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Rheology and Crystallisation of

Filling Fats for Confectionery Products

Thesis submitted for the Degree of Doctor of Philosophy

Department of Food and Nutritional Sciences

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DECLARATION

I confirm that this is my own work and the use of all material from other sources has been properly and fully acknowledge.

Intan Cidarbulan Matita

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ABSTRACT

As the continuous phase in which other ingredients are distributed, filling fat plays an important role in determining the sensory attributes such as hardness, flavour release and texture of filled confectionery products. For this reason, knowledge on the rheological properties and crystallisation behaviour of filling fat is crucial. Two filling fats, which mainly differ in POP (1,3-dipalmitoyl-2-oleoyl-glycerol) and SOS (1,3-distearoyl-2-oleoyl-glycerol) triacylglycerol compositions, were used in this study. Rheological properties of the two filling fats, namely Creamelt 601 and Creamelt 701, were first studied using oscillatory rheology under static and slow cooling conditions to obtain genuine information about the crystallisation behaviour in which a viscoelastic system was evident for both filling fats. Oscillatory rheology was also used to simulate certain manufacturing conditions in which the filling fats are used to show the effects of processing conditions on the crystallisation behaviour of the filling fats. Application of shear during the cooling process at high and low temperatures indicated an enhancement of the crystallisation process and damage to the crystal aggregates respectively. Nevertheless, there was no evidence of a significant impact of the point at which shear was applied on the final rheological properties. The viscoelastic properties of the filling fats at every step of the processing conditions were assessed using an oscillation frequency sweep. Development of a weak crystal network was shown by a reduced frequency-dependent profile as soon as the filling fats reached the first crystallisation temperature. Similarly, the application of shear at higher temperatures showed enhancement of the crystallisation process by secondary nucleation and rearrangement of the crystal network whereas at lower temperatures, shear application may result in destruction of the network. In addition, other crystallisation techniques of analysis such as x-ray diffraction (XRD), differential scanning calorimetry (DSC) and polarised light microscopy (PLM) were also used to study the crystallisation behaviour of the filling fats.

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LIST OF ABBREVIATIONS

CBE	Cocoa butter equivalent
DSC	Differential scanning calorimetry
G'	Elastic modulus
G"	Viscous modulus
G*	Complex modulus
IV	Iodine value
LVR	Linear viscoelastic range
OOO	1,2,3-trioleoyl-glycerol
pNMR	Pulsed nuclear magnetic resonance
PLM	Polarised light microscopy
PLP	1,3-dipalmitoyl-2-linoleoyl-glycerol
PMF	Palm mid fraction
POP	1,3-dipalmitoyl-2-oleoyl-glycerol
POO	1-palmitoyl-2,3-dioleoyl-glycerol
PPO	1,2-dipalmitoyl-3-oleoyl-glycerol
PPP	1,2,3-tripalmitoyl-glycerol
PPS	1,2-dipalmitoyl-3-stearoyl-glycerol
POS	1-palmitoyl-2-oleoyl-3-stearoyl-glycerol
PSS	1-palmitoyl-2,3-distearoyl-glycerol
SFC	Solid fat content
SOS	1,3-distearin-2-oleoyl-glycerol
SOO	1-stearoyl-2,3-dioleoyl-glycerol
SSS	1,2,3-tristearoyl-glycerol
S-U-S	Saturated-unsaturated-saturated
TAG	Triacylglycerol
XRD	X-ray diffraction

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction

Coatings and fillings are two examples of applications in which confectionery fats are used. The coating is the component that surrounds the centre filling substance (Figure 1.1). The coatings and fillings in confectionery products mostly have the same purpose; both act as flavour contributors and continuous media that hold the other ingredients together (Vereecken *et al.*, 2007). Nevertheless, there are main requirements in which the coatings and fillings differ. Coatings must be firm, dry to the touch at ambient temperature and non-greasy. At mouth temperature, complete and quick-melting sensations are desirable for excellent flavour release and non-waxy aftertaste (Pease, 1985). In general, fillings must also have the quick-melting mouthfeel, fast flavour release and smooth aftertaste. Furthermore, the texture requirement for the fillings is different depending on the consumer product properties. Soft, medium and hard texture properties are commonly found in traditional fillings whereas an aerated texture is popular in modern fillings.
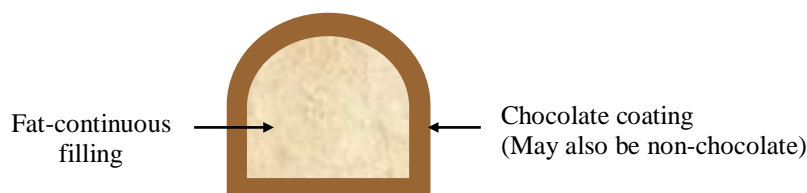
Figure 1.1 Illustration of coating and filling in chocolate-coated praline (Talbot and Slager, 2007)

Fillings can be prepared as a mixture of various ingredients. However, two main ingredients of filling formulations are vegetable fat and sugar. Fat content up to 60% can be found in the

filling although 30-35% is generally accepted (Talbot, 2008). In filled confectionery products, fillings are thought to have more influence on the properties of the final product than coatings because fillings are, in general, present at a higher percentage of the total product than the surrounding shell and also have a higher fat content. In a filled chocolate for example, the filling generally represents 60% of the total product, with 40% fat content, while the chocolate shell accounts for 40% of the total product, with 30% fat content (Talbot, 2009). As the continuous phase in which the other ingredients are distributed in, the filling fat plays an important role in determining the sensory attributes such as hardness, flavour release and mouthfeel of the final product. It was found that 1% fat addition to chocolate with 28% fat content decreases the plastic viscosity by half. The effect of fat addition is even stronger in chocolate with fat content below 23% (Afoakwa *et al.*, 2007). Filling fats are vegetable fat-based products that are frequently used in the confectionery industry, especially in chocolates, pralines, biscuits and wafers. The fats are made by blending two or more vegetable fats that have been previously hydrogenated and/or fractionated and refined. Confectionery fats were categorised by Talbot and Slager (2007) into two main groups, namely lauric and non-lauric, in which the latter was divided into temper and non-temper categories (Figure 1.2). Based on this classification, the two filling fats used in this study belong to the non-lauric, temper group.

```
                        ┌─────────────────────┐
                        │ Confectionery Fats  │
                        └─────────────────────┘
                  ┌──────────────┴──────────────┐
          ┌───────────────┐              ┌───────────────┐
          │  Non-lauric   │              │    Lauric     │
          └───────────────┘              └───────────────┘
```

