucleation (Larson, 1981; Metin and Hartel, 2005; Basso et al., 2010). While questions remain as to how exactly this occurs (Randolph and Larson, 1988), some mechanisms suggest secondary nucleation proceeds by micro-attrition where primary crystals fragment under high shear, feeding the secondary nuclei (Walstra, 2003). Following primary and secondary nucleation and crystal formation is crystal growth where liquid-state TAGs continue to incorporate onto crystal surfaces and heat of fusion is absorbed until an equilibrium is reached (Tavare, 1995; Hartel, 2001, 2013). The energy that is released from heat of fusion may also facilitate the transition of lower order crystals into higher states. Eventually these crystals agglomerate to form a network in which the remaining liquid phase is entrapped (Metin and Hartel, 2005; Kellens et al., 2007).

Following the formation of stable nuclei, growth and association of crystallized lamellae result in the formation of crystal nanoplatelets (Acevedo and Marangoni, 2010; Acevedo et al., 2011;

Marangoni and Wesdorp, 2013). These CNPs organize into stack-like structures by means of molecular van der Waals forces which then go on to assemble into crystallites that cluster via colloidal van der Waals forces to form crystals. Aggregation and sintering (formation of solid bridges) between crystals results in the formation of a 3D fat crystal network (Pink et al., 2013; Tarabukina et al., 2009).

Fat crystal networks are easily characterized using polarized light microscopy (PLM) in bulk systems (Chen et al., 2002; Tang and Marangoni, 2007). Microscopy is used to better understand food structure and interactions at the molecular level and how they impact macroscopic properties (Flint, 1994; Stanley et al., 1998; Aguilera and Stanley, 1999; Morris, 2007). For fat- and oil-based products, factors such as fat crystal morphology and size distribution of non-fat ingredients are important considerations for product developers (Rousseau, 2007). While this distribution may sometimes be difficult to obtain, sizing by either optical microscopy, gravimetry via sieve stacks, or laser diffraction is possible for objects larger than a few μm .

2.4 Compositional and Processing Effects

The crystallization pathway is heavily influenced by both composition and processing, e.g., cooling rate (q), $\dot{\gamma}$, etc. (Hernqvist, 1984; Awua, 2002; Campos et al., 2002; Dhonsi and Stapley, 2006; Kellens et al., 2007; Pérez-Martínez et al., 2007). For example, TAG composition can impact polymorphism where larger diversity favours metastable β' crystal formation (Rousseau, 2007). Furthermore, while $\dot{\gamma}$ can complicate polymorphic assignment based on morphology, the rate of crystallization is increased through its ability to facilitate mass transfer and higher ordered polymorphs proliferate (Sato, 2001; Mazzanti et al., 2003; De Graef et al., 2006; Tarabukina et

al., 2009). Mass transfer of TAGs during crystal growth is hypothesized to occur either through diffusion, convection, or a combination of the two (Mullin, 1993). The rate of crystal growth (R_c) has been explained by the simple model:

$$R_c = \frac{k(\sigma - 1)}{\eta} \quad (8)$$

where k is a constant and σ is the degree of supersaturation (Mathlouthi and Genotelle, 1995). Assuming σ is constant, R_c is reduced as η of the system increases.

Large kinetic factors, e.g., large q , favour the formation of many small crystals composed of lower ordered polymorphs according to Ostwald's Step Rule (Bennett et al., 1965; Sato, 2001). DSC has been used to verify this where a small exotherm corresponding to the α polymorph was observed under a larger degree of supercooling (Hartel, 2001). Liquid-state TAGs quickly incorporate within growing crystals under these conditions, resulting in lamellar occlusions and disorganization (Bennett et al., 1965). Transition of these crystals to a higher ordered polymorph may be on the order of months as a result of steric hindrance and large energy barrier (Sato, 1993; Hartel, 2001).

Larger but fewer crystals of higher polymorphic order form under smaller q (Litwinenko et al., 2002; Metin and Hartel, 2005). Furthermore, these conditions minimize thermal gradients (Kleinert, 1961) and allow the complete formation of a lamella before another layer begins (Marangoni, 2005). While this may seem favourable, one potential caveat to a small q is the coexistence of different polymorphs, which may weaken the overall crystal network (Fessas et al., 2005). The effect of q on fat crystallization has been studied further (Baldino et al., 2010).

Cooling endpoint (T_e) is also an important consideration in processing. For example, α crystals form readily in some oils, e.g., PO, at a T_e under 20 °C due to the monotropic behaviour of TAGs. In addition to β' crystals forming directly from the melt, α crystals simultaneously transition into β' crystals (Fernandes et al., 2013). At T_e values above 20 °C, β' crystals form exclusively from the melt (Litwinenko et al., 2002; De Graef et al., 2006; Foubert et al., 2006). A deep understanding of how processing methods may impact fat crystallization is necessary to industry in order to maintain final product quality (Herrera and Hartel, 2000; Altimiras et al., 2007; Pérez-Martínez et al., 2007; Afoakwa et al., 2008c; Debaste et al., 2008).

3 Palm Oil in Application

Confectionery fats may be split into lauric fats, such as coconut oil and palm kernel oil that are high in lauric acid content, and into non-lauric fats, such as CB, PO, and shea butter. While the handling of lauric fats are quite easy, they are very high in saturated fat content and may not be suitable for some applications, such as confectionery fillings. Furthermore, industry has been continuously seeking non-hydrogenated fats to improve the nutritional profile of their products in response to restricted usage of hydrogenated vegetable oils. PO has quickly risen to the top of fat alternatives in industry due to its natural semi-solid consistency and affordability, however, its physicochemistry is far lesser known than other fats. Here, the production and chemistry of PO is reviewed.

3.1 Production and Use

It has been highly recommended to replace saturated fats with poly- and monounsaturated fats in order to control lipoprotein levels (Hruby and Hu, 2016). This is in part due to the association

between cardiovascular disease and saturated FAs found in animal-based fats (Neaton and Wentworth, 1992), however, positional distribution of these FAs within TAGs must also be taken into account (Karupaiah and Sundram, 2007). While this generally encourages consumption of plant-based oils, tropical oils are considerably higher in saturation (Edem, 2002; Gee, 2007). This prompted nutritional campaigns in the 1980s which resulted in much of the present-day aversion to PO by consumers (Allen, 2002; Gratzner, 2005; McNamara, 2010).

Malaysia is currently the largest producer and exporter of PO and palm by-products, followed by Indonesia (Sambanthamurthi et al., 2000). It is cheaper to manufacture than any other plant-based oil (Edem, 2002) and has chemical and thermal properties ideal for applications including a natural semi-solid consistency, relatively slow melting properties, and low foaming properties (Okuy and Oke, 1984; Kheiri, 1987; Cottrell, 1991). Uses of PO and its fractions are listed in Figure 8.

PO is considered a viable substitute for partially hydrogenated oils because of its natural semi-solid consistency and low tendency to become rancid when compared to other plant-based oils (Maache-Rezzoug et al., 1998; Sambanthamurthi et al., 2000; Tarrago-Trani et al., 2006; Aftab et al., 2013). Several commercial products including biscuits, cream fillings, breads and meat patties, have already been successfully formulated with PO and do not contain the *trans* fats that are produced from either partial hydrogenation or high temperature deodorization (Fritsche and Steinhart, 1998; Basiron, 2001; Sherazi et al., 2009). Furthermore, consumption of a palm-based diet was recently shown to reduce low-density lipoprotein levels (Voon et al., 2011). Despite young consumers seeking fat-reduced options when purchasing food (Do et al., 2008), this has

traditionally not been an issue with confections since they are typically consumed in small quantities as treats (Rössner, 1997).

3.2 Composition

Palmitic and oleic acid are the main FAs found in ripe drupe of oil palm, at 44 % and 39-40 % respectively, followed by linoleic, stearic, and myristic acid (Table 4) (Basiron, 2001; Gee, 2007). To date, no other plant-based oil is found to be as rich in palmitic acid as palm (Sambanthamurthi et al., 2000).

TAGs account for over 95 % of PO content with POP being most abundant (Sambanthamurthi et al., 2000; Gee, 2007). While there exists regioselectivity of unsaturated FAs at the *sn*-2 position, approximately 7-10 % of TAGs in PO are trisaturated. Minor components of natural PO include DAGs, MAGs, FFAs, phosphatides, sterols, and trace metals (Siew and Ng, 1995; Sambanthamurthi et al., 2000). The DAG and TAG composition of commercial PO is listed in Table 5 and consists mainly of PPO/POP and OOP/OPO at 27.6 and 22.5 %, respectively (West and Rousseau, 2016).

Carotenoids, e.g., β -carotene, aid with vision and are responsible for the dark amber colour in raw PO (Edem, 2002). These carotenoids are destroyed after the raw oil has been refined, bleached, and deodorized (Cottrell, 1991). PO is also one of the richest natural sources for tocotrienols (Goh et al., 1985; Sundram and Top, 1994), a subclass of vitamin E that has gained much interest in its role in the circulatory regulation of cholesterol (Sen et al., 2007; Stanley, 2008).

3.3 Processing

Unrefined PO is relatively unstable and must be processed for industrial use (Dian et al., 2006; Kellens et al., 2007). This instability comes from the relatively high FFA content of PO, i.e., 5 wt.% (Gee, 2007; Murphy, 2007). The mishandling of natural PO, such as exposure to high temperatures, propagates its acidification, darkening, and uncontrolled enzymatic peroxidation (Chong, 1993; Sambanthamurthi et al., 2000; Edem, 2002). Consumption of these peroxides may lead to health problems including hyperlipidemia, platelet aggregation, and tissue damage (Osim et al., 1992, 1994).

MAGs, FFAs and other minor components are removed from natural PO following initial processing (Gee, 2007; Kellens et al., 2007). The oil may then be fractionated according to its saturation to produce oils that are functionally optimized for specific applications. For example, higher-melting TAGs can be separated from a melt by selective crystallization using a controlled temperature protocol (Hartel, 2001).

Based on temperature-based fractionation, the two main fractions of PO are palm stearin and palm olein (Tshiamala, 2013). Common palm olein melts at 18 °C to 20 °C and accounts for two-thirds of PO on a weight basis. This liquid fraction is formulated into dressings and cooking oils (Gunstone and Norris, 1983; Stanley, 2008). DAGs co-fractionate into palm olein and produce a eutectic mixture that is responsible for early onset crystallization and clouding (Siew and Ng, 1996; Gee, 2007; Kellens et al., 2007). Palm stearin composes the remainder of PO and melts at 48 °C to 50 °C. This fraction is often used in margarines and shortenings (Gunstone and Norris, 1983; Stanley, 2008). The higher-melting TAGs in palm stearin are responsible for its

higher crystallization rate compared to palm olein and ability to form β crystals (Che Man et al., 1999). Palm olein and stearin may be further fractionated to produce either mid-fractions used for CB equivalents and other confectionery applications or double-fractionated oils such as super olein, which is used in tocotrienol-rich supplements (Kellens et al., 2007; Stanley, 2008). The physical properties of PO and its fractions are listed in Table 6 (Edem, 2002).

Interesterification is another processing method used to treat PO in order to change its physical properties without changing the FA profile. For example, melting profile, mouthfeel, and spreadability are changed using this technique (Gibon et al., 2007; Kellens et al., 2007). Chemical interesterification completely randomizes the position of FAs along the glycerol skeleton whereas enzymatic interesterification exhibits different degrees of regioselectivity depending on the choice of enzyme (Costales-Rodríguez, et al., 2009). Crossbreeding of the African oil palm with trees naturally lower in saturated content, such as *Elaeis oleifera*, i.e., American oil palm, has also been pursued as a means to generate PO with lower saturated FA content (Sambanthamurthi et al., 2000).

3.4 Recrystallization

PO, in addition to other fats and oils, continues to undergo morphological and spatial rearrangement during long-term storage as a result of recrystallization. The crystalline phase undergoes orientation and perfection to increase van der Waals interactions and minimize the free energy of the system (Fennema et al., 1973; Hartel, 1998; Russell et al., 1999; Sonoda et al., 2004; Metin and Hartel, 2005). While this is believed to proceed through solid-state transitions, the mechanism is not fully understood and is independent of both temperature and humidity

(Fennema et al., 1973; Tshiamala, 2013). It is theorized that recrystallization is driven by either differences in local equilibria of different sized crystals, i.e., Ostwald ripening, the fusion of adjacent crystals, i.e., accretion, or the minimization of crystal surface-to-volume ratios (Kuczynski, 1989; Hartel, 2001).

Recrystallization is of particular concern to the food industry because of its impact on product quality and shelf life. Common examples of recrystallization include the hardening of fondants and ‘freezer burn’ of ice cream (Hartel, 2001; Basso et al., 2010). Palm-based products are particularly prone to recrystallization upon storage. For example, margarine may harden, resulting in a gritty product with poor spreadability due to undesired crystal growth (Omar et al., 2005). The tendency for PO to recrystallize is a result of its DAG content causing slow crystallization rates, although this mechanism is not fully understood. MAGs have been added to PO to aid in nucleation and increase the rate of crystallization (Tshiamala, 2013). They tend to form α crystals which readily undergo $\alpha \rightarrow \beta'$ transition (Krog, 2001). While phase diagrams have been used to understand and control crystallization in bulk systems, for complex foods, this is quite complicated (Fennema et al., 1973; Hartel, 2001).

Recrystallization of CB in chocolate, which is responsible for fat bloom, is believed to occur from the growth of pre-existing surface crystals (Jewell, 1972; Timms, 1984; Ziegleder and Moser, 1996; Ziegleder and Schwingshandl, 1998; Tietz and Hartel, 2000; Aguilera et al., 2004; Lonchamp and Hartel, 2004). This may be further promoted by a concentration gradient of TAGs (Lovegren et al., 1976; Talbot, 1994, 1999; Sonwai and Rousseau, 2008; James and Smith, 2009). In addition to β_2 crystals, non-fat ingredients further restrict volumetric flow (Q) of

liquid-state TAGs by creating a more tortuous path to the surface (Rousseau, 2007). Q is described by Darcy's law:

$$Q = \frac{B * A_c}{\eta} * \frac{\Delta P}{L} \quad (9)$$

where B is the permeability coefficient, A_c is the cross-sectional area in which flow occurs, ΔP is change in the pressure of the system, and L is the distance in which this pressure change takes place (Dibildox-Alvarado et al., 2004; Marangoni and Wesdorp, 2013).

Using porosimetry (Loisel et al., 1997), well- and over-tempered couverture containing 32 wt. % CB was determined to contain pores at 1 vol.% and 4 vol.%, respectively. These pores are believed to form by contraction upon cooling (Sonwai and Rousseau, 2008). They typically measured 6 μm in diameter by 3 μm in depth according to atomic force microscopy (AFM) with hundreds being randomly distributed within a square centimetre (Rousseau, 2006). It has been theorized that TAGs migrate through these pores by either the aforementioned diffusion equation (Miquel et al., 2001; Ghosh et al., 2002; Galdámez et al., 2009), capillary action (Aguilera et al., 2004; Marty et al., 2005) or pressure-dependent convective flow (Dahlenborg et al., 2015).