Temper	Non-temper		

```
 ┌──────────────┐   ┌──────────────────┐      ┌──────────────────┐
 │ Cocoa butter │   │   Hydrogenated   │      │   Oils e.g.      │
 │              │   │   Fractionated   │      │   coconut oil,   │
 │              │   │   palm, soya, etc│      │   palm kernel oil│
 └──────────────┘   └──────────────────┘      └──────────────────┘

 ┌──────────────┐   ┌──────────────────┐      ┌──────────────────┐
 │ Fractionated │   │   Low-trans or   │      │   Hydrogenated   │
 │ palm, shea,  │   │   No-trans       │      │   palm kernel oil│
 │ etc          │   │   alternatives   │      │                  │
 └──────────────┘   └──────────────────┘      └──────────────────┘

                                              ┌──────────────────┐
                                              │   Fractionated   │
                                              │   palm kernel oil│
                                              └──────────────────┘
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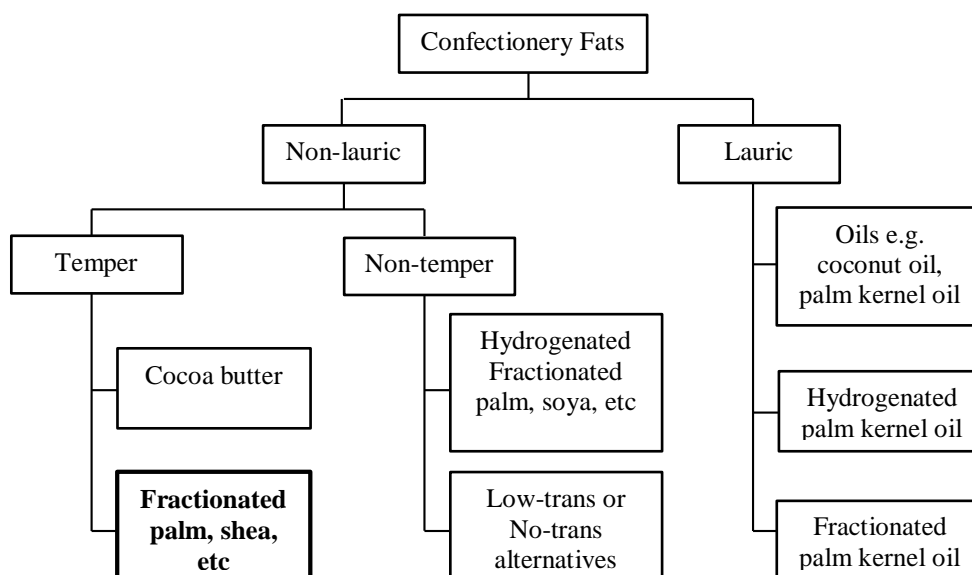
Figure 1.2 Confectionery fats classifications (Talbot and Slager, 2007)

Vegetable fats are commonly used in various confectionery applications to adjust the sensory characteristics of the end products that are strongly determined by the mechanical strength of the fat crystal network (Marangoni and Narine, 2002). Consequently, vegetable fats are considered as key ingredients in many confectionery products. Since almost all vegetable fats used in these products are present in a crystallised form, understanding of the crystallisation behaviour of the vegetable fats is crucial, as some adjustments during production processes might be needed when different types of vegetable fat with different crystallisation behaviour are handled. There are several techniques available for studying the crystallisation behaviour of fats, of which rheology is one, along with other well-known techniques like differential scanning calorimetry (DSC), pulsed nuclear magnetic resonance (pNMR), X-ray diffraction analysis (XRD) and microscopy. Rheology has been used to provide information at many levels from quality control to research and product development areas. During quality control, the specification for products leaving the factory commonly includes rheological characteristics. A good quality product will only lead to customer satisfaction if it behaves

satisfactorily during processing operations such as spreading, shearing, pouring or pumping. Rheology can be used to simulate these processing and post-processing conditions by selecting the relevant mode and rate of deformation. Thus, more efficient simulations of processing conditions on a small scale and inexpensive consumer testing can be achieved by rheological testing.

1.2 Confectionery Filling Fat

Confectionery filling and coating fats mostly fall in the same overall category. Filling fat, however, is generally softer than a coating fat. Traditionally, butter and cream were used in manufacturing of confectionery products like toffees and caramels. However, they are not the most convenient materials to use as they require particular storage condition and water content removal (Stewart and Timms, 2002). Today, the types of vegetable fat used for confectionery products, such as fillings, widely range from 100% liquid oils like hazelnut oil to hard fats like cocoa butter. The filling fat is made by blending two or more vegetable fats that have been previously hydrogenated and/or fractionated and refined. Along with palm kernel oil and fractionated palm oil, blends of hazelnut oil and cocoa butter (with 60:40 or 80:20 hazelnut oil:cocoa butter ratio) are found in typical filling fats composition (Talbot, 2009). In a report on a study of crystallisation behaviour, microstructure and macroscopic properties of filling fats, Vereecken *et al.* (2007) mentioned that the general requirements for a typical filling fat include quick melting behaviour, absence of sandiness and enough solid fat content (SFC) to prevent fat migration. Cocoa butter is certainly an excellent choice for many confectionery applications due to its sharp melting profile. It solidifies at room temperature and melts completely at body temperature. Considering the expensive price and

the inconsistent availability of good quality cocoa butter, confectionery manufacturers often take the option of using replacements or extenders of cocoa butter using other vegetable fats sources like palm and shea (Figure 1.3). Commercially available vegetable fats that have similar triacylglycerol composition to that of cocoa butter are known as cocoa butter equivalents or CBE (Gunstone, 2011).

Figure 1.3 Palm fruit (left) and shea nut (right)

1.2.1 Types of Filling Fat

With increasing demand for different types of fillings, confectionery manufacturers are trying to be innovative in producing better products. Consequently, manufacturers strive for the most suitable fat for different types of applications, which will fulfil customers' requirements. Talbot (2008) categorised filling fats into three different types, namely polymorphic (temper) fat, non-polymorphic (non-temper) non-lauric fat and lauric fat. Nevertheless, classification of filling fats is not restricted to this approach as other methods of classification may be used. Filling fats can also be grouped based on their application as categorised by Wennermark (1992) and Mohos (2010). The polymorphic type of filling fat is widely used for confectionery fillings due to its performance similarity and compatibility with cocoa butter or CBE. They are based on similar triacylglycerol compositions and exhibit similar polymorphic

behaviours, reducing the occurrence of a softening effect caused by fat migration from the filling into the shell. Also, with increasing market requirements of *trans*-fat removal, this type of filling fat is based on non-hydrogenated fat. Figure 1.4 shows the measured solid fat content of cocoa butter, CBE and soft CBE and an interpretation of the SFC curve in regards to some sensory properties such as hardness, heat resistance and waxiness (Torbica *et al.*, 2006). Non-polymorphic, non-lauric filling fat is frequently used in products requiring aeration. This is due to the fact that the partially hydrogenated and often fractionated non-lauric oils have an excellent ability to retain the air held inside the filling as crystallisation takes place. Coconut and palm kernel oil are the two most common oils rich in lauric acid. When used in conjunction with cocoa butter, in fillings as well as in coatings, compatibility must be checked to ensure that the manufacturer is fully aware of the possibility of fat migration and eutectic formation which will lead to blooming and softening of the product.

(A)   (B)
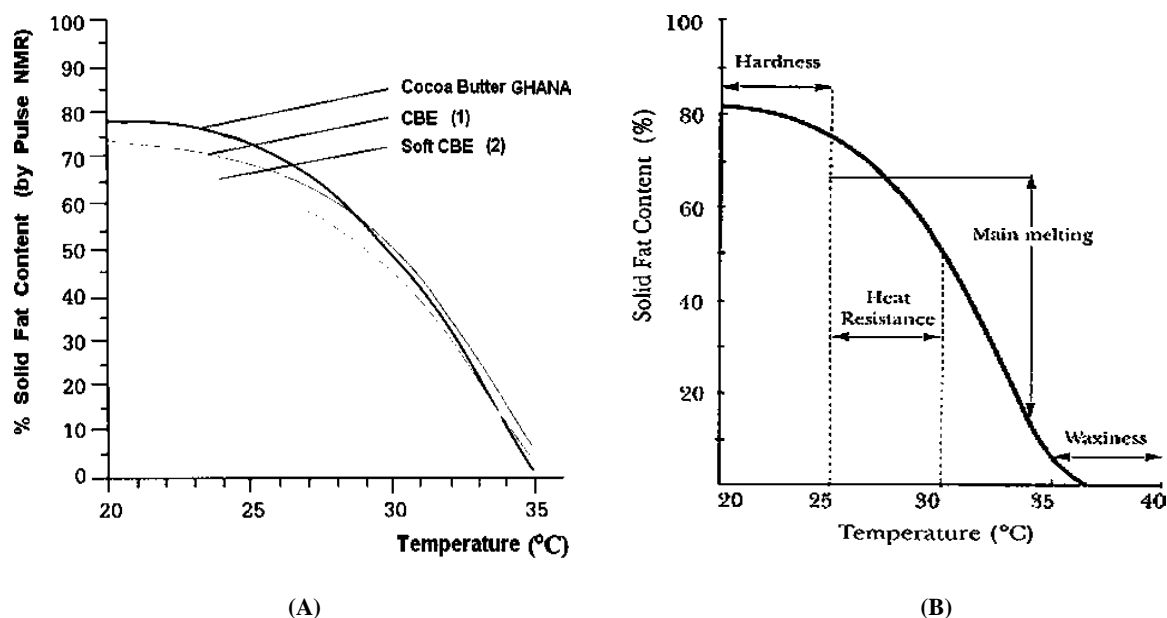
Figure 1.4 Solid fat content (SFC) curves of cocoa butter, cocoa butter equivalent (CBE) and soft CBE (A); interpretation of SFC curve in regards to some sensory properties (Torbica et al., 2006)

1.2.2 Production of Filling Fat