In the case of pralines where fillings are made of low-melting oils, e.g., triolein-rich hazelnut gianduja, it is theorized recrystallization occurs via two steps. First, as the shell contracts during storage, liquid-state TAGs migrate from the filling to the surface as cone-like wells (Beckett, 2000). Afterward, these structures grow and harden, serving as hosts for β_1 crystals (Smith and Dahlman, 2005; Smith et al., 2007; Sonwai and Rousseau, 2008, 2010; Dahlenborg et al., 2011). The solubilization between shell and filling TAGs disrupts the phase behaviour of the shell and exacerbates recrystallization (Bigalli, 1988; Cebula and Ziegleder, 1993; Couzens and Wille,

1997; Walter and Cornillon, 2002; Dahlenborg, 2014). TAG migration into the shell also lowers its density, causing it to swell, and hardens the filling (Minson, 1990; Ziegleder and Moser, 1996; Miquel et al., 2001; Miquel and Hall, 2002; Altimiras et al., 2007; Barron, 2007). This swelling effect has previously been explored using confocal chromatic displacement sensors (Svanberg et al., 2012). Additional issues in pralines fillings with high water activity, e.g., water- or alcohol-based, include sugar bloom and mould growth from improper storage (Minifie, 1989; Larumbe et al., 1991).

While it is believed that storage of CB-based confections at 18 °C or cooler inhibits solid-state transitions, warmer conditions lead to its rapid onset (Cebula and Ziegleder, 1993; Ali et al., 2001; Lonchampt and Hartel, 2004; Schenk and Peschar, 2004). For example, the recrystallization of well-tempered chocolate into β_1 crystals was observed after 30 days when stored at 30 °C (Table 7) (James and Smith, 2009). Storage temperature fluctuations may further promote recrystallization (Hachiya et al., 1989), e.g., recrystallization was exacerbated when storage temperatures fluctuated as little as one degree (Hettich, 1966).

MF and its fractions have been shown to inhibit CB recrystallization (Metin, 1997). This was theorized to occur through either slowing down the $\beta_2 \rightarrow \beta_1$ transition by disrupting β_1 lamellar packing (Cook, 1964) or by lowering overall SFC from the eutectic mixture that is formed with CB (Hartel, 1996). Since MF may reduce the SFC via eutectic effects, crystallization rate and crystal size may be reduced as well, potentially softening the product. Higher-melting fractions of MFs may offer a solution to this because they inhibit recrystallization while better preserving SFC (Lohman and Hartel, 1994; Pajin and Jovanovic, 2005). In one study, it was observed that

addition of MF at 5.0 % and 7.5 % reduced the presence of wells, however, sensory properties, e.g., firmness, snap, melting, were just maintained at 5.0 % (Sonwai and Rousseau, 2010). MF has been used to prolong the shelf life of confections because of this in addition to its affordability and accordance to the Food and Drug Administration Code of Federal Regulations (Bricknell and Hartel, 1998).

Sugar crystallinity has also been shown to impact fat recrystallization. For example, the appearance of fat bloom was visually reduced formulations with amorphous sugar compared to crystalline powder sugar. This was attributed to amorphous sugar having a smoother surface, and therefore fewer active sites to facilitate recrystallization. Furthermore, its rounded shape may create a more tortuous, closely packed system in comparison to the randomly shaped particulates of powder sugar, which may ultimately hinder oil migration that is responsible for β_1 crystal growth (Bricknell and Hartel, 1998).

Other methods used to study recrystallization have included trained sensory panels (Quevedo et al., 2013), whiteness index (Bricknell and Hartel, 1998; Quevedo et al., 2013), area-scale fractal analysis (Brown et al., 1993), computer vision (Briones and Aguilera, 2005), Raman (Dahlenborg et al., 2012), magnetic resonance imaging (Miquel and Hall, 2002), AFM (Hodge and Rousseau, 2002; Rousseau, 2006), optical profilometry (Rousseau et al., 2010), and scanning electron microscopy (James and Smith, 2009; Wang et al., 2010).

4 Characterization of particle interactions in confectionery products

Fat confectionery texture and crystallization largely depend on the combination of the physical properties of the continuous fat phase, ϕ , and, importantly, interactions between the ingredients

themselves. For example, the particle size distribution and surface area of sugar as well as the emulsifier type and concentration all impact the processability and sensory attributes of fat-continuous products. Similarly, moisture can have a damaging effect as it causes the clustering of non-fat ingredients, resulting in poor mixing. The characterization of particle interactions is thus critical to the establishment of the factors governing the optimized production of confectionery products. To this day, there remains a significant dearth of information in this regard, owing largely to the lack of appropriate research tools. A nascent research avenue in food science is that of force spectroscopy, which offers the unique prospect of determining the extent of particle interactions in confectionery products rich with non-fat ingredients.

4.1 Force Spectroscopy

Scientists have romanced the idea of pulling on both sides of a single chemical bond and measuring the force that is required for bond disruption. Such single-molecule experiments have been possible since 1990s using a technique known as force spectroscopy (Noy and Friddle, 2013). While not as developed as its imaging ability, AFM-based force spectroscopy can be used to measure both inter- and intramolecular forces (Miles and McMaster, 1995; Morris, 2007; Han and Serry, 2008). AFM force spectroscopy can yield information such as the conformation and elasticity of proteins (Janshoff et al., 2000), intermolecular interactions in biological and synthetic compounds (Mate et al., 1987; Meyer and Amer, 1988; Florin et al., 1994; Noy et al., 1995; Rief, 1997; Li et al., 1999; Ortiz and Hadziioannou, 1999; Merkel et al., 1999; Janshoff et al., 2000; Wang et al., 2002; Zhang and Zhang, 2003), particulate interactions in colloidal systems (Ducker et al., 1991), localized surface interactions (Frisbie et al., 1994) and ligand-

receptor binding affinities (Florin et al., 1994). Recently, it was used to assess the impact of emulsifiers on CB colloidal properties (Middendorf et al., 2015, 2016).

AFM was developed in 1986 (Binnig et al., 1986), which employs a probe that is scanned across a sample where atomic forces between tip and sample are monitored and sample topography can be analyzed (Hodge and Rousseau, 2002; Rousseau, 2007). Cantilever deflection is measured by the ‘beam bounce’ method where a semiconductor diode laser is reflected off the cantilever back and into a position-sensitive split photodiode detector (Figure 9). The electrical signal that is received by the detector is measured in volts and is proportional to deflection of the cantilever (Cappella and Dietler, 1999).

With AFM-derived force spectroscopy, the tip-sample distance is reduced until ‘jump-to-contact’ has been achieved. Whereas in ‘contact’ mode a feedback loop exists to obtain an image, this is notably absent in force spectroscopy (Han and Serry, 2008). The tip-sample distance is then increased and interactive forces are measured. Tip-sample interactions can be repeatedly measured either at the same surface location or at different locations in order to obtain an interaction map (Owen, 2004). As an alternative to tip retraction, the tip can be intentionally pushed into the sample in a method called nano-indentation (Han and Serry, 2008). Viscoelastic properties typically reserved for rheology, such as Young’s modulus, may be obtained. Tip retraction following indentation causes the cantilever to deflect toward the surface because of adhesion (Owen, 2004).

Force spectroscopy is commonly represented as simple curves where the x-axis is the tip-sample distance and y-axis is cantilever deflection measured in either volts or Newtons for raw or

calibrated signals, respectively. Information that can be extracted from these plots include hardness, elasticity, and rupture bond length (Figure 10). These forces may be estimated using the Lennard-Jones potential (V):

$$V = 4\varepsilon \left\{ \left(\frac{\sigma}{z_r} \right)^{12} - \left(\frac{\sigma}{z_a} \right)^6 \right\} \quad (10)$$

where ε is the depth of the potential well, σ is the distance when force is zero, and z_r and z_a are the distance dependences resulting from repulsive and attractive forces, respectively (Seo and Jhe, 2008). In order to calibrate force with the raw voltage that is detected by the PSPD, the spring constant of the cantilever must first be quantified by one of several methods (Han and Serry, 2008). The first method to derive the constant (k) considers the formula:

$$k = 2\pi^3 l^3 w \sqrt{\frac{\rho^3}{E}} f_0^3 \quad (11)$$

where l is length, w is width, ρ is density, E is Young's modulus, and f_0 is resonance frequency of the cantilever (Han and Serry, 2008). Another method uses a second cantilever with a pre-calibrated spring constant. When this cantilever is pushed against cantilevers of unknown spring constants, these constants can be determined (Hinterdorfer et al., 1996). A third method exposes the cantilever to thermal fluctuations, whereby a thermal noise spectrum is obtained and fitted against a Lorentzian function in order to determine the spring constant (Owen, 2004).

Once the spring constant of the cantilever is known, the deflection distance for a known change in voltage must be determined. This is done by deflecting the cantilever against a clean hard

surface. Because of the large force that is exerted on the cantilever, this is often performed post-experiment. After the distance is known, force can be calculated using Hooke's Law:

$$F = kx \quad (12)$$

where k is spring constant and x is distance determined by the PSPD. Calibration of the cantilever must be performed every time it is mounted to the instrument or between days where conditions such as temperature or humidity differ because deflection may vary. Since deflection values are typically between 0.1 nm to 100 nm, cantilevers with spring constants between 0.01 N·m⁻¹ and 1.00 N·m⁻¹ are most ideal for force spectroscopy studies (Noy and Friddle, 2013).

4.2 Tip Functionalization

The surface of either tip or sample may be intentionally exposed to chemical or biological species in order to study specific molecular-molecular interactions (Han and Serry, 2008; Tang et al., 2008). In sample functionalization, self-assembling monolayers, e.g., silanes on glass surfaces or thiols on gold surfaces, are formed. In tip functionalization, molecules of interest are often adsorbed from solution. Overlaying the interaction-specific force-distance curves with imaging results in chemical or affinity maps - a technique called volume spectroscopy (Morris, 2007; Han and Serry, 2008).

The easiest way to functionalize tips uses short rigid linker molecules followed by spacers, such as alkyl thiols or short-chained polyethylene glycols (PEGs), in order to reduce unwanted tethering. PEGs are often ideal for spacers because of they are both chemically and physically inert and allow tethered molecules to reorient (Ebner et al., 2007). Molecules of interest are diluted with the spacer at 2 mol % upon which the linker is introduced. An issue in AFM force

spectroscopy with functionalized tips is distinguishing specific from non-specific forces with the sample (Noy and Friddle, 2013). Considerations for approaching functionalization in force spectroscopy has been previously reviewed (Blanchette et al., 2008).

Despite little reported on the application of AFM force spectroscopy in food science (Middendorf et al., 2015, 2016), biological topics of interest have included the folding and assembling of macromolecules, construction of biomolecular sensors, and virus-host affinity (Han and Serry, 2008). For example, globular properties exhibit structural anisotropy where the direction of pulling shows unique conformational properties (Figure 11) (Carrion-Vazquez et al., 2003; Dietz et al., 2006; Gao et al., 2011; Graham and Best, 2011; Jagannathan et al., 2012; Ward and Sweeney, 2012). It is suggested that specific fingerprints of unique unfolding events can be obtained, resulting from secondary and tertiary conformations (Rief, 1997; Bustamante et al., 2000; Eckel et al., 2003). In another study, graphite demonstrated different stabilities when pulled either parallel or perpendicular to its polycyclic rings (Zheng et al., 2013). Measurements of polysaccharide chains were also studied using force spectroscopy (Rief et al., 1997; Zhang and Marszalek, 2006).

5 Conclusions

The quality of fat-based confections is not only derived from the physical properties of the continuous fat phase, but also its chemistry with the non-fat ingredients contained within. The amount of fat affects the wetting of non-fat ingredients, behaving as a lubricant and maintaining proper fluidity, while the non-fat ingredients provide surfaces off of which fat can nucleate but may also change viscosity and mobility of the species that contribute to crystal growth. The way

in which these ingredients interact strongly influences the path of fat crystallization and ultimately perception of the product by the consumer. Hardness and spreadability are just two such properties that depend heavily on this relationship. An understanding of ingredient interactions is viable to the success of any product and has become particularly important in confectionery applications, particularly those that are palm-based, where quality is largely determined through trial and error. This opens the door to untapped venues of food science, such as force spectroscopy, where specific ingredient-ingredient interactions can be characterized and used to better predict how such systems will crystallize.

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References

- Ab Latip, R., Lee, Y. -Y., Tang, T. -K., Phuah, E. -T., Lee, C. -M., Tan, C. -P. and Lai, O. -M. (2013). Palm-based diacylglycerol fat dry fractionation: Effect of crystallisation temperature, cooling rate, and agitation speed on physical and chemical properties of fractions. *PeerJ*. **1**:e72.
- Acevedo, N. C. and Marangoni, A. G. (2010). Characterization of the nanoscale in triacylglycerol crystal networks. *Cryst. Growth Des.* **10**(8):3327-3333.
- Acevedo, N. C., Peyronel, F. and Marangoni, A. G. (2011). Characterization and engineering of the nanoscale in triglyceride crystal networks. *Curr. Opin. Colloid Interface Sci.* **16**:374-383.
- Afoakwa, E. O., Paterson, A. and Fowler, M. (2007a). Factors influencing rheological and textural qualities in chocolate: A review. *Trends Food Sci. Technol.* **18**(6):290-298.
- Afoakwa, E. O., Paterson, A. and Fowler, M. (2007b). Effects of particle size distribution and composition on rheological properties of dark chocolate. *Eur. Food Res. Technol.* **226**(2):1259-1268.
- Afoakwa, E. O., Paterson, A., Fowler, M. and Veira, J. (2008a). Characterization of melting properties in dark chocolate from varying particle size distribution and composition using differential scanning calorimetry. *Food Res. Int.* **41**(7):751-757.
- Afoakwa, E. O., Paterson, A., Fowler, M. and Ryan, A. (2008b). Flavor formation and character in cocoa and chocolate: A critical review. *Crit. Rev. Food Sci. Nutr.* **48**(9):1-18.

Afoakwa, E. O., Paterson, A., Fowler, M. and Vieira, J. (2008c). Effects of tempering and fat crystallization behaviours on microstructure, mechanical properties, and appearance in dark chocolate systems. *J. Food Eng.* **89**(2):128-136.

Afoakwa, E. O., Paterson, A., Fowler, M. and Vieira, J. (2009). Influence of tempering and fat crystallization behaviours on microstructural and melting properties in dark chocolate systems. *Food Res. Int.* **42**(1):200-209.

Aftab, A. K., Sherazi, S. T. H., Rubina, S., Razia, S., Ambrat and Arfa, Y. (2013). Consequence of fatty acids profile including trans fat in chocolate and pastry samples. *Int. Food Res. J.* **20**(2):601-605.

Aguilera, J. M., Michel, M. and Mayor, G. (2004). Fat migration in chocolate: Diffusion or capillary flow in a particulate solid?—A hypothesis paper. *J. Food Sci.* **69**(7):169-174.

Aguilera, J. M. and Stanley, D. W. (1999). Microstructural Principles of Food Processing and Engineering. Aspen Publishers, Gaithersburg, Maryland, U.S.A.

Ahmed, J. and Ramaswamy, H. S. (2006). Viscoelastic properties of sweet potato puree infant food. *J. Food Eng.* **74**(3):376-382.

Aidoo, H., Sakyi-Dawson, E., Abbey, L., Tano-Debrah, K. and Saalia, F. K. (2012). Optimisation of chocolate formulation using dehydrated peanut-cowpea milk to replace dairy milk. *J. Sci. Food Agric.* **92**(2):224-231.

- Aidoo, R. P., Depypere, F., Afoakwa, E. O. and Dewettinck, K. (2013). Industrial manufacture of sugar-free chocolates: Applicability of alternative sweeteners and carbohydrate polymers as raw materials in product development. *Trends Food Sci. Technol.* **32**(2):84-96.
- Ali, A., Selamat, J., Che Man, Y. B. and Suria, A. M. (2001). Effect of storage temperature on texture, polymorphic structure, bloom formation and sensory attributes of filled dark chocolate. *Food Chem.* **72**(4):491-497.
- Allen, S. L. (2002). In the Devil's Garden: A Sinful History of Forbidden Food. Random House, New York City, New York, U.S.A.
- Altimiras, P., Pyle, L. and Bouchon, P. (2007). Structure–fat migration relationships during storage of cocoa butter model bars: Bloom development and possible mechanisms. *J. Food Eng.* **80**(2):600-610.
- Awua, P. K. (2002). Cocoa Processing and Chocolate Manufacture in Ghana. David Jamieson and Associates Press, Essex, U.K.
- Baixauli, R., Sanz, T., Salvador, A. and Fiszman, S. M. (2007). Influence of the dosing process on the rheological and microstructural properties of a bakery product. *Food Hydrocolloid.* **21**(2):230-236.
- Baldino, N., Gabriele, D. and Migliori, M. (2010). The influence of formulation and cooling rate on the rheological properties of chocolate. *Eur. Food Res. Technol.* **231**(6):821-828.
- Barron, I. F. (2007). The expansion of wafer and its relation to the cracking of chocolate and 'bakers' chocolate coatings. *Int. J. Food Sci. Technol.* **12**(1):73-84.

Basiron, Y. (2001). New palm-based products. *J. Oleo Sci.* **50**(5):295-303.

Basso, R., Ribeiro, A., Masuchi, M., Gioielli, L., Goncalves, L., Cardoso, L. and Grimaldi, R. (2010). Tripalmitin and monoacylglycerols as modifiers in the crystallisation of palm oil. *Food Chem.* **122**(4):1185-1192.

Beckett, S. T. (1994). Control of particle size reduction during chocolate grinding. **In:** Proceedings of the Pennsylvania Manufacturing Confectioners' Association Production Conference, pp. 136-143. PMCA, Center Valley, Pennsylvania, U.S.A.

Beckett, S. T. (1999). Preparation of Chocolate with Limonene to Reduce Fat Content. United States Patent 6,200,625.

Beckett, S. T. (2000). The Science of Chocolate. Royal Society of Chemistry, Cambridge, U.K.

Beckett, S. T. (2008). Industrial Chocolate Manufacture and Use. Blackwell Science, Oxford, U.K.

Bennett, A., Hamilton, D., Maradudin, A., Miller, R. and Murphy, J. (1965). Crystals: Perfect and Imperfect. Walker and Co., New York City, New York, U.S.A.

Bigalli, G. L. (1988). Practical aspects of the eutectic effect on confectionery fats and their mixtures. *Manuf. Confect.* **68**:65-80.

Binnig, G., Quate, C. F. and Gerber, C. (1986). Atomic force microscope. *Phys. Rev. Lett.* **56**(9):930-933.

- Blanchette, C. D., Loui, A. and Ratto, T. V. (2008). Tip functionalization: Applications to chemical force spectroscopy. **In:** Handbook of Molecular Force Spectroscopy, pp. 185-203. Noy, A., Ed., Springer Science+Business Media, New York City, New York, U.S.A.
- Bomba, P. C. (1993). Shelf life of chocolate confectionery products. **In:** Shelf Life Studies of Foods and Beverages, pp. 341-351. Charalambous, G., Ed., Elsevier, London, U.K.
- Bourne, M. (2002). Food Texture and Viscosity: Concepts and Measurements. Academic Press, San Diego, California, U.S.A.
- Bouzas, J. and Brown, B. D. (1995). Interacting affecting microstructure, texture, and rheology of chocolate confectionery products. **In:** Ingredient Interactions: Effects on Food Quality, pp. 451-528. Gaonkar, A. G., Ed., Marcel and Dekker, New York City, New York, U.S.A.
- Bowser, A. (2006). Crystallization of cocoa butter. *Manuf. Confect.* **86**:115-118.
- Bresson, S., El Marssi, M. and Khelifa, B. (2005). Raman spectroscopy investigation of various saturated monoacid triglycerides. *Chem. Phys. Lipids* **134**(2):119-129.
- Bresson, S., El Marssi, M. and Khelifa, B. (2006). Conformational influences of the polymorphic forms on the CO and C–H stretching modes of five saturated monoacid triglycerides studied by Raman spectroscopy at various temperatures. *Vib. Spectrosc.* **40**(2):263-269.
- Bresson, S., Rousseau, D., Ghosh, S., El Marssi, M. and Faivre, V. (2011). Raman spectroscopy of the polymorphic forms and liquid state of cocoa butter. *Eur. J. Lipid Sci. Technol.* **113**(8):992-1004.

Bricknell, J. and Hartel, R. W. (1998). Relation of fat bloom in chocolate to polymorphic transition of cocoa butter. *J. Am. Oil Chem. Soc.* **75**(11):1609-1615.

Briones, V. and Aguilera, J. M. (2005). Image analysis of changes in surface colour of chocolate. *Food Res. Int.* **38**(1):87-94.

Brown, C. A., Charles, P. D., Johnsen, W. A. and Chesters, S. (1993). Fractal analysis of topographic data by the patchwork method. *Wear* **161**(1-2):61-67.

Bustamante, C., Smith, S. B., Liphardt, J. and Smith, D. (2000). Single-molecules studies of DNA mechanics. *Curr. Opin. Struct. Biol.* **10**(3):279–285.

Cacciuto, A., Auer, S. and Frenkel, D. (2004). Onset of heterogeneous crystal nucleation in colloidal suspensions. *Nature* **428**(6981):404-406.

Campbell, L. (1967). Fat bloom on Chocolate and Its Control by Tempering. British Food Manufacturing Industries Research Association, Leatherhead, U.K.

Campos, R., Narine, S. S. and Marangoni, A. G. (2002). Effect of cooling rate on the structure and mechanical properties of milk fat and lard. *Food Res. Int.* **35**(10):971-981.

Cappella, B. and Dietler, G. (1999). Force-distance curves by atomic force microscopy. *Surf. Sci. Rep.* **34**(1-2):1-104.

Carrion-Vazquez, M., Li, H., Lu, H., Marszalek, P. E., Oberhauser, A. F. and Fernandez, J. M. (2003). The mechanical stability of ubiquitin is linkage dependent. *Nat. Struct. Biol.* **10**(9):738-743.

Carrion-Vazquez, M., Marszalek, P. E., Oberhauser, A. F. and Fernandez, J. M. (1999). Atomic force microscopy captures length phenotypes in single proteins. *Proc. Natl. Acad. Sci. U.S.A.* **96**(20):11288-11292.

Carvalho-da-Silva, A. M., Van Damme, I., Taylor, W., Hort, J. and Wolf, B. (2013). Oral processing of two milk chocolate samples. *Food Funct.* **4**(3):461-469.

Carvalho-da-Silva, A. M., Van Damme, I., Wolf, B. and Hort, J. (2011). Characterisation of chocolate eating behaviour. *Physiol. Behav.* **104**(5):929-933.

Cebula, D. J. and Ziegler, G. (1993). Studies of bloom formation using x-ray diffraction from chocolates after long-term storage. *Eur. J. Lipid Sci. Technol.* **95**(9):340-343.

Chhabra, R. P. (2006). Bubbles, Drops, and Particles in Non Newtonian Fluids. CRC Press, Boca Raton, Louisiana, U.S.A.

Che Man, Y. B., Haryati, T., Ghazali, H. M. and Asbi, B. A. (1999). Composition and thermal profile of crude palm oil and its products. *J. Am. Oil Chem. Soc.* **76**(2):237-242.

Chen, C. E., Lai, O. M., Ghazali, H. M. and Chong, C. L. (2002). Isothermal crystallization kinetics of refined palm oil. *J. Am. Oil Chem. Soc.* **79**(4):403-410.

Chevalley, J. (1999). Chocolate flow properties. In: *Industrial Chocolate Manufacture and Use*, pp. 182-200. Beckett, S. T., Ed., Blackwell Science, Oxford, U.K.

Chong, C. L. (1993). Effects of mesocarp bruising on the rate of free fatty acid release in oil palm fruits. *Int. Biodeterior. Biodegradation* **31**(1):65-70.

Clayton, P. and Conn, H. (2005). Carbohydrate Substitutes. International Patent Cooperation Treaty, Number WO 2005/006891 A1.

Cook, L. R. (1964). Chocolate Production and Use. Harcourt Brace, New York City, New York, U.S.A.

Costales-Rodríguez, R., Gibon, V., Verhé, R. and De Greyt, W. (2009). Chemical and enzymatic interesterification of a blend of palm stearin:soybean oil for low *trans*-margarine formulation. *J. Am. Oil Chem. Soc.* **86**(7):681-697.

Cottrell, R. C. (1991). Nutritional aspects of palm oil. *Am. J. Clin. Nutr.* **53**(4 Suppl):989S-1009S.

Couzens, P. J. and Wille, H. J. (1997). Fat migration in composite confectionery products. *Manuf. Confect.* **77**:45-47.

D'Souza, V., DeMan, J. M. and DeMan, L. (1990). Short spacings and polymorphic forms of natural and commercial solid fats: A review. *J. Am. Oil Chem. Soc.* **67**(11):835-843.

Da Silva, E., Bresson, S. and Rousseau, D. (2009). Characterization of the three major polymorphic forms and liquid state of tristearin by Raman spectroscopy. *Chem. Phys. Lipids* **157**(2):113-119.

Dahlenborg, H. (2014). Fat Bloom on Chocolate Confectionery Systems. Lund University, Lund, Sweden.

Dahlenborg, H., Millqvist-Fureby, A. and Bergenståhl, B. (2015). Effect of particle size in chocolate shell on oil migration and fat bloom development. *J. Food Eng.* **146**:172-181.

Dahlenborg, H., Millqvist-Fureby, A., Bergenståhl, B. and Kalnin, D. J. E. (2011). Investigation of chocolate surfaces using profilometry and low vacuum scanning electron microscopy. *J. Am. Oil Chem. Soc.* **88**(6):773-783.

Dahlenborg, H., Millqvist-Fureby, A., Brandner, B. D. and Bergenståhl, B. (2012). Study of the porous structure of white chocolate by confocal Raman microscopy. *Eur. J. Lipid Sci. Technol.* **114**(8):919-926.

De Cock, N. (2011). Structure Development in Confectionery Products: Importance of Triacylglycerol Composition. Ghent University, Ghent, Belgium.

De Graef, V., Depypere, F., Minnaert, M. and Dewettinck, K. (2011). Chocolate yield stress as measured by oscillatory rheology. *Food Res. Int.* **44**(9):2660-2665.

De Graef, V., Dewettinck, K., Verbeken, D. and Foubert, I. (2006). Rheological behaviour of crystallizing palm oil. *Eur. J. Lipid Sci. Technol.* **108**(10):864-870.

Debaste, F., Kegelaers, Y., Liégeois, S., Ben Amor, H. and Halluin, V. (2008). Contribution to the modelling of chocolate tempering process. *J. Food Eng.* **88**(4):568-575.

DeMan, J. M. (1992). X-ray diffraction spectroscopy in the study of fat polymorphism. *Food Res. Int.* **25**(6):471-476.

Denker, M., Parat-Wilhelms, M., Drichelt, G., Paucke, J., Luger, A., Borchert, K., Hottman, W. and Steinhart, H. (2006). Investigation of the retronasal flavour release during the consumption of coffee with additions of milk constituents by ‘Oral Breath Sampling.’ *Food Chem.* **98**(2):201-208.

Dhonsi, D. and Stapley, A. G. F. (2006). The effect of shear rate, temperature, sugar, and emulsifier on the tempering of cocoa butter. *J. Food Eng.* **77**(4):936-942.

Dian, N. L., Sundram, K. and Idris, N. A. (2006). DSC study on the melting properties of palm oil, sunflower oil, and palm kernel olein blends before and after chemical interesterification. *J. Am. Oil Chem. Soc.* **83**(8):739-745.

Dibildox-Alvarado, E., Rodrigues, J. N., Gioielli, L. A., Toro-Vazquez, J. F. and Marangoni, A. G. (2004). Effects of crystalline microstructure on oil migration in a semisolid fat matrix. *Cryst. Growth Des.* **4**(4):731-736.

Dietz, H., Berkemeier, F., Bertz, M. and Rief, M. (2006). Anisotropic deformation response of single protein molecules. *Proc. Natl. Acad. Sci. U.S.A.* **103**(34):12724–12728.

Do, T. -A. L., Hargreaves, J. M., Wolf, B., Hort, J. and Mitchell, J. R. (2007). Impact of particle size distribution on rheological and textural properties of chocolate models with reduced fat content. *J. Food Sci.* **72**(9):E541-E552.

Do, T. -A. L., Vieira, J., Hargreaves, J. M., Wolf, B. and Mitchell, J. R. (2008). Impact of limonene on the physical properties of reduced fat chocolate. *J. Am. Oil Chem. Soc.* **85**(10):911-920.

- Doká, O., Prágai, E., Bicanic, D., Kylcsár, R. and Ajtony, Z. (2013). Colorimetry and photoacoustic spectroscopy as a suitable tool for determination of fat-free cocoa solids in dark chocolates. *Eur. Food Res. Technol.* **236**(6):963-968.
- Du, H., Kim, C., Corradini, M. G., Ludescher, R. D. and Rogers, M. A. (2014). Micro-viscosity of liquid oil confined in colloidal fat crystal networks. *Soft Matter* **10**(43):8652-8658.
- Ducker, W. A., Senden, T. J. and Pashley, R. M. (1991). Direct measurement of colloidal forces using an atomic force microscope. *Nature* **353**(6341):239-241.
- Ebner, A., Wildling, L., Kamruzzahan, A. S., Rankl, C., Wruss, J., Hahn, C. D., Hölzl, M., Zhu, R., Kienberger, F., Blaas, D., Hinterdorfer, P. Gruber, H. J. (2007). A new, simple method for linking of antibodies to atomic force microscopy tips. *Bioconj. Chem.* **18**(4):1176-1184.
- Eckel, R., Ros, R., Ros, A., Wilking, S. D., Sewald, N. and Anselmetti, D. (2003). Identification of binding mechanisms in single molecule-DNA complexes. *Biophys. J.* **85**(3):1968-1973.
- Edem, D. O. (2002). Palm oil: Biochemical, physiological, nutritional, hematological, and toxicological aspects: A review. *Plant Foods Hum. Nutr.* **57**(3-4):319-341.
- Engelen, L., De Wijk, R. A., Van Der Bilt, A., Prinz, J. F., Janssen, A. M. and Bosman, F. (2005). Relating particles and texture perception. *Physiol. Behav.* **86**(1-2):111-117.
- Engelen, L. and Van Der Bilt, A. (2008). Oral physiology and texture perception of semisolids. *J. Texture Stud.* **39**(1):83-113.

Eremina, T. A., Kuznetsov, V. A., Eremin, N. N., Okhrimenko, T. M., Furmanova, N. G., Efremova, E. P. and Rak, M. (2005). On the mechanism of impurity influence on growth kinetics and surface morphology of KDP crystals. II. Experimental study of influence of bivalent and trivalent impurity ions on growth kinetics and surface morphology of KDP crystals. *J. Cryst. Growth* **273**(3-4):586-593.