Confectionery fats especially chocolates have, historically, been based mostly on cocoa butter and milkfat (Stewart and Timms, 2002). Cocoa butter is extracted from the cocoa bean by a number of processing steps including fermentation, drying, roasting, peeling, grinding and pressing (Gordon, 1994). In chocolate application, particularly milk chocolate, most of the cocoa butter is deodorised to sterilise the fat and remove any unwanted flavours (Stewart and Timms, 2002). Since milkfat is incorporated into confectionery products such as powder or crumb, it is processed by spray drying a pre-concentrated milk to produce milk powder or creating a mixture of cocoa mass (cocoa liquor obtained from cocoa beans) and sweetened condensed milk and drying the mixture (Shukla, 1994). Today, both cocoa butter and milkfat are still present in many confectionery fillings. However, the use of vegetable oil sources like palm oil as confectionery fats has been increasing for many years. For use in confectionery products, quick melting behaviour, stability and reasonably hard consistency of fats are three main requirements that can be achieved by using fats containing shorter chain fatty acids, or by fat modification processes (Minifie, 1980). These techniques were principally applied to expand the application of vegetable fats in confectionery products. The production of vegetable fat based confectionery fats principally involves blending, fractionation and/or hydrogenation as modification tools before the refining process. Fractionation of palm oil was traditionally done to obtain stearin and olein fractions. Today, intermediate fractions of palm oil known as palm mid fraction (PMF) are obtained and commonly used in the production of typical palm-based CBE as seen in Figure 1.5 (Traitler and Dieffenbacher, 1985). PMF can be obtained through double fractionation of palm oil (Pease, 1985). In combination with 20-30 % shea oil fraction, PMF is suitable to be used in chocolate production (Gunstone, 2011).

Palm Oil   Vegetable Tallow

↓   ↓

Degummed   Degummed

Neutralized   Neutralized

Bleached   Bleached

↓   ↓

Fractionated   Fractionated

Mixed

↓

Deodorized

↓

CBE
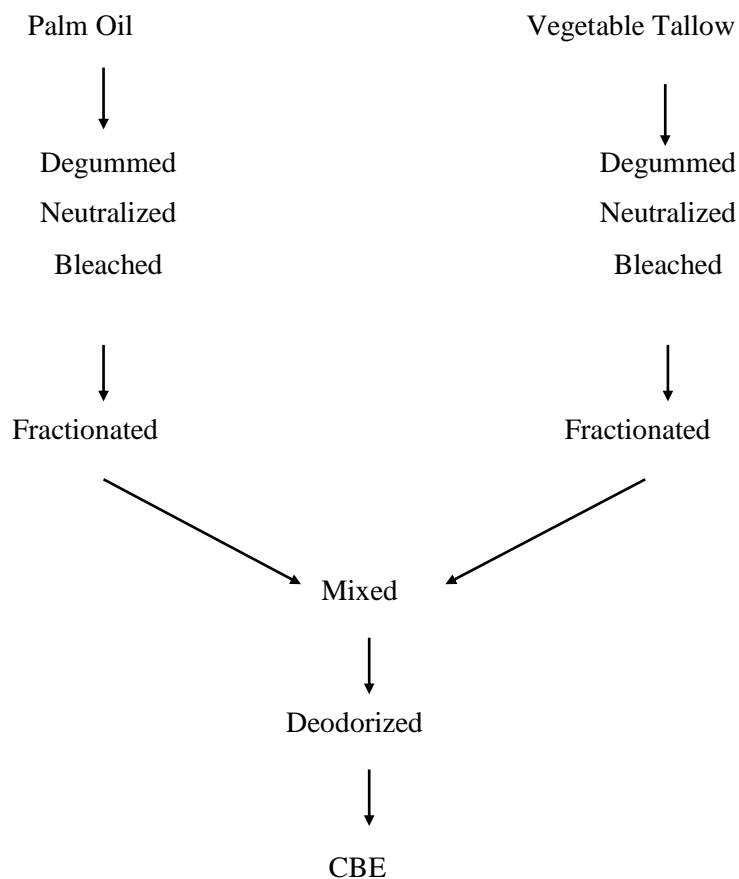
Figure 1.5 Production of a typical palm-based cocoa butter equivalent (CBE) (Traitler and Dieffenbacher, 1985)

1.2.2.1 Modification

Depending on the application, vegetable fat suppliers often have to modify the physical characteristics of the fats to suit various applications. Modification can be done through several methods, namely blending, fractionation, inter-esterification and hydrogenation. Blending is certainly the cheapest method available and widely used in many applications. Conversely, hydrogenation and inter-esterification are considered to be rather costly methods due to the capital investment required and the cost of catalyst and hydrogen. These former method is used to alter the melting profile of the fat by the addition of hydrogen to carbon-carbon double bonds in unsaturated fatty acids while the latter involves interchange of the

esterified fatty acids using catalyst (Dijkstra, 2002; Huyghebaert *et al.*, 1994). Fractionation is another widely used method to separate the fats or oils into two or more mixtures of glycerides, creating two new products, an olein and a stearin, that are considerably different from the starting material. Stearin is the higher melting fraction, which is separated once it is crystallised from the melt. Conversely, the olein is the lower-melting fraction that can also be used for a further fractionation process (Rajah, 1994). The two filling fats used in this study are modified using fractionation and blending methods. Figure 1.6 shows a general diagram of typical palm oil products from a fractionation process.

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                        ┌─────────────┐
                        │  Palm Oil   │
                        │  IV 51-53   │
                        └──────┬──────┘
              ┌────────────────┴────────────────┐
       ┌──────┴──────┐                   ┌───────┴──────┐
       │   Olein     │                   │ Hard Stearin │
       │  IV 32-36   │                   │  IV 32-36    │
       └──────┬──────┘                   └───────┬──────┘
        ┌─────┴─────┐                      ┌──────┴──────┐
  ┌─────┴────┐ ┌────┴────┐           ┌─────┴────┐  ┌─────┴─────┐
  │Super Olein│ │Soft PMF │           │Soft Stearin│ │Super Stearin│
  │ IV 64-66  │ │IV 42-48 │           │ IV 70-72  │  │ IV 17-21   │
  └─────┬────┘ └────┬────┘           └──────────┘  └───────────┘
  ┌─────┴────┐ ┌────┴────┐
  │ Top Olein│ │Hard PMF │
  │ IV 70-72 │ │IV 32-36 │
  └──────────┘ └─────────┘
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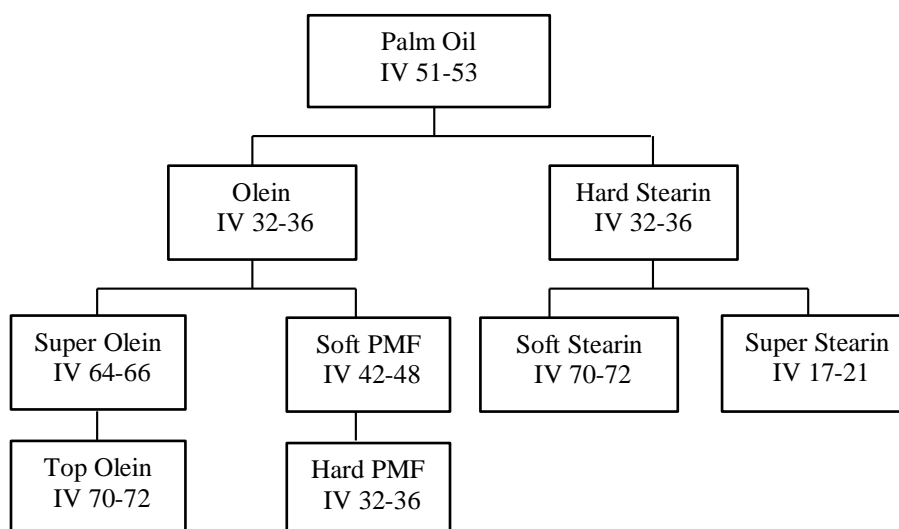
Figure 1.6 General diagram of palm oil fractions with iodine value (IV) as an indication of unsaturation degree of fatty acids. The higher the IV, the softer the fat and vice versa (Hernandez and Huertas, 2005)

1.2.2.2 Refining

Once the desired specifications are achieved, different fat fractions are blended and refined to remove some of undesirable components and impurities such as moisture, free fatty acids, metals, odoriferous substances and colours. For crude vegetable oil like palm oil, two methods of refining can be used, namely physical refining and chemical refining. The main

difference between the two methods lies in the manner of free fatty acids removal (Basiron, 2005). In chemical refining, the free fatty acids are removed through the neutralisation process. Flow diagrams of both methods are illustrated by Basiron (2005) as seen in Figure 1.7. The filling fats used in this study were produced by the manner shown in Figure 1.8. The purpose and procedure of each step is summarised in Table 1.1.

Table 1.1 Processing of a typical confectionery filling fat (Werleman, 2013a)

Refining steps	Purpose	Procedure
Neutralisation	Free fatty acids removal	Addition of sodium hydroxide to warm oil to form water soluble soap
		Soap stock drainage and washing off oil with hot water to remove any trace of soap
		Water removal from the oil by drying
		Addition of Citric acid or phosphoric acid solution to preheated oil (90-95°C)
Bleaching	Colour components, trace metals, phospholipids, polycyclic aromatic hydrocarbon, and residual soap removal	Mixing for 15 min before further addition of 0.15% water under rapid stirring for 15 min
		Addition of 0.35-0.5% bleaching earth and mixing for 20 min
		Oil drying at 100 mbar pressure for 30-45 min preceding filtration of the bleaching earth
Deodorisation	Volatile impurities removal	Required temperature: 180-230°C
		Under vacuum (2-6 mbar) and steam for agitation
		Run for 90-150 min
		Cooling to 40-60°C by passing it through and external heat exchanger and saturating it with nitrogen during transfer to storage tank
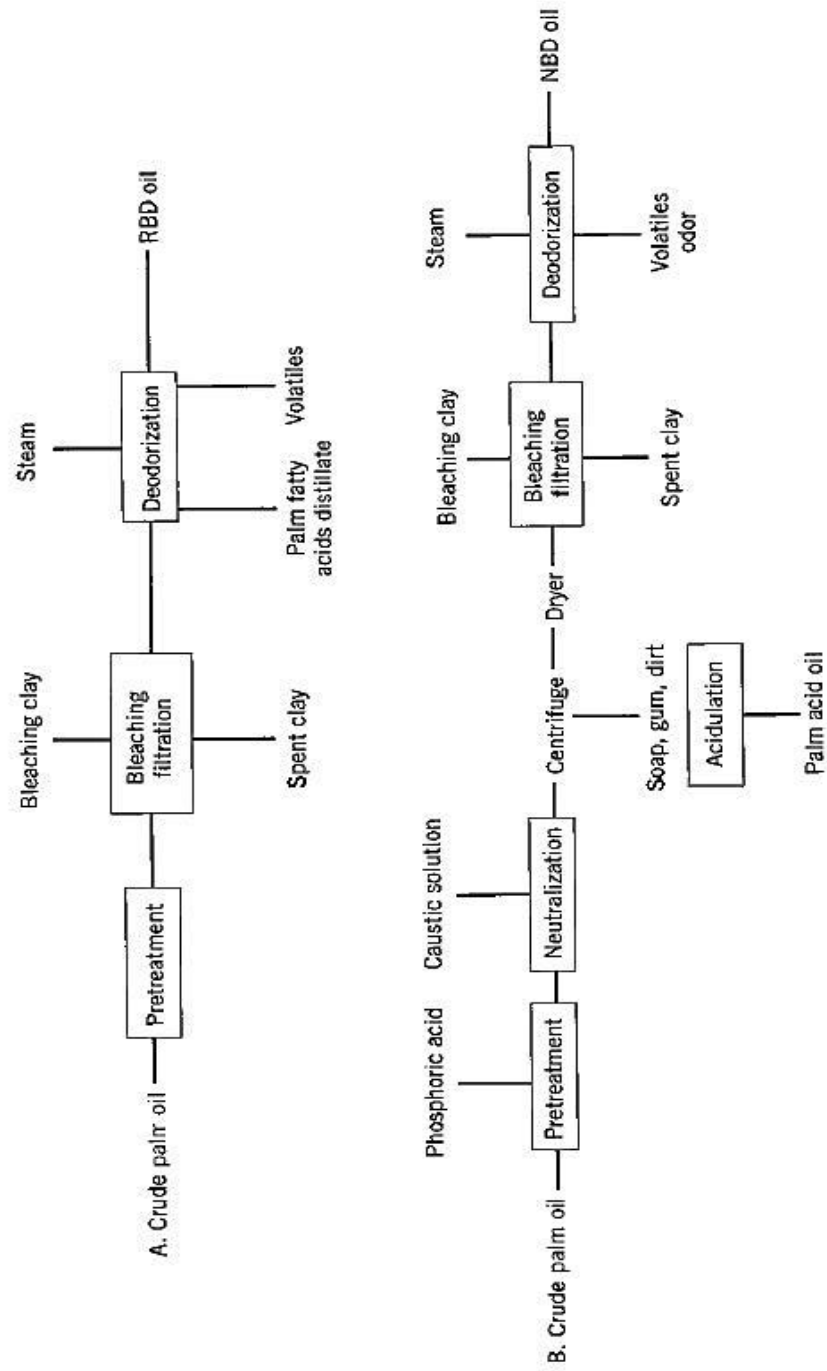
Figure 1.7 Flow diagrams of physical (A) and chemical (B) refining methods of crude palm oil (Basiron, 2005)
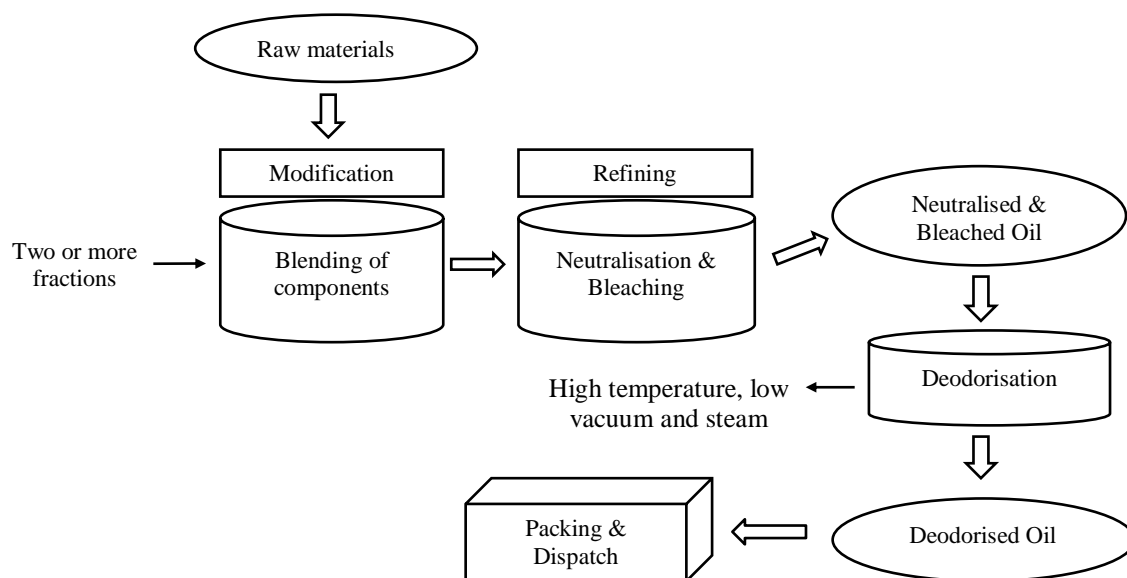
Figure 1.8 Schematic of general manufacturing process of confectionery filling fat (Werleman, 2013a)

1.3 Basic Properties of Fat Crystal Network

Fat crystallisation is known to be rather complex due to the molecular composition and large number of combinations in the arrangement of the three fatty acids on the glycerol molecule. The physical properties of a triacylglycerol molecule are simplest when the same fatty acids are attached to all the three positions (monoacid triacylglycerols). Complexity increases when two or more different fatty acid chains are attached (di- or tri-acid triacylglycerols) resulting in at least two distinct isomers. In addition, the natural ability of triacylglycerol molecules to crystallise into different crystal forms, depending on the processing conditions, also affects the crystallisation behaviour (Ueno *et al.*, 1997). In the confectionery industry, sensory attributes of the end products are determined by the underlying fat crystal network. Narine and Marangoni (2005) developed a schematic structural hierarchy of fat crystal networks to provide insight into the interrelationship between factors like composition, processing conditions, structure and mechanical properties during crystal network formation (Figure 1.9).

Rheology, Mechanical Strength, Sensory → **Fat**

Macroscopic >0.2mm

Crystal Network

Solid Fat Content →

↑ Heat, mass, momentum transfer

Microstructure 0.25-200μm

Crystal Clusters

↑ Heat, mass, momentum transfer

Polymorphism →

Crystals

↑ Heat, mass, momentum transfer

Nanostructure 0.4-250nm

Molecular Structure →
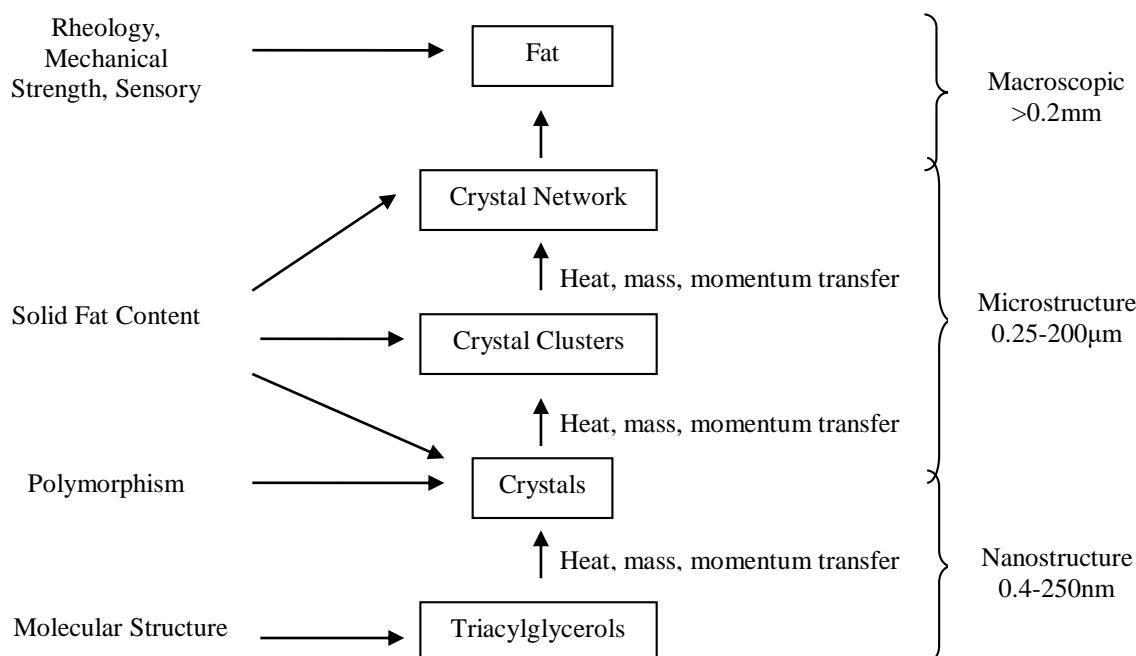
Triacylglycerols

Figure 1.9 Schematic structural hierarchy of fat crystal network (Narine and Marangoni, 2005)

1.3.1 Triacylglycerols (TAGs)