Fennema, O. R., Powrie, W. D. and Marth, E. H. (1973). Low-Temperature Preservation of Foods and Living Matter. Marcel Dekker, New York City, New York, U.S.A.

Fernandes, V. A., Müller, A. J. and Sandoval, A. J. (2013). Thermal, structural and rheological characteristics of dark chocolate with different compositions. *J. Food Eng.* **116**(1):97-108.

Fessas, D., Signorelli, M. and Schiraldi, A. (2005). Polymorphous transitions in cocoa butter: A quantitative DSC study. *J. Therm. Anal. Calorim.* **82**(3):691-702.

Flint, O. (1994). Food Microscopy. Bios Scientific, Oxford, U.K.

Florin, E. L., Moy, V. T. and Gaub, H. E. (1994). Adhesion forces between individual ligand-receptor pairs. *Science* **264**(5157):415–417.

Foubert, I., Dewettink, K., Janssen, G. and Vanrolleghem, P. A. (2006). Modelling two-step isothermal fat crystallization. *J. Food Eng.* **75**(4):551-559.

Foubert, I., Fredrick, E., Vereecken, J., Sichien, M. and Dewettinck, K. (2008). Stop-and-return DSC method to study fat crystallization. *Thermochim. Acta* **471**(1-2):7-13.

Frisbie, C. D., Rozsnyai, L. F., Noy, A., Wrighton, M. S. and Lieber, C. M. (1994). Functional group imaging by chemical force microscopy. *Science* **265**(5181):2071-2074.

Fritsche, J. and Steinhart, H. (1998). Analysis, occurrence, and physiological properties of trans fatty acids (TFA) with particular emphasis on conjugated linoleic acid isomers (CLA): A review. *Eur. J. Lipid Sci. Technol.* **100**(6):190-210.

Galdámez, J. R., Szlachetka, K., Duda, J. L. and Ziegler, G. R. (2009). Oil migration in chocolate: A case of non-Fickian diffusion. *J. Food Eng.* **92**(3):261–268.

Gao, Y., Sirinakis, G. and Zhang, Y. (2011). Highly anisotropic stability and folding kinetics of a single coiled coil protein under mechanical tension. *J. Am. Chem. Soc.* **133**(32):12749-12757.

Garside, J. (1987). General principles of crystallization. In: Food Structure and Behaviour, pp. 35-49. J. M. V. Blanshard, J. M. V. and Lillford, P., Eds., Academic Press, London, U.K.

Gee, P. T. (2007). Analytical characteristics of crude and refined palm oil and fractions. *Eur. J. Lipid Sci. Technol.* **109**(4):373-379.

Ghorbel, D., Saïdi, I., Ben Slema, M. and Gharsallah, M. (2011). Adjusting flow properties of molten milk chocolate by means of emulsifiers and fat. *J. Food Process Eng.* **34**(2):317-331.

Ghosh, V., Ziegler, G. R. and Anantheswaran, R. C. (2002). Fat, moisture, and ethanol migration through chocolates and confectionary coatings. *Crit. Rev. Food Sci. Nutr.* **42**(6):583-626.

Gibon, V., De Greyt, W. and Kellens, M. (2007). Palm oil refining. *Eur. J. Lipid Sci. Technol.* **109**(4):315-335.

Glicerina, V., Balestra, F., Dalla Rosa, M. and Romani, S. (2013). Rheological, textural, and calorimetric modifications of dark chocolate during process. *J. Food Eng.* **119**(1):173-179.

Goh, S. H., Choo, Y. M. and Ong, S. H. (1985). Minor carotenoids of palm oil. *J. Am. Oil Chem. Soc.* **62**(6):237-240.

Götz, J., Balzer, H. and Hinrichs, R. (2005). Characterisation of the structure and flow behaviour of model chocolate systems by means of NMR and rheology. *Appl. Rheol.* **15**(2):98–111.

Graham, T. G. W. and Best, R. B. (2011). Force-induced change in protein unfolding mechanism: Discrete or continuous switch? *J. Phys. Chem. B* **115**(6):1546-1561.

Gratzer, W. (2005). *Terrors of the Table: The Curious History of Nutrition*. Oxford University Press, New York City, New York, U.S.A.

Gresti, J., Bugaut, M., Maniongui, C. and Bezard, J. (1993). Composition of molecular species of triacylglycerols in bovine milk fat. *J. Dairy Sci.* **76**(7):1850-1869.

Gunstone, F. D. and Norris, F. A. (1983). *Lipids in Foods: Chemistry, Biochemistry, and Technology*. Pergamon Press, Oxford, U.K.

Hachiya, I., Koyano, T. and Sato, K. (1989). Seeding effects on solidification behavior of cocoa butter and dark chocolate. II. Physical properties of dark chocolate. *J. Am. Oil Chem. Soc.* **66**(12):1763-1770.

Han, W. and Serry, F. M. (2008). *Force Spectroscopy with the Atomic Force Microscope*. Agilent Technologies, U.S.A.

Hartel, R. W. (1996). Applications of milk-fat fractions in confectionery products. *J. Am. Oil Chem. Soc.* **73**(8):945-953.

Hartel, R. W. (1998). Mechanisms and kinetics of recrystallization in ice cream. **In:** Properties of Water in Foods: ISOPOW VI, pp. 287-319. Reid, D., Ed., Blackie, London, U.K.

Hartel, R. W. (1999). Chocolate: Fat bloom during storage. *Manuf. Confect.* **79**:89-99.

Hartel, R. W. (2001). Crystallization in Foods. Aspen Publishers, Gaithersburg, Maryland, U.S.A.

Hartel, R. W. (2013). Advances in food crystallization. *Annu. Rev. Food Sci. Technol.* **4**:277-292.

Hayakawa, M. and DeMan, J. M. (1982). Interpretation of cone penetrometer consistency measurements of fats. *J. Texture Stud.* **13**(2):201-210.

Haylock, S. J. and Dodds, T. M. (1999). Ingredients from milk. **In:** Industrial Chocolate Manufacture and Use, pp. 57-77. Beckett, S. T., Ed., Blackwell Science, Oxford, U.K.

Hernqvist, L. (1984). On the structure of triglycerides in the liquid state and fat crystallization. *Fett. Wiss. Technol.* **86**(8):297-300.

Hernqvist, L. (1988a). Crystal structure of fats and fatty acids. **In:** Crystallization and Polymorphism of Fats and Fatty Acids, pp. 97-138. Garti, N. and Sato, K., Eds., Marcel Dekker, New York City, New York, U.S.A.

Hernqvist, L. (1988b). Chocolate temper. **In:** Industrial Chocolate Manufacture and Use, pp. 159-171. Beckett, S. T., Ed., Blackie and Son, Glasgow, U.K.

Herrera, M. L. and Hartel, R. W. (2000). Effect of processing conditions on the crystallization kinetics of fats. *J. Am. Oil Chem. Soc.* **77**(11):1177-1187.

Hettich, A. (1966). Experimental basis for the definition of ‘proper’ chocolate temper. *Manuf. Confect.* **46**:29-36.

Himawan, C., Starov, V. M. and Stapley, A. F. (2006). Thermodynamic and kinetic aspects of fat crystallization. *Adv. Colloid Interface Sci.* **122**(1-3):3-33.

Hinterdorfer, P., Baumgartner, W., Gruber, H. J., Schlicher, K. and Schindler, H. (1996). Detection and localization of individual antibody-antigen recognition events by atomic force microscopy. *Proc. Natl. Acad. Sci. U.S.A.* **93**(8):3477-3481.

Hodge, S. M. and Rousseau, D. (2002). Fat bloom formation and characterization in milk chocolate observed by atomic force microscopy. *J. Am. Oil Chem. Soc.* **79**(11):1115-1121.

Hruby, A. and Hu, F. B. (2016). Saturated fat and heart disease: The latest evidence. *Lipid Technol.* **28**(1):7-12.

Hu, Y., Liang, J. K., Myerson, A. S. and Taylor, L. S. (2005). Crystallization monitoring by Raman spectroscopy: Simultaneous measurement of desupersaturation profile and polymorphic form in flufenamic acid systems. *Ind. Eng. Chem. Res.* **44**(5):1233-1240.

Huyghebaert, A. and Hendrickx, H. (1971). Polymorphism of cocoa butter shown by differential scanning calorimetry. *Lebensm. Wiss. Technol.* **79**:59-63.

Jagannathan, B., Elms, P. J., Bustamante, C. and Marqusee, S. (2012). Direct observation of a force-induced switch in the anisotropic mechanical unfolding pathway of a protein. *Proc. Natl. Acad. Sci. U.S.A.* **109**(44):17820-17825.

James, B. J. and Smith, B. G. (2009). Surface structure and composition of fresh and bloomed chocolate analysed using x-ray photoelectron spectroscopy, cryo-scanning electron microscopy, and environmental scanning electron microscopy. *Lebensm. Wiss. Technol.* **42**(5):929-937.

James, P. F. (1982). Nucleation in glass-forming systems: A review. **In**: Nucleation and Crystallization in Glasses, pp. 1-48. Simmons, J. H., Uhlmann, D. R. and Beall, G. H., Eds., American Ceramic Society, Columbus, Ohio, U.S.A.

Jamieson, P. (2008). The sugarfree toolbox: Bulk ingredients and intense sweeteners. *Manuf. Confect.* **88**:33-46.

Janshoff, A., Neitzert, M., Obersörfer, Y. and Fuchs, H. (2000). Force spectroscopy of molecular systems: Single molecule spectroscopy of polymers and biomolecules. *Angew. Chem. Int. Ed.* **39**(18):3212-3237.

Jeffery, M. S. (1993). Key functional properties of sucrose in chocolate and sugar confectionery. *Food Technol.* **47**:141-144.

Jewell, G.G. (1972). Some observations on bloom on chocolate. *Int. Choc. Rev.* **27**(6):161-162.

Johansson, D. and Bergenståhl, B. (1992). The influence of food emulsifiers on fat and sugar dispersions in oils. I. Adsorption, sedimentation. *J. Am. Oil Chem. Soc.* **69**(8):705-717.

Kaiser, J. M., Gestel, A. V. and Vercauteren, J. (1998). Reduced-Fat Confectioneries Comprising Emulsifying Agent Combinations and Preparation Thereof. Patent WO 1999045790 A1.

Karupaiah, T. and Sundram, K. (2007). Effects of stereospecific positioning of fatty acids in triacylglycerol structures in native and randomized fats: A review of their nutritional implications. *Nutr. Metab.* **4**:16.

Kellens, M., Gibon, V., Hendrix, M. and De Greyt, W. (2007). Palm oil fractionation. *Eur. J. Lipid Sci. Technol.* **109**(4):336-349.

Khan, R. S. and Rousseau, D. (2006). Hazelnut oil migration in dark chocolate: Kinetic, thermodynamic and structural considerations. *Eur. J. Lipid Sci. Technol.* **108**(5):434-443.

Kheiri, S. A. (1987). End uses of palm oil: Human food. **In:** Palm Oil, pp. 71-83. Gunstone, F. D., Ed., John Wiley and Sons, Chichester, U.K.

Kilcast, D. and Clegg, S. (2002). Sensory perception of creaminess and its relationship with food structure. *Food Qual. Prefer.* **13**(7-8):609-623.

Kleinert, J. (1961). Studies on the formation of fat bloom and methods of delaying it. *Rev. Int. Choc.* **16**:201-219.

Kobayashi, M. (1988). Vibrational spectroscopic aspects of polymorphism and phase transition of fats and fatty acids. **In:** Crystallization and Polymorphism of Fats and Fatty Acids, pp. 139-187. Garti, N. and Sato, K., Eds., Marcel Dekker, New York City, New York, U.S.A.

Krog, N. (2001). Crystallization properties and lyotropic phase behaviour of food emulsifiers. **In:** Crystallization and Polymorphism of Fats and Fatty Acids, pp. 505-526. Garti, N. and Sato, K., Eds., Marcel Dekker, New York City, New York, U.S.A.

Kroger, M., Meister, K. and Kava, R. (2006). Low-calorie sweeteners and other sugar substitutes: A review of the safety issues. *Compr. Rev. Food Sci. Food Saf.* **5**(2):35-47.

Kronenberger, A. (2006). Atomic force microscopy: Basics and Applications. Jacobs University Bremen, Bremen, Germany.

Krüger, C. (1999). Sugar and bulk sweetener. **In:** Industrial Chocolate Manufacture and Use, pp. 36-56. Beckett, S. T., Ed., Blackwell Science, Oxford, U.K.

Kuczynski, G. C. (1989). Towards the understanding of the process of sintering. **In:** Sintering'85, pp. 3-16. Kuczynski, G. C., Uskokovic, D. P. and Palmour III, H., Eds., Plenum Press, New York City, New York, U.S.A.

Kulozik, U., Tolkach, A., Bulca, S. and Hinrichs, J. (2003). The role of processing and matrix design in development and control of microstructures in dairy food production: A survey. *Int. Dairy J.* **13**(8):621-630.

Land, T. A., Martin, T. L. and Potapenko, S. (1999). Recovery of surfaces from impurity poisoning during crystal growth. *Nature* **399**(6735):442-445.

Larson, M. A. (1981). Secondary nucleation: an analysis. *Chemical Engineering Communications*, 12, 161–169.

Larsson, K. (1966). Classification of glyceride crystal forms. *Acta Chem. Scand.* **20**(8):2255-2260.

Larsson, K. (1994). *Lipids: Molecular Organization, Physical Functions, and Technical Application*. The Oily Press, Dundee, U.K.

Larsson, K., Quinn, P., Sato, K. and Tiberg, F. (2006). *Lipids: Structure, Physical Properties, and Functionality*. The Oily Press, Bridgewater, U.K.

Larumbe, A., González, H. H. L., Resnik, S. L. and Chirife, J. (1991). Moisture migration and mold growth in a composite chocolate product. *Lebensm. Wiss. Technol.* **24**(4):307-309.

Lee, S., Heuberger, M., Rousset, P. and Spencer, N. D. (2002). Chocolate at sliding interface. *J. Food Sci.* **67**(7):2712-2717.

Lee, S. -W. (2004). Relaxation characteristics of poly(vinylidene fluoride) and ethylene-chlorotrifluoroethylene in the transient uptake of aromatic solvents. *Korean J. Chem. Eng.* **21**(6):1119-1125.

Lee, W. E. and Pangborn, R. M. (1986). Time-intensity: The temporal aspects of sensory perception. *Food Technol.* **40**:71-82.

- Li, H. B., Rief, M., Oesterhelt, F., Gaub, H. E., Zhang, X. and Shen, J. C. (1999). Single-molecule force spectroscopy on polysaccharides by AFM-nanomechanical fingerprint of alpha-(1,4)-linked polysaccharides. *Chem. Phys. Lett.* **305**(3-4):197-201.
- Liang, B. and Hartel, R. W. (2004). Effects of milk powders in milk chocolate. *Journal of Dairy Science* **87**(1):20-31.
- Litwinenko, J. W., Rojas, A. M., Gerschenson, L. N. and Marangoni, A. G. (2002). Relationship between crystallization behaviour, microstructure, and mechanical properties in a palm oil-based shortening. *J. Am. Oil Chem. Soc.* **79**(7):647-654.
- Liu, Y., Meng, Z., Zhang, F., Shan, L. and Wang, X. (2010). Influence of lipid composition, crystallization behaviour and microstructure on hardness of palm oil-based margarines. *Eur. Food Res. Technol.* **230**(5):759-767.
- Lohman, M. H. and Hartel, R. W. (1994). Effect of milk fat fractions on fat bloom in dark chocolate. *J. Am. Oil Chem. Soc.* **71**(3):267-276.
- Loisel, C., Keller, G., Lecq, G., Bourgaux, C. and Ollivon, M. (1998). Phase transitions and polymorphism of cocoa butter. *J. Am. Oil Chem. Soc.* **75**(4):425-439.
- Loisel, C., Lecq, G., Ponchel, G., Keller, G. and Ollivon, M. (1997). Fat bloom and chocolate structure studied by mercury porosimetry. *J. Food Sci.* **62**(4):781-788.
- Lonchamp, P. and Hartel, R. W. (2004). Fat bloom in chocolate and compound coatings. *Eur. J. Lipid Sci. Technol.* **106**(4):241-274.