Complex triacylglycerol mixtures are naturally present in fats. TAGs are esters formed from a single glycerol molecule, which has three alcohol (-OH) functional groups combined with three fatty acids (Figure 1.10). Different TAG molecules contain different fatty acids and variations in how these fatty acids are positioned on the glycerol backbone. TAG that contains long carbon chain fatty acids with no double bonds are highly saturated and high in melting point due to close and uniform packing between the individual chains in the crystal network. Conversely, shorter chains or carbon chains that contain double bonds, which are unsaturated, are lower in melting point. Figure 1.11 shows the molecular structure of some saturated and unsaturated fatty acids. The melting points of these fatty acids and pure TAGs are provided in Table 1.2 and Table 1.3. Different length and degree of saturation of the fatty acids will influence the melting and crystallisation properties, which in turn determine the end product quality.

$$\underset{\substack{CH_2-OH \\ | \\ CH_2-OH \\ | \\ CH_2-OH}}{} + \underset{\substack{OH-\overset{O}{\overset{||}{C}}-C_nH_{2n+1} \\ OH-\overset{O}{\overset{||}{C}}-C_nH_{2n+1} \\ OH-\overset{O}{\overset{||}{C}}-C_nH_{2n+1}}}{} \longrightarrow \underset{\substack{CH_2-O-\overset{O}{\overset{||}{C}}-C_nH_{2n+1} \\ | \\ CH_2-O-\overset{O}{\overset{||}{C}}-C_nH_{2n+1} \\ | \\ CH_2-O-\overset{O}{\overset{||}{C}}-C_nH_{2n+1}}}{} + 3\,H_2O$$

Glycerol   +   3 Fatty acids $\longrightarrow$ Triglyceride   +   3 H_2O
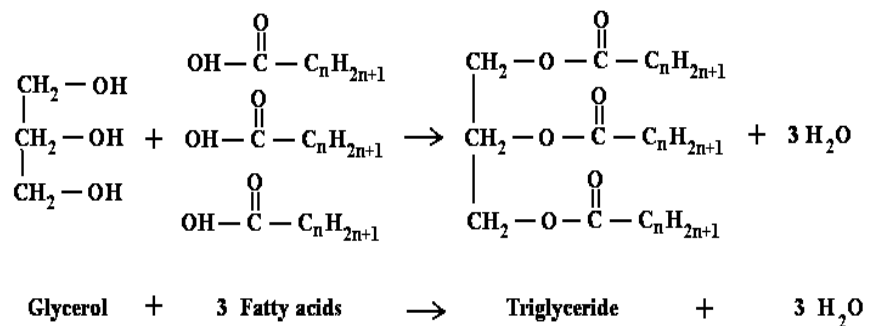
Figure 1.10 Structure and formation of triacylglycerols (Matusz, 2009)

Lauric acid

Palmitic acid

Stearic acid

Oleic acid

Linoleic acid
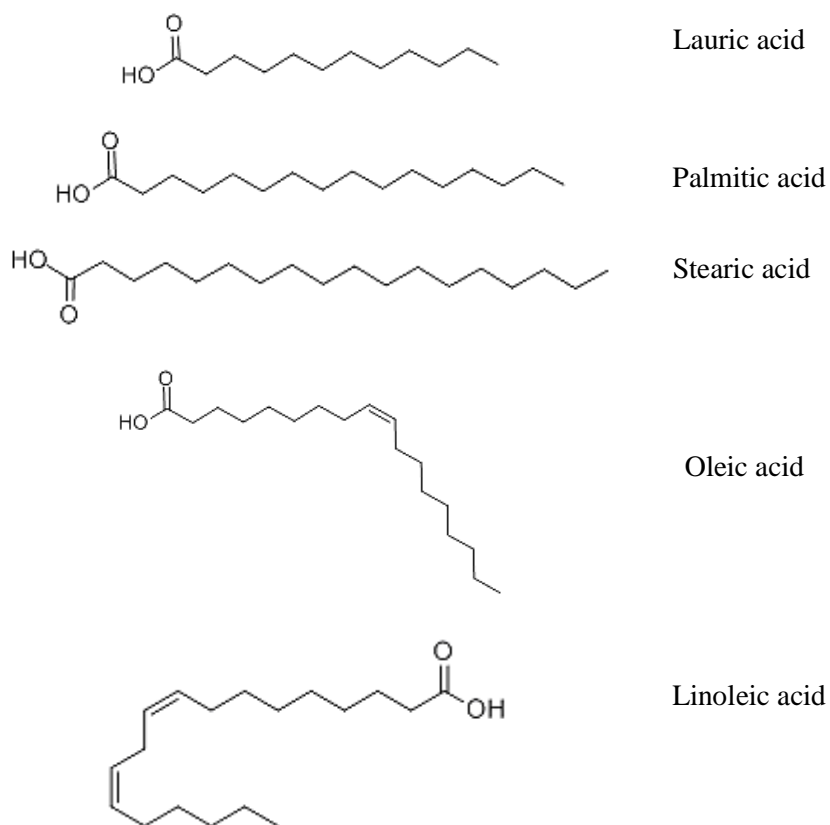
Figure 1.11 Molecular structure of some saturated and unsaturated fatty acids (ChemicalBook, 2008)

Table 1.2 Melting points of common saturated and unsaturated fatty acids (ChemicalBook, 2008)

Saturation	Fatty acid	Chain	Melting point [°C]
Saturated	Lauric	C12:00	44-46
	Palmitic	C16:00	61-62.5
	Stearic	C18:00	67-72
Unsaturated	Oleic	C18:1	13-14
	Linoleic	C18:2	-5

Table 1.3 Melting points of some pure triacylglycerols (Stauffer, 1996; Talbot, 2009)

Triacylglycerol	Abbreviation[*]	Melting point [°C]
1,2,3-tripalmitoyl-glycerol	PPP	66
1,2,3-tristearoyl-glycerol	SSS	73
1,2,3-trioleoyl-glycerol	OOO	5
1-palmitoyl-2,3-dioleoyl-glycerol	POO	18
1,3-dipalmitoyl-2-oleoyl-glycerol	POP	37
1-palmitoyl-2-oleoyl-3-stearoyl-glycerol	POS	37
1-stearoyl-2,3-dioleoyl-glycerol	SOO	23
1,3-distearin-2-oleoyl-glycerol	SOS	43

[*]P = palmitic acid (C16:0), S = stearic acid (C18:0), O = oleic acid (C18:1, cis)

POP (1,3-dipalmitoyl-2-oleoyl-glycerol), POS (1-palmitoyl-2-oleoyl-3-stearoyl-glycerol) and SOS (1,3-distearin-2-oleoyl-glycerol) are the three main TAGs present in cocoa butter and CBE components. Table 1.4 presents the content of these main TAGs in common fats in comparison to the two filling fats used in this study, namely Creamelt 601 and Creamelt 701. As a palm- and shea-fraction based filling fat, it is normal to expect the TAG profiles of Creamelt 601 and Creamelt 701 to reflect a combination of the pure palm and shea fractions depending on the formulation. The higher content of POP in Creamelt 701 indicates the presence of more palm fraction component in the formulation, while the higher SOS content in Creamelt 601 indicates the greater amount of shea fraction component.

Table 1.4 Main triacylglycerol compositions [%] of different confectionery fats

Triacylglycerol	Cocoa butter[*]	Palm mid fraction[*]	Shea butter[*]	Shea stearin[*]	Fractionated palm oil filling fat[**]	Creamelt 601[***]	Creamelt 701[***]
POP	16	66	3	1	47	41	55
POS	37	12	6	7	9	9.5	10
SOS	26	3	42	74	n/a	15	4

*Gunstone (2011); **Vereecken *et al.* (2007); *** Werleman (2012)

Bell et al. (2007) studied the effects of trans fatty acids on the crystallisation behaviour of different fat blends used in commercial shortenings that may consequently affect the performance of bakery products. Upon shear crystallisation, the viscoelastic behaviour of the fat blends changed from viscoelastic solid to viscoelastic liquid. The rate of change was faster for a fat blend with high trans fatty acid content compared to the low-trans one. This is seen from the rheological frequency sweep data in Figure 1.12, where frequency dependent behaviour (as an indication of a viscoelastic liquid system) was exhibited by a high-trans blend by the time the crystallisation temperature (31°C) was reached. According to Pajin *et al.* (2007), the effect of *trans* fatty acids in the fat composition on functionality of final products was also seen in confectionery product application. The crystallisation rates of three filling fat samples used in nougat fillings were studied using dynamic rheological measurement. The crystallisation onset at around 17°C was observed with a rapid increase in viscosity values (Figure 1.13). Although the onset temperatures were similar, steeper viscosity increase was seen in *trans*-containing filling fats with higher solid fat content (S1 and S2) and a more gradual increase was seen for low-*trans* lauric filling fat (S3). These mentioned studies emphasised the significance of TAG composition in the achievement of the desired product quality.
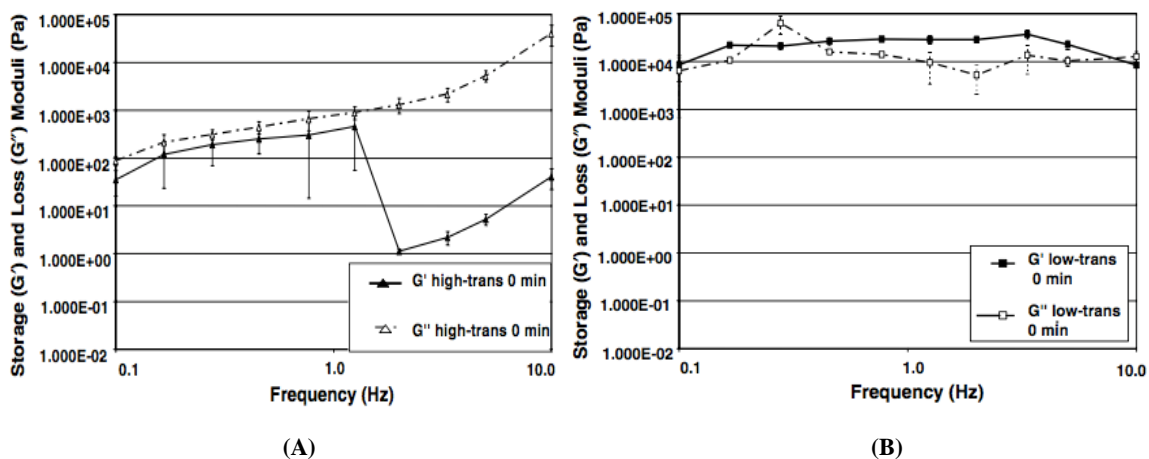
Figure 1.12 Frequency sweep of high- (A) and low-*trans* (B) fat blends crystallised at 0 min of stirring at 31°C. Sudden drop in elastic modulus (G') (A) may be caused by liquid slip between large number of small crystals (Bell et al., 2007)
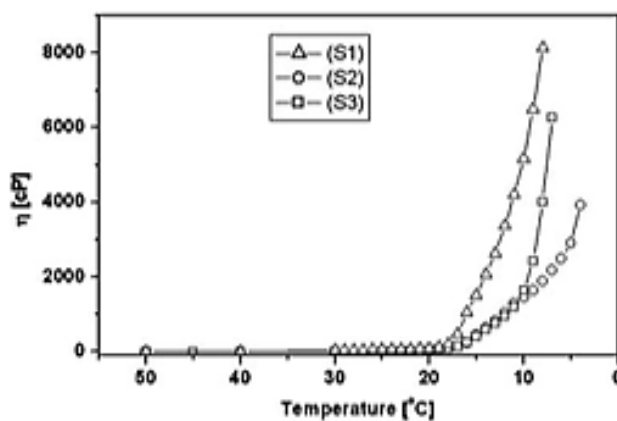
Figure 1.13 Crystallisation rate of *trans* (S1 and S2) and low-*trans* lauric (S3) filling fats as measured by dynamic rheological measurement of apparent viscosity (ŋ) and temperature (Pajin et al., 2007)

1.3.2 Crystallisation Mechanisms

Triacylglycerol molecules are free to rotate around each single bond in the liquid state. The formation of ordering with nearby chains results in crystallisation of the triacylglycerol molecules. This physical change occurs via two main processes namely nucleation and crystal growth. The crystallisation behaviour of fats is of industrial significance in the

processing of fat crystal based products and in the separation of fats with different chemical and physical properties from raw materials (Sato, 2001). Due to the industrial importance, there have been many studies on the crystallisation behaviour of fat materials. As described by Narine and Marangoni (1999a), development of a fat crystal network starts with nucleation of triacylglycerol molecules into a certain polymorphic form followed by continuous crystal growth and aggregation that results in larger microstructures. Growth of a crystal network is completed when a permanent three-dimensional network has been successfully created. A mechanism for nucleation of the TAGs was proposed by Metin and Hartel (2005) as illustrated in Figure 1.14. When nucleation starts, formation of the crystalline phase from the liquid, results in organisation of the molecules. In a lipid system, rapid cooling of the liquid phase leads to the formation of a less stable polymorph caused by the structural organisation in the liquid phase. Under slow cooling conditions, there is sufficient time to allow the molecules to arrange into a more ordered structure of a more stable polymorph. In either case, the lamellae formed eventually create a three-dimensional crystal network. This crystal network formation is influenced by both the lipid composition and the crystallisation conditions, such as cooling rate, crystallisation temperature and agitation (Metin and Hartel, 2005).

Time
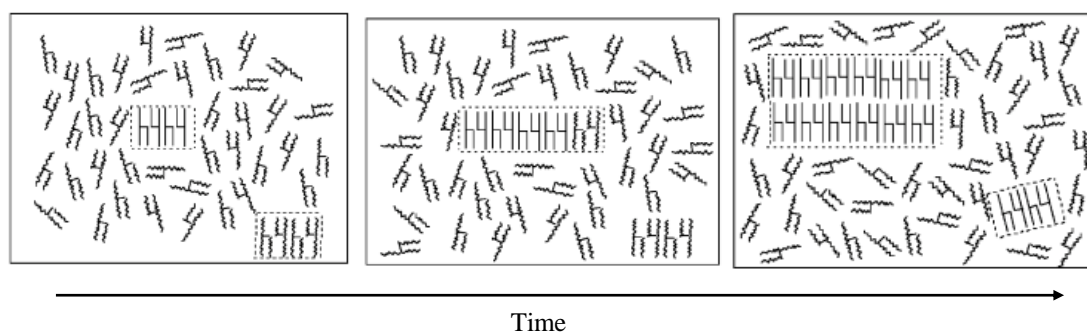
Figure 1.14 Proposed mechanism for nucleation of triacylglycerols (TAGs) by Metin and Hartel (2005), where straight chains indicate crystallised TAGs and bent chains indicate fluid TAGs

1.3.2.1   Nucleation

The nucleation process or formation of the initial crystalline state from the liquid melt appears due to ordering of the molecules into a crystal network. At a certain temperature, the smallest formed crystalline phase is called the nucleus (Metin and Hartel, 2005). These small crystals will continuously melt and reform until the interaction energy between molecules overcomes the Brownian effects to finally form stable nuclei with a specific arrangement (Narine and Marangoni, 1999a). Figure 1.15A shows formation of nuclei from the isothermal crystallisation of palm oil at around 18°C (Chen *et al.*, 2002). The nucleation process started with primary nucleation followed by secondary nucleation. Two types of primary nucleation mechanism, namely homogenous and heterogeneous nucleation, may occur. Nucleation is mostly stimulated by the presence of foreign particles (heterogeneous) such as crystal seeds or dust that reduces the surface energy, providing an arrangement for nucleation. Without foreign surface interruption (homogeneous), the nucleation process is slow and hardly seen. Simultaneously, secondary nucleation occurs as the microscopic crystalline elements detach from the surface of the crystallising components (Metin and Hartel, 2005).

1.3.2.2   Crystal growth

At this stage, TAGs from the fat melt start to arrange and integrate into existing crystals or nuclei. These crystals will then grow and aggregate into clusters of larger microstructures until a three-dimensional network is formed (Figure 1.15B). Under stationary conditions, fat molecules meet each other due to Brownian motion and aggregate as a result of Van der Waals forces (Kloek, 1998). The crystal growth is affected by three variables, namely degree of supercooling, rate of molecular dispersion into the crystal surface and time needed for incorporation of TAG molecules into the growing crystal network. Crystal growth of a

multicomponent fat is considered to be particularly low due to the rather small supersaturation degree for each TAG molecule and the competitive environment of similar molecules competing for the same position in the network. Timms (1994) explained that with a rapid cooling rate crystals will grow relatively slowly, forming a large number of smaller and less perfect crystals due to the faster rate of molecular dispersion into the crystal surface with new molecules attaching to existing surfaces despite not being perfectly aligned.

(A)   **(B)**
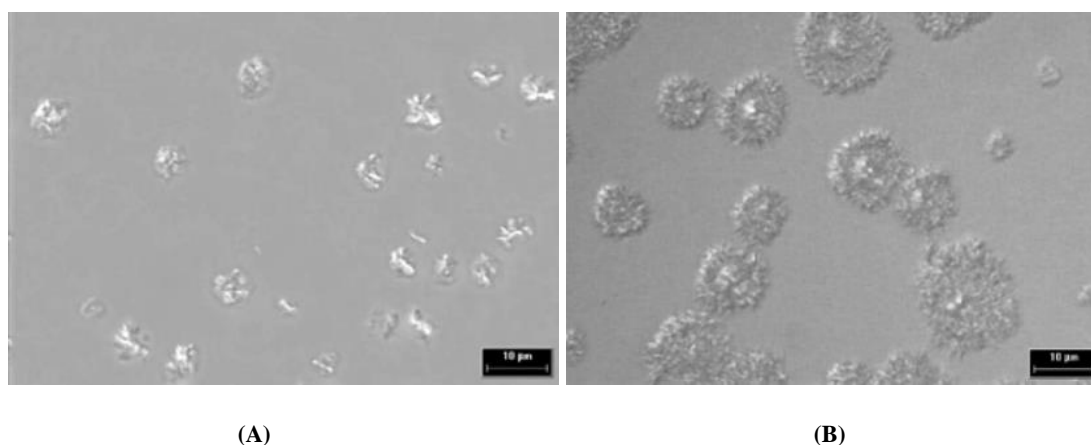
Figure 1.15 Microscopic images of nucleation (A) and crystal growth (B) stages during isothermal crystallisation of palm oil at around 18°C. The bar represent 10µm (Chen et al., 2002)

Another crystallisation mechanism was proposed by Timms (2007) in which post-growth events are described (Figure 1.16). As nucleation and growth occur simultaneously, smaller crystals that are unstable will re-dissolve during the ripening stage. This stage continues for an indefinite period until larger and more stable crystals exist in the supersaturated liquid. These crystals will, then, agglomerate with each other and form clusters of spherulitic crystals. At the end of the crystallisation process, separation between the solid fat (stearin) and liquid oil (olein) was seen. The ripening process was also mentioned in the proposed crystallisation mechanism by Hartel (2008) as a recrystallisation stage that often occurs

during storage. A generalised diagram, showing the transition from liquid to crystalline state was illustrated by Hartel (2008) as shown in Figure 1.17.
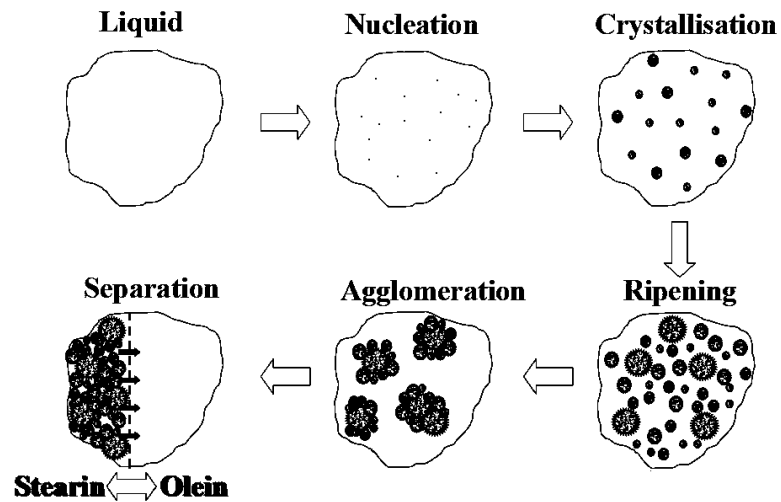
Figure 1.16 Fat crystallisation mechanism as proposed by Timms (2007). Dark-coloured spheres represent crystals that nucleate, grow and agglomerate from the melt (illustrated as white surrounding) to form a crystal network
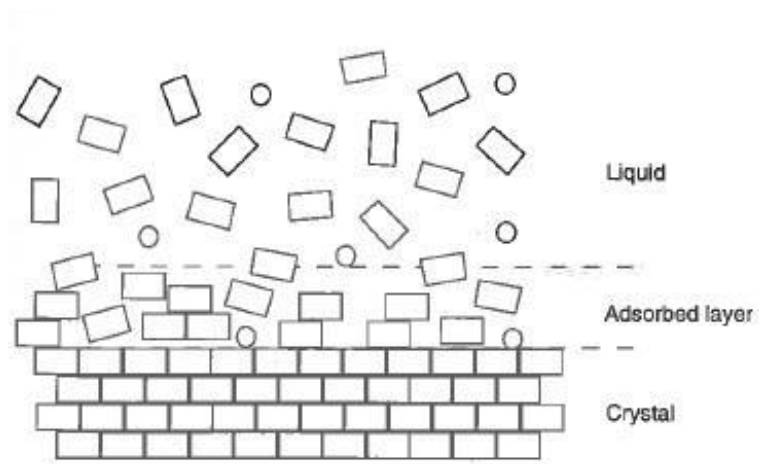
Figure 1.17 Schematic representation of a growing crystal surface with squares and circles to represent simultaneous aggregating and nucleating crystals, respectively (Hartel, 2008)

1.3.3 Polymorphism

Fats exhibit polymorphism due to their ability to form different crystal forms. Other than phase behaviour and rheological properties, polymorphism is one of the three factors that contribute to the physical characteristics of a fat-based food product. Influenced by the processing conditions and based on the chain packing structure, fat crystals typically exist in three principal polymorphs namely α, β' and β. These may exhibit sub-forms having minor differences. Thus, cocoa butter exhibits more than four polymorphs. Koyano and Sato (2002) summarised the main physical properties of each polymorph according to stability, density, melting point and morphology (Table 1.5). Melting point, density and stability are directly related to each other; melting point and density increase as the stability of the polymorph increases. Depending on the crystallisation conditions, the more stable polymorphs usually develop as a result of transformation from the less stable ones. During processing, polymorphic transformation can be forced by manipulating conditions such as tempering and shearing (Himawan *et al.*, 2006). All three polymorphs may also form directly from the melt. When this happens, crystallisation of these polymorphs is determined by the nucleation rate, which is inversely proportional to the thermal stability of the crystals. Each polymorphic form is classified by short-spacing data from X-ray diffraction analysis.

Table 1.5 Main physical properties of typical polymorphs (Koyano and Sato, 2002)

Polymorph	Stability	Density	Melting point	Morphology
α	Least stable	Lowest	Lowest	Amorphous-like
β'	Metastable	Intermediate	Intermediate	Rectangular
β	Most stable	Highest	Highest	Needle shaped

Table 1.6 outlines the short-spacing diffraction patterns found by (Larsson, 1966), deMan (1992) and Himawan *et al.* (2006) for each corresponding polymorph. With the increased usage of palm oil in foods such as confectionery products, Yap *et al.* (1989) examined the polymorphic stability of palm oil and palm oil products for which stability in the β' polymorph was reported. In addition to the palm oil and palm oil products, Ishikawa *et al.* (1997) also studied the polymorphic behaviour of modified palm oils obtained from a random inter-esterification process. This study showed the possible transformation into the β polymorph of the modified palm oil depending on the trisaturated TAG content and storage time. However, the most stable polymorph was also predominantly β'.

Table 1.6 Short spacing data references of different polymorphs

Polymorph	Larsson (1966)	deMan (1992)	Himawan *et al.* (2006)
α	Strong spacing near 0.415 nm	Single spacing at 0.415 nm	Spacing near 0.42 nm
β'	Strong spacing near 0.42 and 0.38 nm or near 0.427, 0.397 and 0.371 nm	Two strong spacings at ~0.38 and 0.42 nm or 3 spacing at ~0.427, 0.397 and 0.42 nm	Two strong spacings at 0.37-0.40 nm and 0.42-0.43 nm
β	Other than criteria α and β'	Very strong spacing at 0.46 or 0.38 nm	Strong spacing near 0.46 nm and ~0.36-0.39 nm

1.3.4 Microstructure

The microstructure of fat crystals is considered to be one of the internal factors that determine the mechanical properties of fat-based products and the sensory impressions that depend on these properties. The study of microstructure in crystalline materials like edible fats is, therefore, important. Marangoni and Narine (2002) identified that the macroscopic mechanical properties of edible fats are directly influenced by the microstructure of the crystal network rather than the final SFC. This may be due to the variations in size, shape and