Lovegren, N. V., Gray, M. S. and Fugee, R. O. (1976). Effect of liquid fat on melting point and polymorphic behaviour of cocoa butter and a cocoa butter fraction. *J. Am. Oil Chem. Soc.* **53**(3):108-112.

Maache-Rezzoug, Z., Bouvier, J. M., Allaf, K. and Patras, C. (1998). Study of mixing in connection with the rheological properties of biscuit dough and dimensional characteristics of biscuits. *Food Eng.* **35**(1):43-56.

Marangoni, A. G. (2005). Fat Crystal Networks. Marcel Dekker, New York City, New York, U.S.A.

Marangoni, A. G. (2012). Chocolate Compositions Containing Ethylcellulose. Patent US 20120183651 A1.

Marangoni, A. G. and Wesdorp, L. H. (2013). Structure and Properties of Fat Crystal Networks. CRC Press, Boca Raton, Florida, U.S.A.

Marty, S., Baker, K., Dibildox-Alvarado, E., Rodrigues, J. N. and Marangoni, A. G. (2005). Monitoring and quantifying of oil migration in cocoa butter using a flatbed scanner and fluorescence light microscopy. *Food Res. Int.* **38**(10):1189-1197.

Marty, S. and Marangoni, A. G. (2009). Effects of cocoa butter origin, tempering procedure, and structure on oil migration kinetics. *Cryst. Growth Des.* **9**(10):4415-4423.

Mate, C. M., McClelland, G. M., Erlandsson, R. and Chiang, S. (1987). Atomic-scale friction of a tungsten tip on a graphite surface. *Phys. Rev. Lett.* **59**(17):1942-1946.

Mathlouthi, M. and Genotelle, J. (1995). Rheological properties of sucrose solutions and suspensions. **In:** Sucrose, Properties and Applications, pp. 126-154. Mathlouthi, M. and Reiser, P., Eds., Blackie, Glasgow, U.K.

Mazzanti, G., Guthrie, S. E., Sirota, E. B., Marangoni, A. G. and Idziak, S. H. J. (2003). Orientation and phase transitions of fat crystals under shear. *Cryst. Growth Des.* **3**(5):721-725.

McNamara, D. J. (2010). Palm oil and health: A case of manipulated perception and misuse of science. *J. Am. Coll. Nutr.* **29**(3 Suppl):240S-244S.

Merkel, R., Nassoy, P., Leung, A., Ritchie, K. and Evans, E. (1999). Energy landscapes of receptor-ligand bonds explored with dynamic force spectroscopy. *Nature* **397**(6714):50-53.

Metin, S. (1997). Crystallization Behaviour and Kinetics of Blends of Cocoa Butter and Milk Fat or Milk Fat Fractions. University of Wisconsin-Madison, Madison, Wisconsin, U.S.A.

Metin, S. and Hartel, R. W. (2005). Crystallization of fats and oils. **In:** Bailey's Industrial Oil and Fat Products, pp. 45-76. Shahidi, F., Ed., John Wiley and Sons, Chichester, U.K.

Meyer, G. and Amer, N. M. (1988). Novel optical approach to atomic force microscopy. *Appl. Phys. Lett.* **53**(12):1045-1047.

Middendorf, D., Juadjur, A., Bindrich, U. and Mischnick, P. (2015). AFM approach to study the function of PGPR's emulsifying properties in cocoa butter based suspensions. *Food Struct.* **4**:16-26.

- Middendorf, D., Bindrich, U., Mischnick, P., Juadjur, A., Franke, K and Heinz, V. (2016). Atomic force microscopy study on the effect of different lecithins in cocoa-butter based suspensions. *Colloids Surf., A* **499**:60-68.
- Miles, M. J. and McMaster, T. J. (1995). Scanning probe microscope in food-related systems. **In:** *New Physicochemical Techniques for the Characterization of Complex Food Systems*, pp. 69-85. Dickinson, E., Ed., Blackie, Glasgow, U.K.
- Minifie, B. W. (1989). *Chocolate, Cocoa, and Confectionery: Science and Technology*. Chapman and Hall, London, U.K.
- Minson, E. (1990). Cookie confectionery combinations. *Manuf. Confect.* **70**:121-126.
- Miquel, M. E., Carli, S., Couzens, P. J., Wille, H. -J. and Hall, L. D. (2001). Kinetics of the migration of lipids in composite chocolate measured by magnetic resonance imaging. *Food Res. Int.* **34**(9):773-781.
- Miquel, M. E. and Hall, L. D. (2002). Measurement by MRI of storage changes in commercial chocolate confectionery products. *Food Res. Int.* **35**(10):993-998.
- Mongia, G. and Ziegler, G. R. (2000). The role of particle size distribution of suspended solids in defining the flow properties of milk chocolate. *Int. J. Food Prop.* **3**(1):137-147.
- Morris, V. J. (2007). Atomic force microscopy techniques for characterising food structure. **In:** *Understanding and Controlling the Microstructure of Complex Foods*, pp. 209-231. McClements, D., J., Ed., CRC Press, Boca Raton, Florida, U.S.A.

Mullin, J. W. (1993). Crystallization. Butterworth, London, U.K.

Murphy, D. J. (2007). Future prospects for oil palm in the 21st century: Biological and related challenges. *Eur. J. Lipid Sci. Technol.* **109**(4):296-306.

Neaton, J. D. and Wentworth, D. (1992). Serum cholesterol, blood pressure, cigarette smoking, and death from coronary heart disease. Overall findings and differences by age for 316,099 white men. Multiple Risk Factor Intervention Trial Research Group. *Arch. Intern. Med.* **152**(1):56-64.

Nopens, I., Foubert, I., De Graef, V., Van Laere, D., Dewettinck, K. and Vanrolleghem, P. (2008). Automated image analysis tool for migration fat bloom evaluation of chocolate coated food products. *Lebensm. Wiss. Technol.* **41**(10):1884-1891.

Nováková, H., Čopíková, J., Maixner, J. and Maryška, M. (2002). The production of clusters in milk chocolate. *Int. J. Food Sci. Technol.* **37**(5):485-495.

Noy, A. and Friddle, R. W. (2013). Practical single molecule force spectroscopy: How to determine fundamental thermodynamic parameters of intermolecular bonds with an atomic force microscope. *Methods* **60**(2):142-150.

Noy, A., Frisbie, C. D., Rozsnyai, L. F., Wrighton, M. S. and Lieber, C. M. (1995). Chemical force microscopy: Exploiting chemically-modified tips to quantify adhesion, friction, and functional group distributions in molecular assemblies. *J. Am. Chem. Soc.* **117**(30):7943-7951.

Noy, A., Vezhenov, D. V. and Lieber, C. M. (2008). Chemical force microscopy nanoscale probing of fundamental chemical interactions. **In:** Handbook of Molecular Force Spectroscopy, pp. 97-122. Noy, A., Ed., Springer Science+Business Media, New York City, New York, U.S.A.

Okiy, D. A. and Oke, O. L. (1984). A study of the effects of salt and pepper on palm oil used as a frying medium. *Food Chem.* **14**(4):251-262.

Olinger, P. M. (1994). New options for sucrose-free chocolate. *Manuf. Confect.* **74**:77-84.

Omar, Z., Let, C. C., Seng, C. C. and Rashid, N. A. (2005). Crystallisation and rheological properties of hydrogenated palm oil and palm oil blends in relation to crystal networking. *Eur. J. Lipid Sci. Technol.* **107**(9):634-640.

Ortiz, C. and Hadziioannou, G. (1999). Entropic elasticity of single polymer chains of poly(methacrylic acid) measured by atomic force microscopy. *Macromolecules* **32**(3):780-787.

Osim, E. E., Owu, D. U., Isong, E. and Umoh, I. B. (1992). Influence of chronic consumption of thermoxidized palm oil on platelet aggregation in the rats. *Discov. Innov.* **4**(1):83-87.

Osim, E. E., Owu, D. U., Isong, E. and Umoh, I. B. (1994). Influence of chronic consumption of thermoxidized and fresh palm oil diets on basal metabolic rate, body weight, and morphology of tissues in rats. *Discov. Innov.* **6**(4):389-396.

Owen, R. (2004). A Practical Guide to AFM Force Spectroscopy and Data Analysis. JPK Instruments Technical Report.

Pajin, B. and Jovanovic, O. (2005). Influence of high-melting milk fat fraction on quality and fat bloom stability of chocolate. *Eur. Food Res. Technol.* **220**(3):389-394.

Pérez-Martínez, D., Alvarez-Salas, C., Charó-Alonso, M., Dibildox-Alvarado, E. and Toro-Vazquez, J. F. (2007). The cooling rate effect on the microstructure and rheological properties of blends of cocoa butter with vegetable oils. *Food Res. Int.* **40**(1):47-62.

Pink, D. A., Quinn, B., Peyronel, F. and Marangoni, A. G. (2013). Edible oil structures at low and intermediate concentrations. I. Modeling, computer simulation, and predictions for x-ray scattering. *J. Appl. Phys.* **114**(23):234901.

Quevedo, R., Valencia, E., Alvarado, F., Ronceros, B. and Bastias, J. M. (2013). Comparison of whiteness index vs. fractal fourier in the determination of bloom chocolate using image analysis. *Food Bioprocess Technol.* **6**(7):1878-1884.

Rak, M., Eremin, N. N., Eremina, T. A., Kuznetsov, V. A., Okhrimenko, T. M., Furmanova, N. G. and Efremova, E. P. (2005). On the mechanism of impurity influence on growth kinetics and surface morphology of KDP crystals—I: defect centres formed by bivalent and trivalent impurity ions incorporated in KDP structure—theoretical study. *J. Cryst. Growth* **273**(3-4):577-585.

Randolph, A. D. and Larson, M. A. (1988). *Theory of Particulate Processes*. Academic Press, New York City, New York, U.S.A.

Rao, M. A. (2007). Influence of food microstructure on food rheology. **In:** *Understand and Controlling the Microstructures of Complex Foods*, pp. 411-424. McClements, D. J., Ed., CRC Press, Boca Raton, Florida, U.S.A.

Rector, D. (2000). Chocolate-controlling the flow. Benefits of polyglycerol polyricinoleic acid. *Manuf. Confect.* **80**:63-70.

Rief, M. (1997). Reversible unfolding of individual titin immunoglobulin domains by AFM. *Science* **276**(5315):1109-1112.

Rief, M., Oesterhelt, F., Heymann, B. and Gaub, H. E. (1997). Single molecule force spectroscopy on polysaccharides by atomic force microscopy. *Science* **275**(5304):1295-1297.

Rössner, S. (1997). Chocolate-divine food, fattening junk or nutritious supplementation? *Eur. J. Clin. Nutr.* **51**(6):341-345.

Rousseau, D. (2006). On the porous mesostructure of milk chocolate viewed with atomic force microscopy. *Lebensm. Wiss. Technol.* **39**(8):852-860.

Rousseau, D. (2007). The microstructure of chocolate. **In:** Understanding and Controlling the Microstructure of Complex Foods, pp. 648-690. McClements, D. J., Ed., CRC Press, Boca Raton, Florida, U.S.A.

Rousseau, D., Hodge, S. M., Nickerson, M. T. and Paulson, A. T. (2005). Regulating the $\beta' \rightarrow \beta$ polymorphic transition in food fats. *J. Am. Oil Chem. Soc.* **82**(1):7-12.

Rousseau, D. and Marangoni, A. G. (1999). The effects of interesterification on physical and sensory attributes of butterfat and butterfat-canola oil spreads. *Food Res. Int.* **31**(5):381-388.

Rousseau, D. and Sonwai, S. (2008). Influence of the dispersed particulate in chocolate on cocoa butter microstructure and fat crystal growth during storage. *Food Biophys.* **3**(2):273-278.

Rousseau, D., Sonwai, S. and Khan, R. (2010). Microscale surface roughening of chocolate viewed with optical profilometry. *J. Am. Oil Chem. Soc.* **87**(10):1127-1136.

Rousset, Ph., Sellappan, P. and Daoud, P. (2002). Effect of emulsifiers on surface properties of sucrose by inverse gas chromatography. *J. Chromatogr. A* **969**(1-2):97-101.

Russell, A. B., Cheney, P. E. and Wantling, S. D. (1999). Influence of freezing conditions on ice crystallization in ice cream. *J. Food Eng.* **39**(2):179-191.

Saberi, A. H., Lai, O. -M. and Miskandar, M. S. (2012). Melting and solidification properties of palm-based diacylglycerol, palm kernel olein, and sunflower oil in the preparation of palm-based diacylglycerol-enriched soft tub margarine. *Food Bioprocess Technol.* **5**(5):1674-1685.

Sambanthamurthi, R., Sundram, K. and Tan, Y. (2000). Chemistry and biochemistry of palm oil. *Prog. Lipid Res.* **39**(6):507-558.

Sato, K. (1988). Crystallization of fats and fatty acids. **In:** Crystallization and Polymorphism of Fats and Fatty Acids, pp. 227-266. Garti, N. and Sato, K., Eds., Marcel Dekker, New York City, New York, U.S.A.

Sato, K. (1993). Polymorphic transformations in crystal growth. *J. Phys. D: Appl. Phys.* **26**(8B):B77-B84.

Sato, K. (2001). Crystallization behaviour of fats and lipids: A review. *Chem. Eng. Sci.* **56**(7):2255-2265.

Sato, K., Arishima, T., Wang, Z. H., Ojima, K., Sagi, N. and Mori, H. (1989). Polymorphism of POP and SOS. I. Occurrence and polymorphic transformation. *J. Am. Oil Chem. Soc.* **66**(5):664-674.

Savage, C. M. and Dimick, P. S. (1995). Influence of phospholipids during crystallization of hard and soft cocoa butters. *Manuf. Confect.* **75**:127-132.

Schantz, B., Linke, L. and Rohm, H. (2003). Effects of different emulsifiers on rheological and physical properties of chocolate. **In:** Proceedings of the 3rd International Symposium on Food Rheology and Structure, pp. 329-333. Fischer, P., Marti, I. and Windhab, E. J., Eds., ETH – Laboratory of Food Process Engineering, Zurich, Switzerland.

Schantz, B. and Rohm, H. (2005). Influence of lecithin–PGPR blends on the rheological properties of chocolate. *Lebensm. Wiss. Technol.* **38**(1):41-45.

Schenk, H. and Peschar, R. (2004). Understanding the structure of chocolate. *Radiat. Phys. Chem.* **71**(3-4):829-835.

Schlichter-Aronhime, J. and Garti, N. (1988). Solidification and polymorphism in cocoa butter and the blooming problems. **In:** Crystallization and Polymorphism of Fats and Fatty Acids, pp. 363-393. Garti, N. and Sato, K., Eds., Marcel Dekker, New York City, New York, U.S.A.

Schoukens, G. and De Clerck, K. (2005). Thermal analysis and Raman spectroscopic studies of crystallization in poly(ethylene 2,6-naphthalate). *Polymer* **46**(3):845-857.

Seguine, E. S. (1991). Tempering: The inside story. *Manuf. Confect.* **5**:117-125.

Sen, C. K., Khanna, S. and Roy, S. (2007). Tocotrienols in health and disease: The other half of the natural vitamin E family. *Mol. Aspects Med.* **28**(5-6):692-728.

Seo, Y. and Jhe, W. (2008). Atomic force microscopy and spectroscopy. *Rep. Prog. Phys.* **71**(1):1-23.

Servais, C., Jones, R. and Roberts, I. (2002). The influence of particle size distribution on the processing of food. *J. Food Eng.* **51**(3):201-208.

Servais, C., Ranc, H. and Roberts, I. D. (2004). Determination of chocolate viscosity. *J. Texture Stud.* **34**(5-6):467-497.

Shah, A. B., Jones, G. P. and Vasiljevic, T. (2010). Sucrose-free chocolate sweetened with *Stevia rebaudiana* extract and containing different bulking agents: Effects on physicochemical and sensory properties. *Int. J. Food Sci. Technol.* **45**(7):1426-1435.

Sherazi, S. T. H., Kandhro, A., Mahesar, S. A., Bhanger, M. I., Talpur, M. Y. and Arain, S. (2009). Application of transmission FT-IR spectroscopy for the trans fat determination in the industrially processed edible oils. *Food Chem.* **114**(1):323-327.

Siew, W. L. and Ng, W. L. (1995). Partition coefficient of diglycerides in crystallization of palm oil. *J. Am. Oil Chem. Soc.* **72**(5):591-595.

Siew, W. L. and Ng, W. L. (1996). Characterisation of crystals in palm olein. *J. Sci. Food Agric.* **70**(2):212-216.

Siew, W. L. and Ng, W. L. (1999). Influence of diglycerides on crystallisation of palm oil. *J. Sci. Food Agric.* **79**(5):722-726.

Smith, K. W., Bhaggan, K., Talbot, G. and Van Malssen, K. F. (2011). Crystallization of fats: influence of minor components and additives. *J. Am. Oil Chem. Soc.* **88**(8):1085-1101.

Smith, K. W., Cain, F. W. and Talbot, G. (2007). Effect of nut oil migration on polymorphic transformation in a model system. *Food Chem.* **102**(3):656-663.

Smith, P. R. and Dahlman, A. (2005). The use of atomic force microscopy to measure the formation and development of chocolate bloom in pralines. *J. Am. Oil Chem. Soc.* **82**(3):165-168.

Sokmen, A. and Gunes, G. (2006). Influence of some bulk sweeteners on rheological properties of chocolate. *Lebensm. Wiss. Technol.* **39**(10):1053-1058.

Sonoda, T., Takata, Y., Ueno, S. and Sato, K. (2004). DSC and synchrotron-radiation x-ray diffraction studies on crystallization and polymorphic behavior of palm stearin in bulk and oil-in-water emulsion states. *J. Am. Oil Chem. Soc.* **81**(4):365-373.

Sonwai, S. and Rousseau, D. (2008). Fat crystal growth and microstructural evolution in milk chocolate. *Cryst. Growth Des.* **8**(9):3165-3174.

Sonwai, S. and Rousseau, D. (2010). Controlling fat bloom formation in chocolate: Impact of milk fat on microstructure and fat phase crystallisation. *Food Chem.* **119**(1):286-297.

Spearing, N. (2007). The Effect of Fat on the Texture of Milk Chocolate. University of Nottingham, Nottingham, U.K.

Stanley, D. W., Aguilera, J. M., Baker, K. W. and Jackman, R. L. (1998). Structure/property relationships of foods affected by processing and storage. **In:** Phase/State Transitions in Foods, pp. 1-56. Rao, M. A. and Hartel, R. W., Eds., Marcel Dekker, New York City, New York, U.S.A.

Stanley, J. C. (2008). The nutritional reputation of palm oil. *Lipid Technol.* **20**(5):112-114.

Stapley, A. G. F., Tewkesbury, H. and Fryer, P. J. (1999). The effects of shear and temperature history on the crystallization of chocolate. *J. Am. Oil Chem. Soc.* **76**(6):677-685.

Subramaniam, P. J. (2000). Confectionery products. **In:** The Stability and Shelf Life of Food, pp. 221-248. Kilcast, D. and Subramaniam, P. J., Eds., Woodhead Publishing, Cambridge, U.K.

Sundram, K. and Top, A. G. M. (1994). Vitamin E from palm oil: Its extraction and nutritional properties. *Palmas* **15**(1):77-82.

Svanberg, L., Ahrné, L., Lorén, N. and Windhab, E. (2011a). Effect of sugar, cocoa particles and lecithin on cocoa butter crystallisation in seeded and non-seeded chocolate model systems. *J. Food Eng.* **104**(1):70-80.

Svanberg, L., Ahrné, L., Lorén, N. and Windhab, E. (2011b). Effect of pre-crystallization process and solid particle addition on microstructure in chocolate model systems. *Food Res. Int.* **44**(5):1339-1350.

Svanberg, L., Ahrné, L., Lorén, N. and Windhab, E. (2013). Impact of pre-crystallization process on structure and product properties in dark chocolate. *J. Food Eng.* **114**(1):90-98.

Svanberg, L., Lorén, N. and Ahrné, L. (2012). Chocolate swelling during storage caused by fat or moisture migration. *J. Food Sci.* **77**(11):E328-E334.

Talbot, G. (1994). Chocolate temper. **In:** Industrial Chocolate Manufacture and Use, pp. 156-166. Beckett, S. T., Ed., Blackie, Oxford, U.K.

Talbot, G. (1999). Vegetable fats. **In:** Industrial Chocolate Manufacture and Use, pp. 307-322. Beckett, S. T., Ed., Blackie, Oxford, U.K.

Tang, D. and Marangoni, A. G. (2007). Structure and function of fat crystals and their role in microstructure formation in complex foods. **In:** Understanding and Controlling the Microstructure of Complex Foods, pp. 67-88. McClements, D. J., Ed., CRC Press, Boca Raton, Florida, U.S.A.

Tang, J., Ebner, A., Ilk, N., Lichtblau, H., Huber, C., Zhu, R., Pum, D., Leitner, M., Pastushenko, V., Gruber, H. J., Sleytr, U. B. and Hinterdorfer, P. (2008). High-affinity tags fused to s-layer proteins probed by atomic force microscopy. *Langmuir* **24**(4):1324-1329.

Tarabukina, E., Jegou, F., Haudin, J. M., Navard, P. and Peuvrel-Disdier, E. (2009). Effect of shear on the rheology and crystallization of palm oil. *J. Food Sci.* **74**(8):405-416.

Tarrago-Trani, M. T., Phillips, K. M., Lemar, L. E. and Holden, J. M. (2006). New and existing oils and fats used in products with reduced trans-fatty acid content. *J. Am. Diet. Assoc.* **106**(6):867-880.

Tavare, N. S. (1995). Industrial Crystallization: Process Simulation Analysis and Design. Plenum Press, New York City, New York, U.S.A.

Tietz, R. A. and Hartel, R. W. (2000). Effects of minor lipids on crystallization of milk fat-cocoa butter blends and bloom formation in chocolate. *J. Am. Oil Chem. Soc.* **77**(7):763-771.

Timms, R. E. (1984). Phase behaviour of fats and their mixtures. *Prog. Lipid Res.* **23**(1):1-38.

Timms, R. E. (2003). *Confectionery Fat Handbook: Properties, Production, and Applications*. The Oily Press, London, U.K.

Tscheuschner, H. D. (1994). Rheological and processing properties of fluid chocolate. *Appl. Rheol.* **94**:83-88.

Tscheuschner, H. D. and Wünsche, D. (1979). Rheological properties of chocolate masses and the influence of some factors. **In:** *Food Texture and Rheology*, pp. 355-368. Sherman, P., Ed., Academic Press, New York City, New York, U.S.A.

Tshiamala, I. T. (2013). *Influence of a Commercial Monoglyceride on the Microstructural Development of Palm Oil*. Ghent University, Ghent, Belgium.

Vaeck, S. V. (1960). Cocoa butter and fat bloom. *Manuf. Confect.* **40**:35-46,71-74.

Van Den Tempel, M. (1979). Rheology of concentrated suspensions. *J. Colloid Interface Sci.* **71**(1):18-20.

Van Der Vaart, K., Depypere, F., De Graef, V., Schall, P., Fall, A., Bonn, D. and Dewettinck, K. (2013). Dark chocolate's compositional effects revealed by oscillatory rheology. *Eur. Food Res. Technol.* **236**(6):931-942.

Van Langevelde, A. J., Driessen, R., Molleman, W., Peschar, R. and Schenk, H. (2001a). Cocoa-butter long spacings and the memory effect. *J. Am. Oil Chem. Soc.* **78**(9):911-918.

Van Langevelde, A. J., Van Malssen, K., Peschar, R. and Schenk, H. (2001b). Effect of temperature on re-crystallization behaviour of cocoa-butter. *J. Am. Oil Chem. Soc.* **78**(9):919-925.

Van Malssen, K. F., Peschar, R. and Schenk, H. (1994). Geometrical aspects of real-time powder diffraction using a normal generator and a linear diode-array detector. *J. Appl. Crystallogr.* **27**(3):302-315.

Van Malssen, K. F., Van Langevelde, A. J., Peschar, R. and Schenk, H. (1999). Phase behaviour and extended phase scheme of static cocoa-butter investigated with real-time x-ray powder diffraction. *J. Am. Oil Chem. Soc.* **76**(6):669-676.

Vernier, F. C. (1998). Influence of Emulsifiers on the Rheology of Chocolate and Suspensions of Cocoa or Sugar Particles in Oil. University of Reading, Reading, U.K.

Verstringe, S., Danthine, S., Blecker, C., Depypere, F. and Dewettinck, K. (2012). Influence of monopalmitin on the isothermal crystallization mechanism of palm oil. *Food Res. Int.* **51**(1):344-353.

Vivar-Vera, G., Torrestiana-Sanchez, B., Monroy-Rivera, J. A. and Brito-De La Fuente, E. (2008). Chonching: Rheological and structural changes of chocolate mass. *Dtsch. Lebensmitt. Rundsch.* **104**(8):376-382.

Voon, P. T., Ng, T. K. W., Lee, V. K. M. and Nesaretnam, K. (2011). Diets high in palmitic acid (16:0), lauric and myristic acids (12:0 + 14:0), or oleic acid (18:1) do not alter postprandial or fasting plasma homocysteine and inflammatory markers in healthy Malaysian adults. *Am. J. Clin. Nutr.* **94**(6):1451-1457.

Walker, J. H. (2009). Bulk chocolate handling. **In:** Industrial Chocolate Manufacture and Use, pp. 169-188. Beckett, S. T., Ed., Blackwell Publishing, Chichester, U.K.

Walstra, P. (2003). Physical Chemistry of Foods. Marcel Dekker, New York City, New York, U.S.A.

Walter, P. and Cornillon, P. (2002). Lipid in two-phase chocolate systems investigated by NMR and DSC. *Food Res. Int.* **35**(8):761-767.

Walton, A. G. (1969). Nucleation in liquids and solutions. **In:** Nucleation, pp. 225-308. Zettlemoyer, A. C., Ed., Marcel Dekker, New York City, New York, U.S.A.

Wang, C., Shi, W. Q., Zhang, W. K., Zhang, X., Katsumoto, Y. and Ozaki, Y. (2002). Force spectroscopy study on poly(acrylamide) derivatives: Effects of substitutes and buffers on single-chain elasticity. *Nano Lett.* **2**(10):1169-1172.

Wang, F., Liu, Y., Shan, L., Jin, Q., Wang, X. and Li, L. (2010). Blooming in cocoa butter substitutes based compound chocolate: Investigations on composition, morphology and melting behavior. *J. Am. Oil Chem. Soc.* **87**(10):1137-1143.

Ward, I. M. and Sweeney, J. (2012). Mechanical Properties of Solid Polymers, John Wiley and Sons, Chichester, U.K.

West, R. and Rousseau, D. (2016). Crystallization and rheology of palm oil in the presence of sugar. *Food Res. Int.* **85**:224-234.

Wille, R. L. and Lutton, E. S. (1966). Polymorphism of cocoa butter. *J. Am. Oil Chem. Soc.* **43**(8):491-496.

Windhab, E. J. (1999). New developments in crystallization processing. *J. Therm. Anal. Calorim.* **57**(1):171-180.

Yoshikawa, S., Kida, H. and Sato, K. (2014). Promotional effects of new types of additives on fat crystallization. *J. Oleo Sci.* **63**(4):333-345.

Yucel, U. and Coupland, J. N. (2011). Ultrasonic attenuation measurements of the mixing, agglomeration, and sedimentation of sucrose crystals suspended in oil. *J. Am. Oil Chem. Soc.* **88**(1):33-38.

Zarić, D. B., Pajin, B. S., Lončarević, I. S., Šoronja Simović, D. M. and Šereš, Z. I. (2012). The impact of the manufacturing process on the hardness and sensory properties of milk chocolate. *Acta Period. Technol.* **43**:139-148.

Zeng, Y. (2000). Impf-und Scherkristallisation von Schokoladen. ETH Zurich, Zurich, Switzerland.

Zettlemoyer, A. C., Ed. (1969). Nucleation. Marcel Dekker, New York City, New York, U.S.A.

Zhang, Q. and Marszalek, P. E. (2006). Identification of sugar isomers by single-molecule force spectroscopy. *J. Am. Chem. Soc.* **128**(17):5596-5597.

Zhang, W. and Zhang, Y. (2003). Single molecule mechanochemistry of macromolecules. *Prog. Polym. Sci.* **28**(8):1271-1295.

Zheng, P., Chou, C. -C., Guo, Y., Wang, Y. and Li, H. (2013). Single molecule force spectroscopy reveals the molecular mechanical anisotropy of the FeS₄ metal center in rubredoxin. *J. Am. Chem. Soc.* **135**(47):17783-17792.

Ziegleder, G. (1997). Fat bloom and migration. *Manuf. Confect.* **77**:43-44.

Ziegleder, G. (2009). Flavour development in cocoa and chocolate. **In**: Industrial Chocolate Manufacture and Use, pp. 169-188. Beckett, S. T., Ed., Blackwell Publishing, Chichester, U.K.

Ziegleder, G., Moser, C. and Geier Greguska, J. (1996). Kinetics of fat migration within chocolate products. Part 1. Principles and analytics. *Eur. J. Lipid Sci. Technol.* **98**(6):196-199.

Ziegleder, G. and Schwingshandl, I. (1998). Kinetics of fat migration within chocolate products. Part 3: Fat bloom. *Eur. J. Lipid Sci. Technol.* **100**(9):411-415.

Ziegler, G. R., Mongia, G. and Hollender, R. (2001). The role of particle size distribution of suspended solids in defining the sensory properties of milk chocolate. *Int. J. Food Prop.* **4**(2):353-370.

Zumbé, A. and Brinkworth, R. (1993). Product and process for producing milk chocolate. United States Patent US 5238698 A.

Table 1. Effect of soy lecithin concentration on τ_0 and η^* of milk chocolate (Schantz and Rohm, 2005).