polymorph of the crystalline structures that result in different textural properties between different fat-based products (Shi *et al.*, 2005). These characteristics of individual crystals as well as the processing conditions will determine the properties of the crystal networks. Processing parameters such as crystallisation temperature and cooling rate are known to influence the microstructural properties of fat crystal networks. In the study of polymorphism and crystallisation kinetics of tripalmitin at different crystallisation temperatures, four different microstructures i.e. grainy, fibrous, feathery and lamellar were exhibited by the β' polymorph (Kellens *et al.*, 1992). Different microstructures were also demonstrated by palm-based shortenings when crystallised at different cooling rates (Litwinenko *et al.*, 2002). The slow cooling conditions resulted in two different networks consisting of smaller, more numerous and needle-like crystals. Under rapid cooling conditions, a more uniform texture was observed. The microstructure of the fat crystal network consisted of crystallites and aggregate structures between 0.25 and 200 μm in size (Narine and Marangoni, 2005). Shi *et al.* (2005) showed the influence of TAG composition on the morphology of the crystal network. Different crystal morphologies for different model lipid systems were seen, depending on the high-melting TAGs present or the saturation degree. For a trisaturated long-chain TAG system, flake-shaped crystals and grain-like aggregates were found whereas a disaturated long-chain TAG system resulted in needle-shaped and irregular spherulite aggregates. Needle-shaped crystals were also formed by a system containing a mixture of tri- and di-saturated long chain TAGs, but regular spherulites were seen in the aggregates. Microstructure analysis of filling fat was done by Vereecken *et al.* (2007) using *trans*-containing and *trans*-free samples from partially hydrogenated palm kernel oil and fractionated palm oil, respectively. Different microstructures of these filling fats were seen after 30 minutes of crystallisation at 5°C which could be due to faster crystallisation of the

trans-containing sample that caused rapid formation of a large number of smaller crystals formation (Figure 1.18).

<div align="center">(A)   (B)</div>
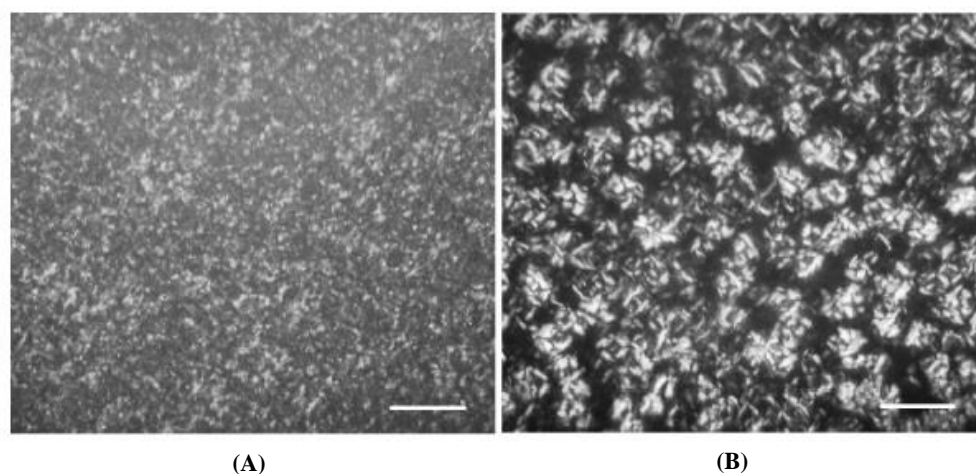
Figure 1.18 Microscopic images (500x magnification) of trans-containing filling fat (A) and trans-free filling fat (B) after 30 mins of crystallisation at 5°C (Vereecken et al., 2007)

1.3.5 Rheological Properties

Mechanical behaviour is the response of a substance when a certain deformation is applied, which can be evaluated via sensory and instrumental methods. The former involves a sensory test and statistical analysis, while the latter uses regimes that mimic reasonable and practical conditions within an instrument. The mechanical evaluation must be done with a correlation to the practical sensory application to obtain meaningful information. Rheology has become one of the tools used to provide mechanical behaviour information at many levels from quality control to product development. Both Muller (1973) and Walters (1980) explained the importance of studying the rheological behaviour of foodstuffs for many purposes, ranging from process engineering and quality control to consumer studies. For example, in chocolate rheology, determination of the rheological properties is useful in the achievement of good quality products since it can describe the effects of changes in fat composition and processing

conditions (Gonçalves and Lannes, 2010). In addition, the rheological properties of food materials can also be used to characterise the composition and structural changes during the production process (Barbosa-Cánovas *et al.*, 1996). Between the product developer and consumer, rheological analysis provides an understanding of consumer perception and its relation to food structure (Borwankar, 1992). Rheological properties during isothermal crystallisation of confectionery fats were studied by De Graef *et al.* (2007) using oscillatory rheology at 10°C. This method allows identification of different crystallisation steps during the crystallisation process including primary crystallisation, microstructural development and development of macroscopic properties of the crystal network. This is shown by the changes in complex modulus and phase angle as seen in Figure 1.19. Furthermore, these changes may also indicate polymorphic transition of α to β' and development of β' crystals from the melt. This, however, was assumed, as no analysis was conducted to confirm the polymorphic behaviour.
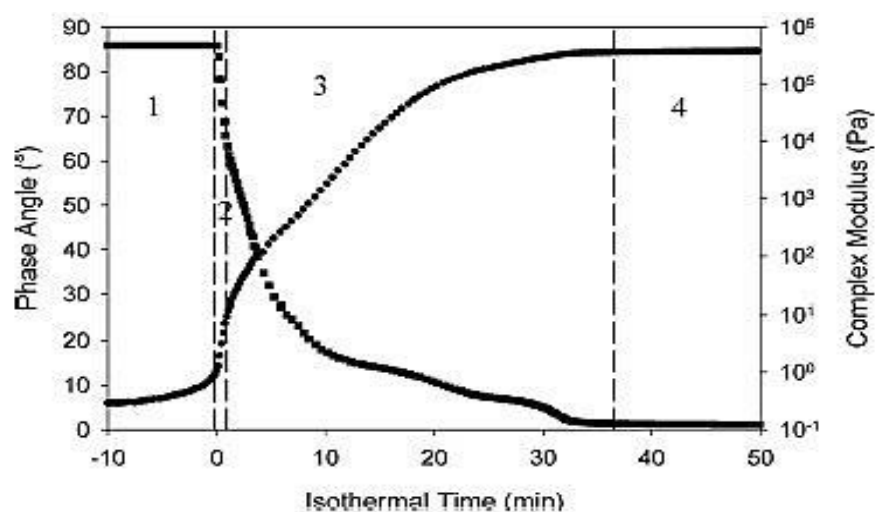
Figure 1.19 Phase angle (■) and complex modulus (•) of a trans-free confectionery fat during crystallisation process including melting period at 70°C (1), nucleation (2), crystal growth (3) and aggregation (4) (De Graef *et al.*, 2007)

1.3.5.1 Basic rheological concepts

Rheology is defined as the study of flow and deformation of matter. It describes the material behaviour under application of force and deformation. This force and deformation are expressed as stress and strain, where stress (σ) is the force acting per unit area and strain (γ) is deformation that can be measured by change in length. The stress is measured in units of Pascals (Pa) and strain is often measured in percentage (%) because it is dimensionless. Mechanical behaviour of a material is basically described by the solid (elastic) and liquid (viscous) characteristics. Foods, however, exhibit a simultaneous combination of both characteristics, creating a viscoelastic system. Since viscoelastic material behaves partly as a viscous material and partly as an elastic material, the resulting response to a sinusoidally varying shear consists of both viscous and elastic components (Vincent, 2012). Wyss *et al.* (2007) showed a schematic figure of stress response to oscillatory strain deformation that indicates different responses for different type of materials (Figure 1.20). For ideal solid material, the stress and strain responses are proportional. Both the stress and applied sinusoidal strain deformation are exactly in phase (phase angle $\delta = 0°$). For a purely viscous fluid, the measured stress is out of phase with the applied strain ($\delta = \pi/2$ or 90°). For viscoelastic material, both in-phase and out-of-phase stress responses are seen as a contribution from both the solid and liquid characteristics, respectively. With respect to the applied strain deformation, the value of δ lies between an ideal solid and pure liquid materials ($0 < \delta < \pi/2$).
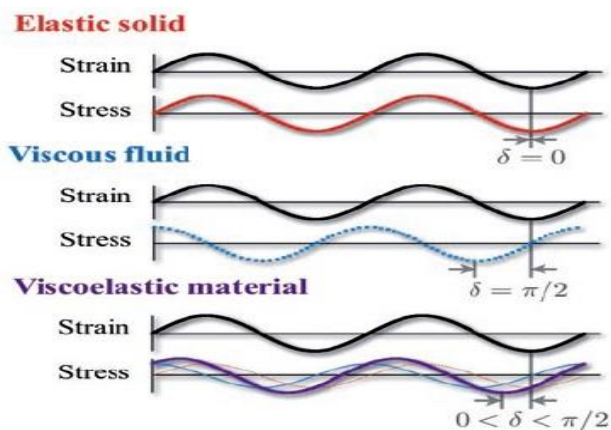
Figure 1.20 Sinusoidal strain and resulting stress of a viscoelastic material (Wyss et al., 2007)

The most commonly used method to study the viscoelastic behaviour of a material is oscillatory rheology. Using this method, the stress response to an applied sinusoidal strain or vice versa is measured. The stress/strain ratio is measured (overall complex modulus, G*) and the viscoelastic nature of the material at the frequency of oscillation is assessed and usually presented as the elastic (G') and the viscous (G") moduli that represent solid and liquid characteristics, respectively. This is based on the evidence that G' is the ratio of the stress that is in-phase with the strain and G" is the ratio of the stress that is 90° out-of-phase with the strain (Ferry, 1980). G' and G" are also known as storage and loss modulus because the value of G' is a measure of energy stored by the material during a deformation process and G" value is a measure of the lost deformation energy (Mezger, 2011). Depending on the dominating modulus value, stronger viscoelastic liquid or solid behaviour is exhibited by the material. When the G" value is higher than G', the material behaves more like a liquid. Conversely, when the G' value is higher than G", more solid-like behaviour is demonstrated (Rao, 1992). Both G' and G" are moduli functions that are mathematically derived from the complex modulus (G*) as described by the equation below (Ferry, 1980).

$$G^* = \sqrt{G'^2 + G''^2}$$

Another rheological method to study the behaviour of viscoelastic material is creep analysis where the slow deformation of a material (creep) is measured under a constant stress application. When a small stress is constantly applied to the viscoelastic material, changing behaviour from elastic components followed by viscoelastic effects is shown by the resulting strain. In sufficient time, the resulting strain will be large enough to deplete the elastic components, showing effects from the viscous components alone. The creep analysis is suitable in predicting the gravitational effects on a material by imitating the sedimentation, sagging and levelling conditions (Bohlin, 1994).