Concentration ($\text{g} \cdot \text{kg}^{-1}$)	τ_0 (Pa)	η^* (Pa·s)
0	79.60	12.30
1	19.50	4.18
2	11.80	3.63
3	10.20	3.28
4	9.00	3.04
5	9.15	2.89
6	9.59	2.79
7	10.30	2.71

Table 2. Effect of CB origin on TAG composition (Marty and Marangoni, 2009).

TAG (%)*	Brazil	China	Ecuador	Ivory Coast	Malaysia	Nigeria
OOL	0.5	1.0	1.1	0.6	1.0	1.8
PLP	1.5	1.1	1.3	1.5	1.2	1.1
OOO	5.1	0.8	1.9	1.8	1.4	1.2
POP	22.8	23.5	24.1	24.3	22.7	23.1
PPP	0.0	0.1	0.0	0.0	0.0	0.1
SOO	7.0	1.7	2.1	2.1	1.8	1.2
POS	34.1	37.0	37.3	37.3	36.9	37.3
PPS	0.1	0.2	0.1	0.1	0.1	0.2
SOS	28.3	33.1	31.1	31.4	33.4	32.5
SSP	0.1	0.4	0.3	0.3	0.4	0.5
SSS	0.6	1.0	0.8	0.7	1.1	1.1

*Oleic (O), linoleic (L), palmitic (P), and stearic (S)

Table 3. Melting point and chain-length packing of CB polymorphs (Afoakwa et al., 2007a).

Polymorph	Melting point (°C)	Chainlength
γ	16-18	2
α	21-22	2
β'_2	25.5	2
β'_1	27-29	2
β_2	34-35	3
β_1	36	3

Table 4. Typical FA composition of PO (Basiron, 2001).

FA	Composition (%)
Palmitic	44.0
Oleic	39.2
Linoleic	10.1
Stearic	4.5
Myristic	1.1
Linolenic	0.4
Arachidic	0.4
Lauric	0.2

Table 5. DAG and TAG composition of PO (West and Rousseau, 2016).

DAG*	Composition (%)	TAG*	Composition (%)
1,3-PO	2.0	PPO/POP	27.6
1,2-PO	1.0	OOP/OPO	22.5
1,3-OO	0.8	POL/PLO/OLP	10.1
1,3-PL	0.7	PPL/PLP	7.8
1,3-PP	0.6	PPP	5.5
1,2-OO	0.5	POS/PSO/SPO	5.2
1,2-PP	0.3	OOO	4.4
1,3-SO	0.2	OOS/OSO	2.6
1,2-SO	0.1	LLP/LPL	2.2
1,3-PS	0.1	OOL/OLO	2.1
1,2-PS	0.1	PPS/PSP	1.1
		PLS/PSL/LPS	0.9
		LLO/LOL	0.5
		LLS/LSL	0.3
		AOP/PAO/OPA	0.3
		OOA/OAO	0.2
		LLL	0.1
		LnLP/LnPL/PLnL	0.1
		SSP/SPS	0.1

*Palmitic (P); oleic (O); linoleic (L); stearic (S); linolenic (Ln); arachidic (A)

Table 6. Physical properties of PO and its fractions (Edem, 2002).

Property	PO	Palm olein	Palm stearin
Melting point (°C)	34.2	21.6	44.5-56.2
Relative density (50 °C/water at 25 °C)	0.89-0.92	0.91-0.92	0.88-0.89
Refractive index	1.46	1.47	1.45
Moisture and impurities (%)	0.10	0.10	0.00-0.15
Iodine value	47.00-55.83	55.00-61.54	21.60-49.40
Saponification value (mg KOH·g ⁻¹)	196.0-208.2	189.0-198.0	193.0-206.0
Unsaponifiable matter (%)	0.010-0.500	0.001-0.500	0.100-1.000

Table 7. Effect of temperature cycles on fat bloom formation (Ali et al., 2001).

Week	Storage temperature (°C)			
	18		30	
	Fat bloom ^a	Cycles ^b	Fat bloom	Cycles
0	-	7	+	6
1	-	7	++	4
2	-	7	++++	2
3	-	7	++++	1
4	-	7	++++	1
5	-	7	++++	1
6	-	7	++++	1
7	-	7	++++	1
8	-	7	++++	1

^a -, no bloom; +, weak bloom; ++, bloom; +++, strong bloom; +++++, intensive bloom

^b one cycle is 30 °C (16 h) and 20 °C (8 h)

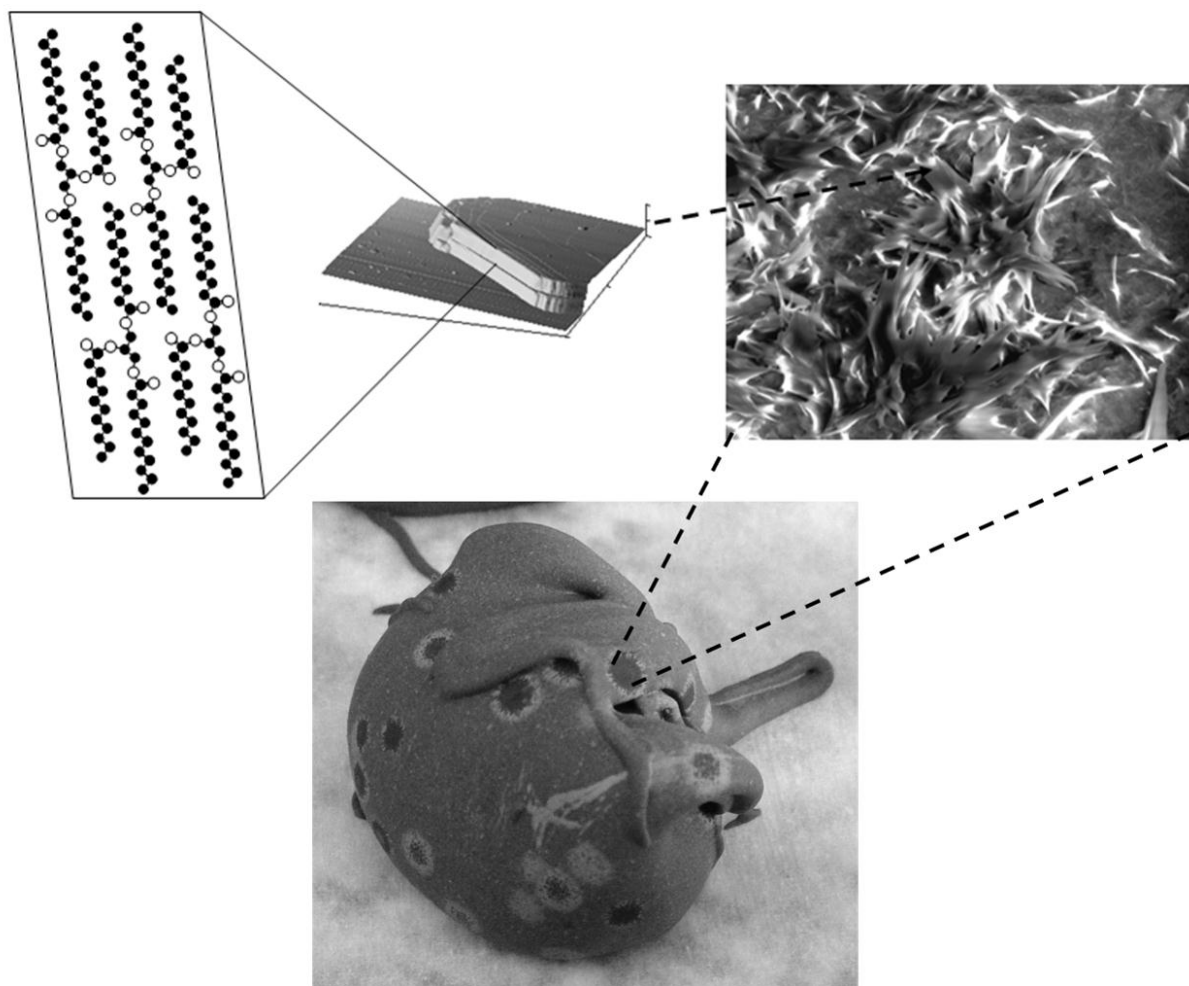


Figure 1. Multiscale interpretation of chocolate from glyceride assembly to fat bloom.

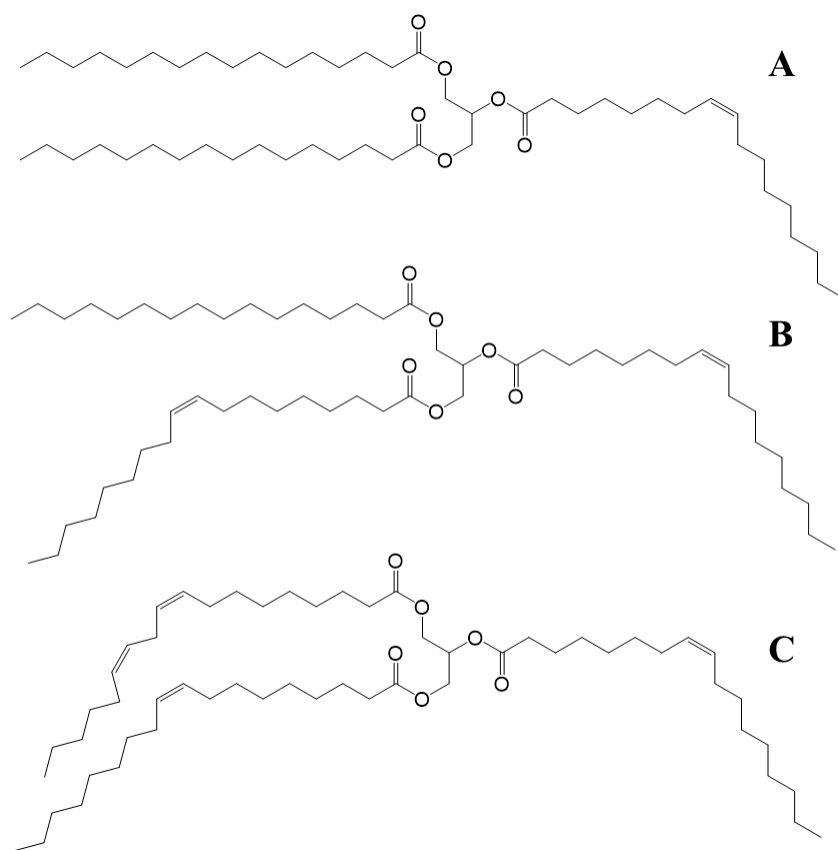


Figure 2. Molecular structure of (A) 1,3-dipalmitoyl-2-oleoylglycerol, (B) 1-palmitoyl-2,3-dioleoylglycerol, and (C) 1-palmitoyl-2-oleoyl-3-linoleoylglycerol.

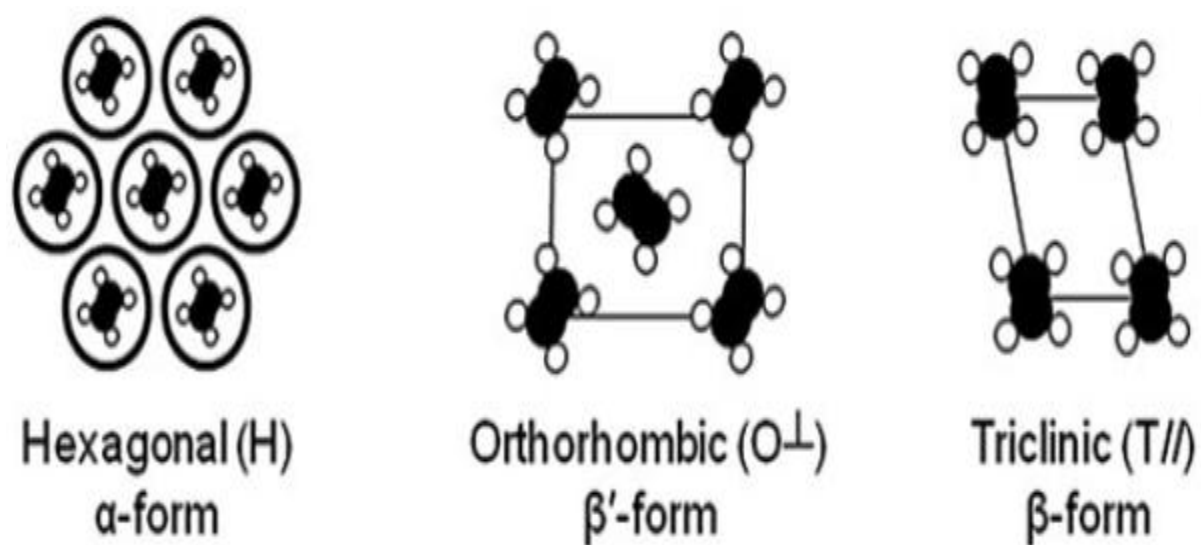


Figure 3. Polymorphic forms of TAGs (Bresson et al., 2011).

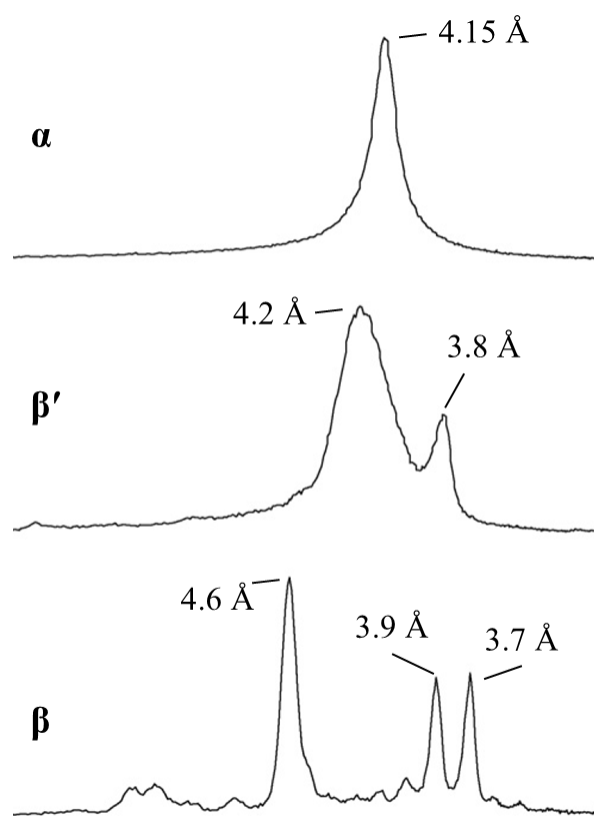


Figure 4. Diffractograms of different tristearin polymorphs (adapted from Da Silva et al., 2009).

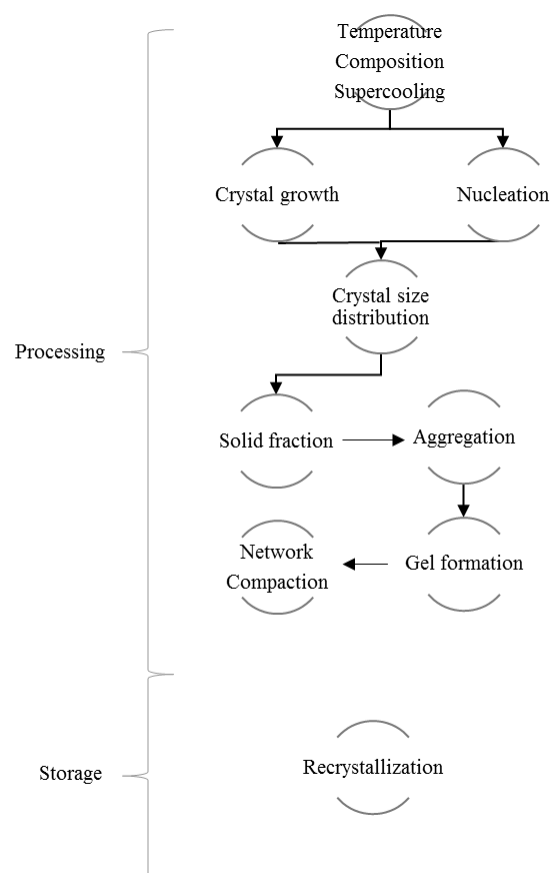


Figure 5. Timeline of events during fat crystallization and storage (adapted from Himawan et al., 2006).

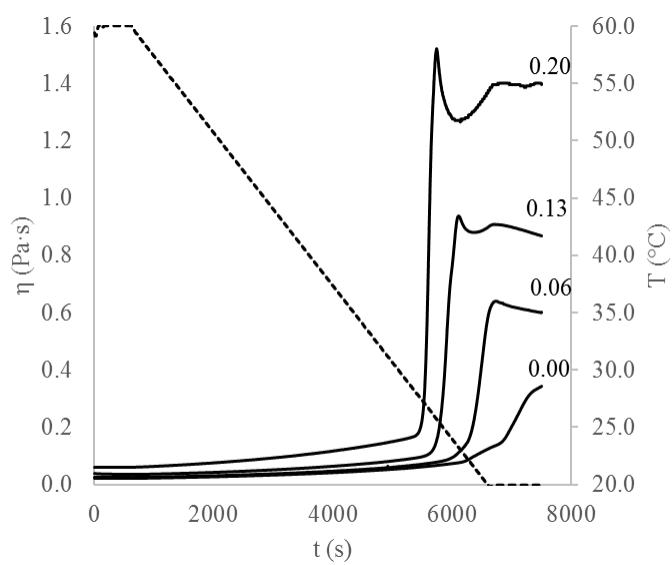


Figure 6. Effect of sugar volume fraction on PO crystallization during cooling (unpublished data).

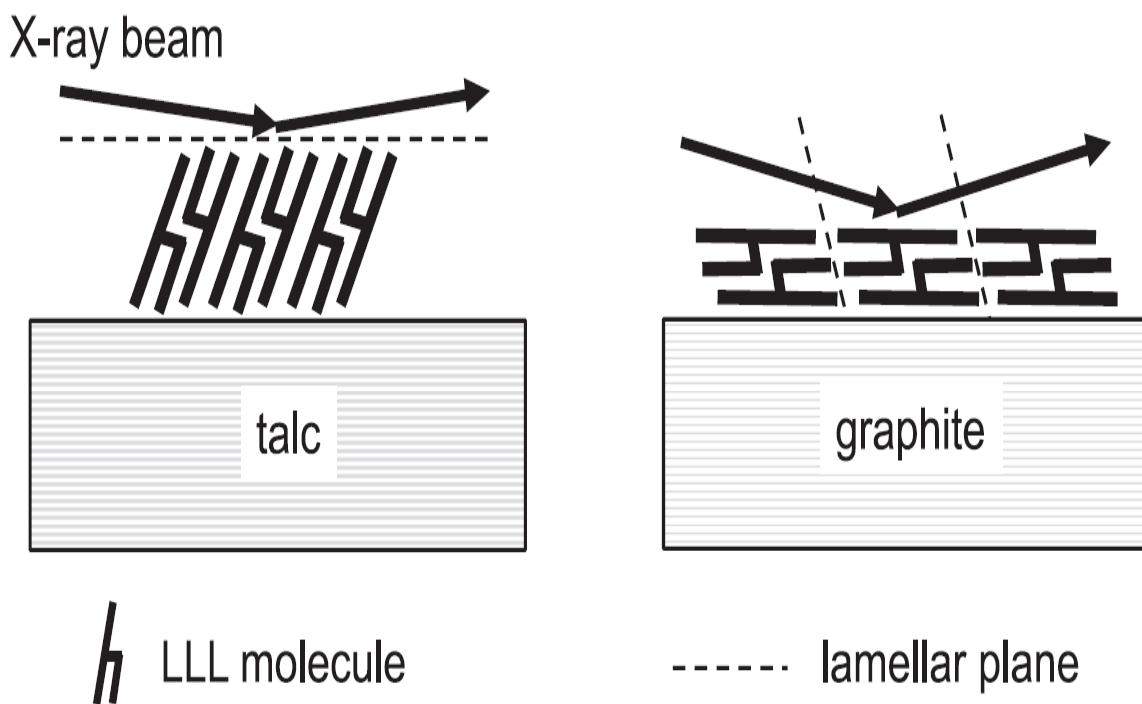


Figure 7. Effect of additive on lamellar orientation of LLL (Yoshikawa et al., 2014).

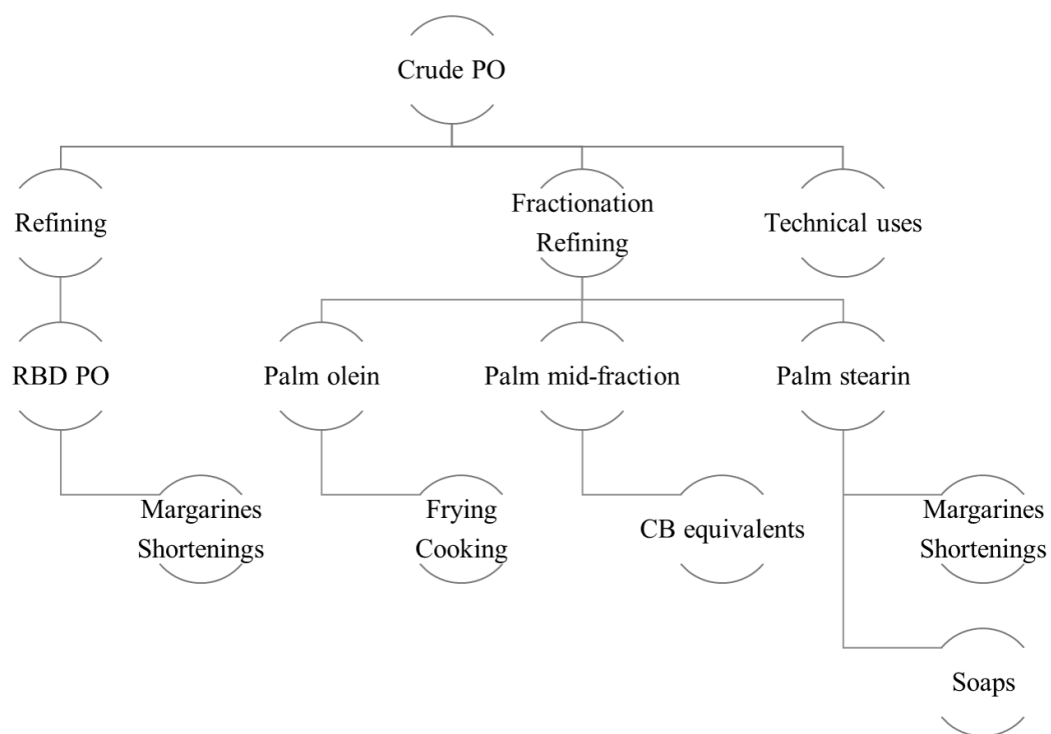


Figure 8. Uses of PO and its fractions (adapted from Basiron, 2001).

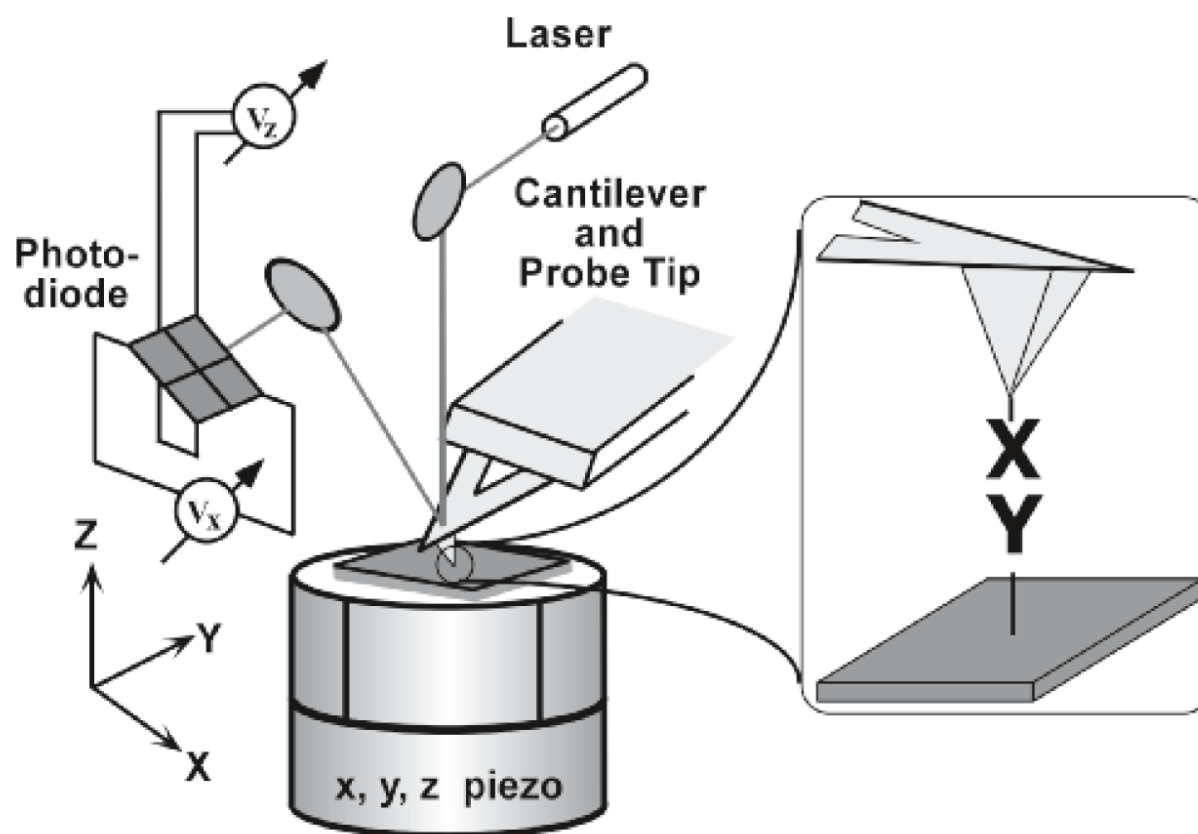


Figure 9. Schematic diagram of AFM (Noy et al., 2008).

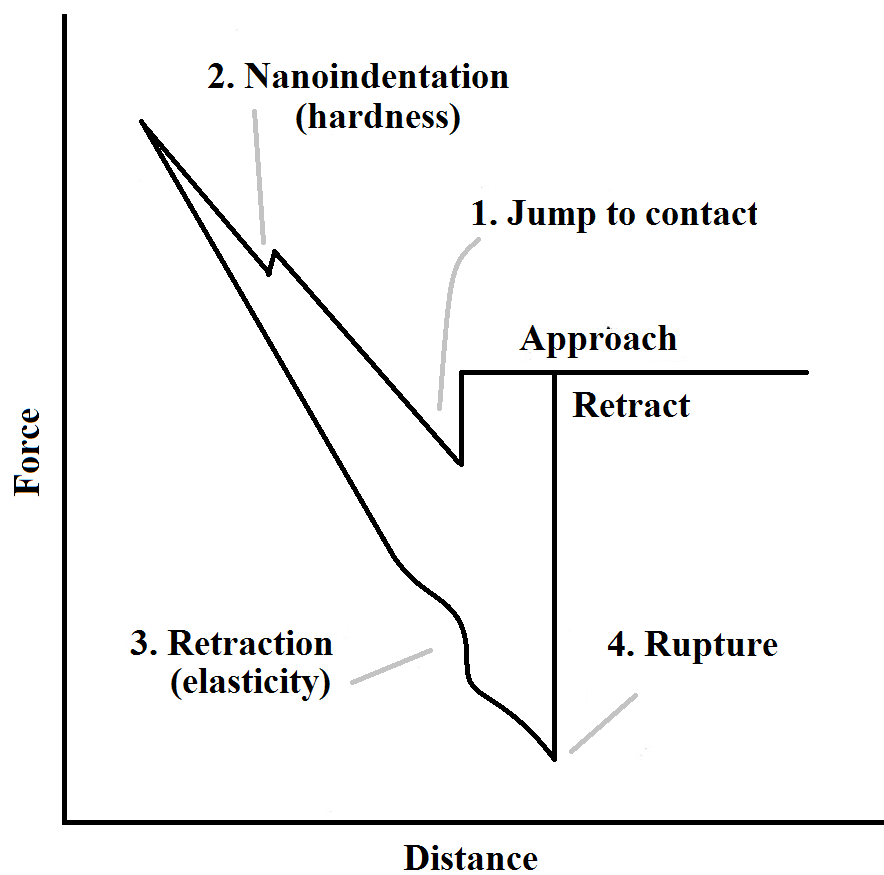


Figure 10. Interpretation of force-distance curves (adapted from Kronenberger, 2006).

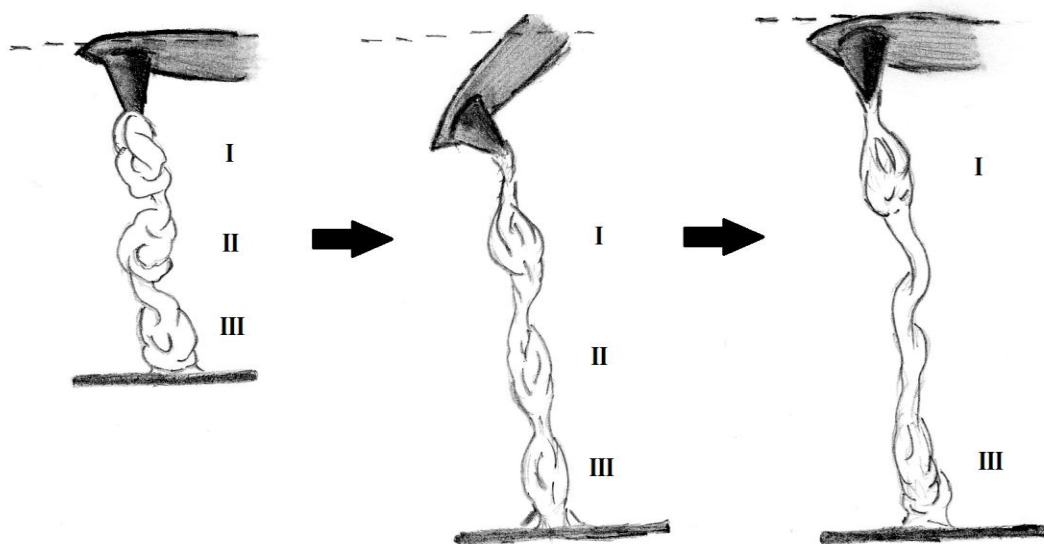


Figure 11. Dimension-dependent conformational analysis of a protein depicting the unfolding of conformation II (adapted from Carrion-Vazquez et al., 1999).