In a fat crystallisation study, oscillatory rheology is suitable for analysis of crystallisation behaviour without irreversibly breaking down the crystal network, allowing data collection from undamaged systems. Furthermore, oscillatory rheology can also be used to indicate crystallisation stages and microstructural development of the fat crystal network during the crystallisation process (De Graef *et al.*, 2006).

1.3.5.2 Rheology in food processing

Rheology has been described as the physical approach to characterise the mechanical behaviour of a material. It can be used to mimic the processing and post-processing conditions by selecting the relevant mode and rate of deformation, achieving more efficient simulations of processing conditions on a small scale and inexpensive consumer testing. Rheology is, therefore, related to sensory evaluation of a product. De Graef *et al.* (2011) reported the benefits of using oscillatory rheology to measure the conventional characteristics of molten chocolate. The rheological analysis was capable of showing effects of variations in fat content on yield value and flow curve of milk chocolate (Figure 1.21).
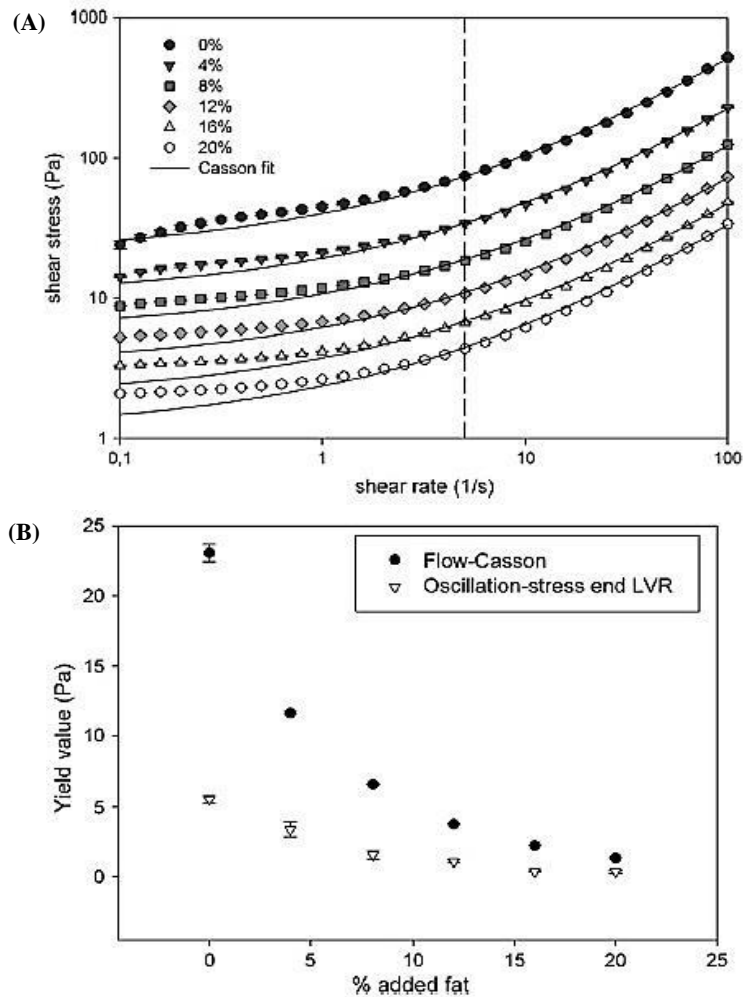
Figure 1.21 Flow curves (A) and yield values (B) of milk chocolate samples with different fat content (De Graef et al., 2011)

Holdgaard (2012) from Palsgaard A/S confectionery and bakery group emphasised the importance of rheology in chocolate production. As a non-Newtonian fluid, chocolate exhibits different viscosities at different applied shear rates (Figure 1.22). For this reason, a single-speed measurement technique will not be able to fully describe the association between the production process and chocolate flow. Thus, more reliable information will be obtained through multiple-speed rheological evaluation methods.
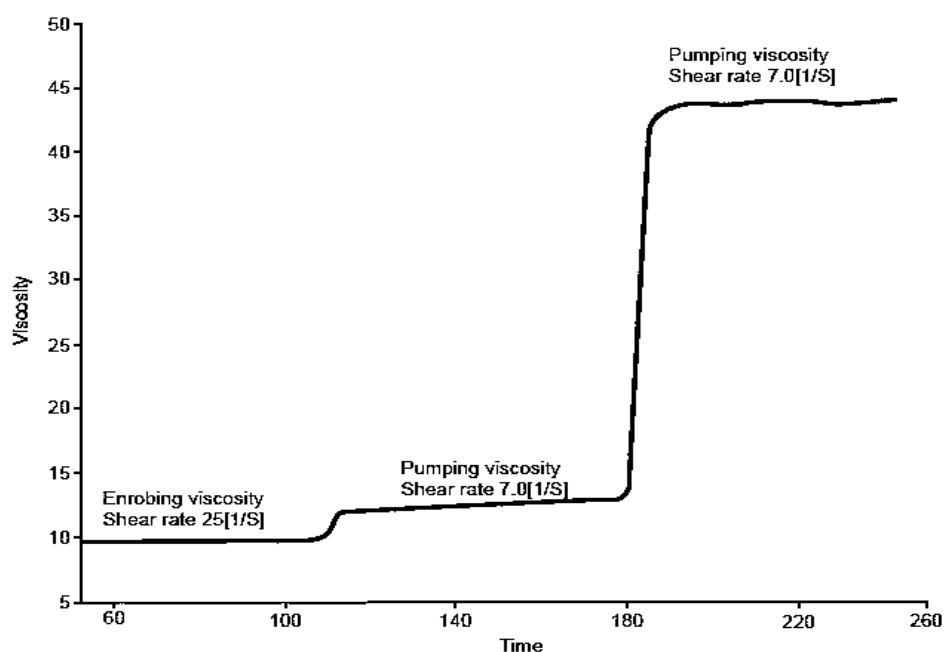
Figure 1.22 Apparent viscosity (Pa s) of chocolate measured at different shear rates for a certain time (s) period. The shear rates imitate blowing movement during enrobing (25 1/s) and pumping movement (7 1/s) (Holdgaard, 2012)

Furthermore, a close relationship between rheological aspects and different steps of the dark chocolate manufacturing process was found by Glicerina *et al.* (2013). This was seen by observing the rheological properties of different samples from each production stage, namely mixing, pre-refining, refining, conching and tempering (Figure 1.23). It was believed that knowledge of the rheological properties at each production step could be useful for processing optimisation as well as final product quality assessment and improvement. In a recent publication, Fischer and Windhab (2011) presented a general overview of the rheological techniques in relation to food processing and the final product. For fat users and manufacturers, a review on the rheology of fats was published many years ago by Scott Blair (1954). However, the use of rheological analysis in the oils and fats area has increased in the past few years. Although a considerable amount of work on the rheology of fats has been reported, it appears that there are no recent reviews of this particular area.

Storage G' (Pa) and loss G'' (Pa) modulus of dark chocolate samples evaluated at 1 Hz.

Campioni	G'	G''
A	16,346 ± 15[b]	2046 ± 23[b]
B	18,593 ± 25[c]	3161 ± 8[c]
C	167086 ± 25[d]	21,473 ± 28[d]
D	5966 ± 24[a]	1406 ± 21[a]
E	4133 ± 20[a]	1396 ± 21[a]

[a-d] Values in the same column followed by different letters differ significantly at $p < 0.05$ level.

Figure 1.23 Obtained rheological properties of dark chocolate samples from different production stages: mixing (A), pre-refining (B), refining (C), conching (D) and tempering (E) (Glicerina et al., 2013)

Although it is not generally accepted as a standard method to follow a crystallisation process, rheological measurement offers supporting information which is complimentary to other crystallisation techniques like pNMR and DSC. In the crystallisation behaviour study of low-*trans* shortenings by Jirasubkunakorn *et al.* (2007), rheological measurements were able to follow the process of crystallisation from liquid phase to crystals that aggregated into a larger crystalline network as shown by the continuous increase of G' as an indicator of the elastic textural properties of the fat (Figure 1.24). Moreover, the use of rheological measurements to follow polymorphism and crystal network development was demonstrated by Toro-Vazquez *et al.* (2004) in a static crystallisation study of cocoa butter (Figure 1.25). When rapid cooling rates were applied, a stationary crystallisation study using rheological measurements was used to simulate cooling tunnel conditions during the production process. In the same study, Toro-Vazquez *et al.* (2004) also used rheological measurements under stirring conditions to investigate the effects of shear rate on crystallisation of cocoa butter. The obtained rheograms indicated encouragement of the crystallisation process by shear as seen in the earlier increase of torque values at 400 rpm shear rate (Figure 1.26).
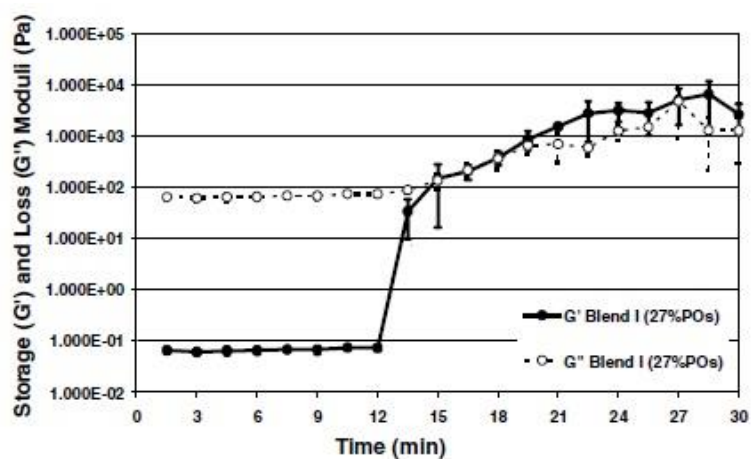
Figure 1.24 Rheological properties of crystallising low-*trans* fat blend containing rapeseed oil and palm oil fractions at 20°C (Jirasubkunakorn et al., 2007)
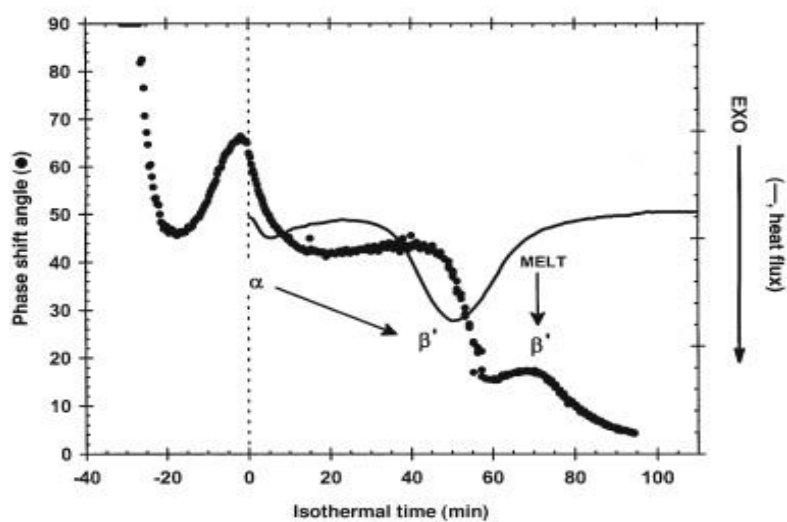
Figure 1.25 Phase angle (●) data combined with crystallisation thermogram (—) by DSC, describing polymorphic changes of cocoa butter crystallised isothermally at 19°C (Toro-Vazquez et al., 2004)
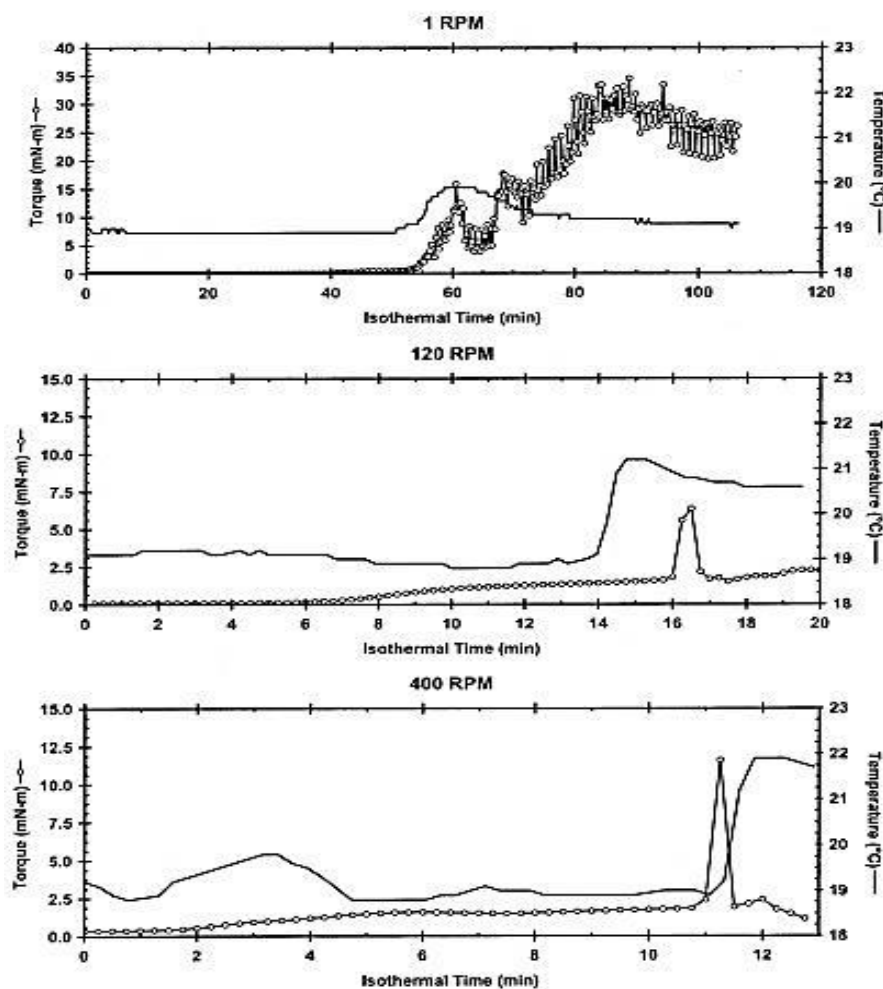
Figure 1.26 Torque (o) and tempering temperature (—) of cocoa butter crystallised at 19°C under different shear rates (1, 120 and 400 RPM) as measured by Toro-Vazquez et al. (2004)

For certain processes such as tempering, shear needs to be applied in order to obtain relevant rheological data. Sonwai and Mackley (2006) used the Rheo-X-ray analysis to investigate the effect of shear on the crystallisation of cocoa butter in which the rheological changes demonstrated an increase in the transformation rate to β polymorph under high shear rate application. In Figure 1.27, earlier increase in pressure (t=6 mins) is seen from sample crystallisation under 600 s^{-1} shear rate, while later pressure increase (t=21 and 31 mins) is observed for samples crystallised under 180 and 84 s^{-1} shear rates respectively.
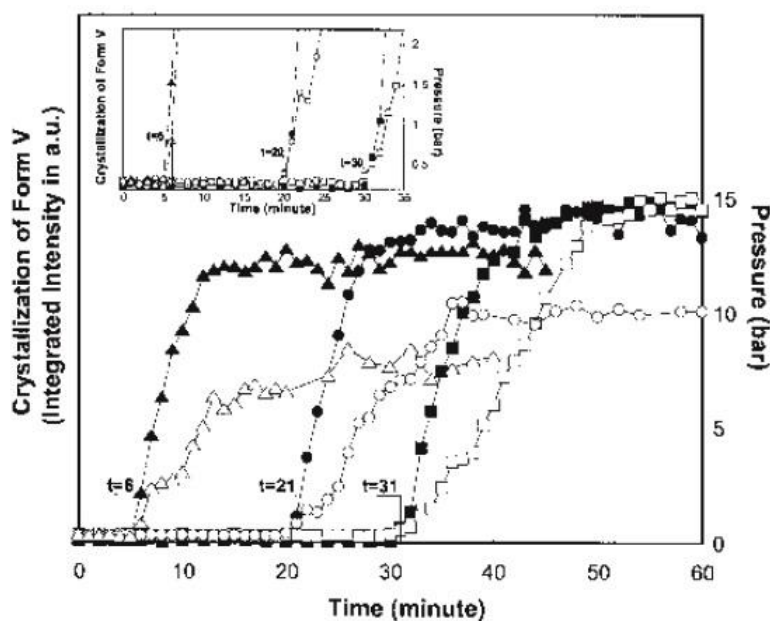
Figure 1.27 Structure change (presented as integrated intensity of small angle X-ray spectra) and corresponding rheological changes (presented as pressure difference) during crystallisation of cocoa butter at 20°C. The crystallisation of Form V is presented by curves with closed symbols (▲, shear rate 600 s⁻¹; ●, shear rate 180 s⁻¹; ■, shear rate 84 s⁻¹), while the pressure difference is represented by curves with open symbol (△, shear rate 600 s⁻¹; ○, shear rate 180 s⁻¹; □, shear rate 84 s⁻¹) (Sonwai and Mackley, 2006)

In the case of palm oil that is known to be stable in the β' polymorph, the application of high shear was shown to accelerate the formation of β' crystals (De Graef *et al.*, 2008). This was indicated by a major increase of rheological parameters (complex modulus and apparent viscosity) that occurred earlier under high shear rate application (Figure 1.28). Also, the obtained rheological data also showed a high degree of structure when a high shear rate was applied as confirmed by the microscopic image at high shear rate, showing more aggregates were present. In another crystallisation study of palm oil under shear by Tarabukina *et al.* (2009), larger size and earlier detection of spherulites as well as faster growth rate at the beginning of the crystallisation process were observed at high shear rate (Figure 1.29).
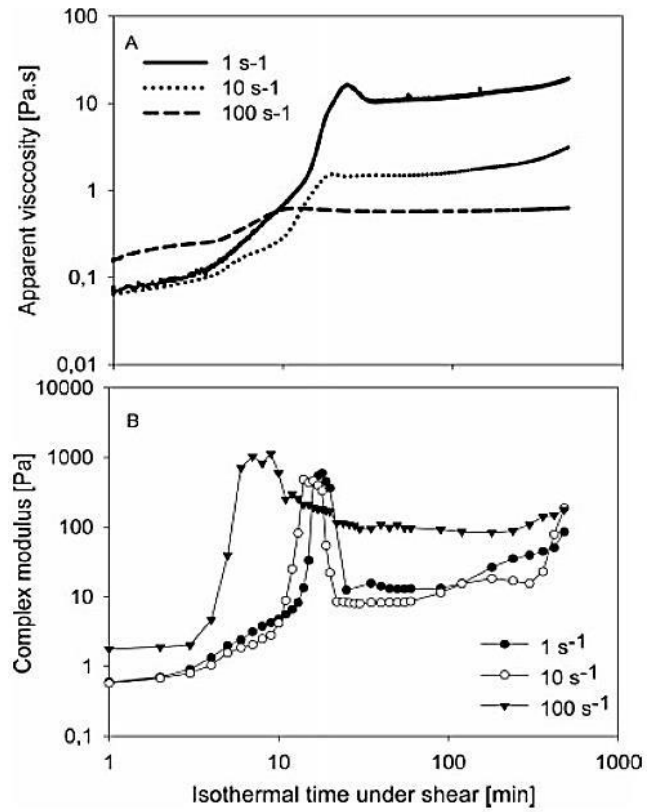
Figure 1.28 Apparent viscosity (A) and complex modulus (B) of palm oil crystallisation under different shear rates of 1, 10 and 100 s^{-1} at 18°C (De Graef et al., 2008)
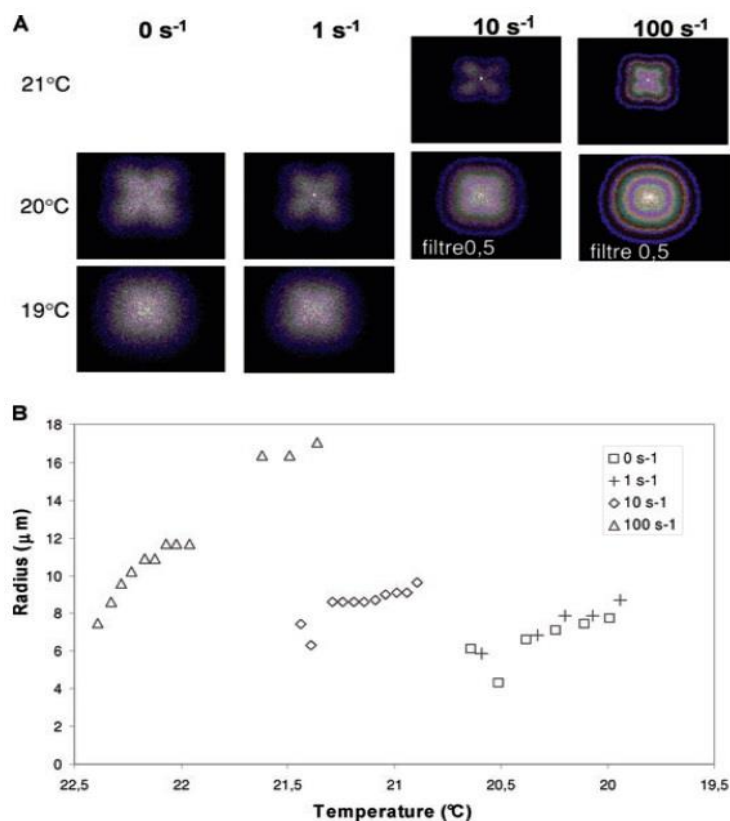
Figure 1.29 Small angle light scattering (A) and spherulites radius observations of palm oil crystallised under different shear rates and temperatures (Tarabukina et al., 2009)

1.4 Project Aim

Being one of the main ingredients in confectionery products, vegetable fat plays an important role in determining the sensory attributes of the final product such as hardness, flavour release and mouth feel. Since it is present in a crystallised form, understanding of the crystallisation behaviour of the selected vegetable fats is, therefore, crucial as some adjustments during production processes might be needed when different types of vegetable fat with different crystallisation behaviour are handled. The isothermal crystallisation behaviour of coating and filling fats for confectionery products were studied by Foubert *et al.* (2006) and Vereecken *et al.* (2007), respectively. However, there was no rheological approach in these two studies. In fact, few papers have explored the rheological aspect of

filling fats exclusively. In chocolate manufacturing process, the right rheological characteristic is needed as it influences the moulding or enrobing step that may result in undesirable product quality (Rutson, 1989). The main purpose of this study is to investigate the rheology of filling fats using the developed rheological method of analysis to characterise the physical properties, which reflects the processing behaviour of two filling fats with different composition and solid fat content. The application of shear was expected to enhance the crystallisation process by increasing the formation of more stable crystals, as seen in previous studies. Oscillatory rheology was chosen as it is believed to deliver valuable information on the crystallisation properties of the filling fats along with the other techniques for crystallisation analysis like DSC, microscopy and XRD. In addition, a major objective of this study was also to use oscillatory rheology to follow the crystal network development of the filling fats under the developed processing conditions and to study the effects of shear on the crystallisation response of the filling fats. Rheological knowledge of the products can be used to advise confectionery fat manufacturers and users on how to treat products containing particular selections of fat.

CHAPTER 2. INSTRUMENTAL TECHNIQUES PRINCIPLES

This chapter describes the general principles of instrumental techniques used in this study. Information on the materials and methods are given separately in Chapter 3.

2.1 Oscillatory Rheology

In this study, imitative rheological measurements were performed to obtain meaningful crystallisation properties of the material being tested in correlation with the real processing situation. The nature of the crystalline material present in food products can be studied using an indirect approach like rheology. This is usually done by looking at the increase in viscosity of the solution during the crystallisation process as this relates to the quantity of crystals present under certain controlled conditions. However, crystal formation and heat release during crystallisation contribute to increases and decreases of viscosity, and these processes may occur at the same time during the crystallisation process. Other rheological measures such as elastic and viscous moduli are, therefore, used in this study to provide a better understanding of the changes occurring. Geometries that are used in conventional experiments include concentric cylinder, cone-and-plate and parallel plate systems. While the bottom plate is fixed and the top geometry oscillates, a time dependent strain or stress is applied to the sample (Figure 2.1).
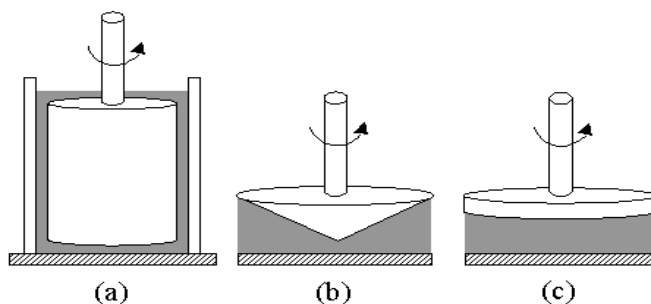
Figure 2.1 Three types of measuring systems for rheological measurements: concentric cylinder (a), cone-and-plate (b) and parallel plate (c) (Garboczi et al., 2014)

In rheological characterisation of food material, more than one type of oscillatory tests need to be done in order to obtain a complete rheological overview (Tabilo-Munizaga and Barbosa-Cánovas, 2005). Characterisation of a viscoelastic material usually starts with a stress or strain sweep test to determine the linear viscoelastic range (LVR) of the material. It is performed under constant frequency and temperature at stress or strain value increasing with time. Determining the LVR will give information about the viscoelastic character of a material. If the elastic property is dominant over the viscous one, the material exhibits solid or gel characteristics. On the contrary, if the viscous property is dominant, the material exhibits liquid characteristic. When G' and G" (see Section 1.3.5.1) are plotted against the preferred strain/stress range, a plateau of the LVR is obtained and a certain stress/strain value within that range is selected for use in the subsequent test mode. When the irreversible deformation region is entered, decreasing curves of G' and G" is indicated. To indicate the processing capability of materials as well as the sensory characteristics, the frequency sweep test is seen as a good characterising tool. The measurement is performed at a range of frequency and constant strain/stress and temperature values. By performing this type of oscillation test, structural strength of the material can be analysed.

2.2 Differential Scanning Calorimetry (DSC)

Thermal analysis is one of the most commonly used techniques in the evaluation of food crystallinity. The most commonly used instrument for this particular food analysis is the differential scanning calorimeter (DSC). It measures the difference in energy flow between a small sample in a DSC pan and an empty reference pan as they are both heated. At a phase transition, the latent heat requires additional energy input (power) to maintain the temperature. Thus, crystallisation exotherms and melting endotherms are observed as a function of either temperature or time. These melting and crystallisation profiles may then be associated with phase changes, including transition states of the material. DSC may not be suitable for direct interpretation of processing conditions due to the static state of the sample during the test. However, the DSC melting profile is frequently used for both investigation of crystal polymorphs and the amount of crystals present in food materials. Upon cooling, a less-stable polymorph will often form initially followed by transformation into more-stable forms. Depending on the cooling rate, both of these events will increase the energy peaks. The total amount of crystalline material formed is related to the area under the energy peak. Due to different melting points of various glycerides, one or two melting endotherms are often found in natural fats (Hartel, 2001). The stop-and-return method of DSC analysis was first introduced by Foubert *et al.* (2008) to provide information on the mechanism of isothermal crystallisation of fats. It is useful when early crystallisation takes place before an isothermal temperature is reached, so the isothermal crystallisation curve does not provide the full information. The basic principle of this technique is illustrated in Figure 2.2. In principle, the melting profiles for different isothermal crystallisation times are determined using this particular technique. Molten sample is cooled to the desired isothermal crystallisation temperature and interrupted at different times followed by reheating steps (Figure 2.2A). The reheating steps result in melting profiles with different peak temperatures (Figure 2.2B) that

can be used to identify the different solid fat fractions developed at the moment the isothermal crystallisation was interrupted and how this changes over time. Furthermore, the melting enthalpy or the area under the melting curves is used to present the amount of crystallised fat during the different isothermal crystallisation. Crystallisation curve can be presented by plotting this data as a function of isothermal crystallisation time (Figure 2.2C).
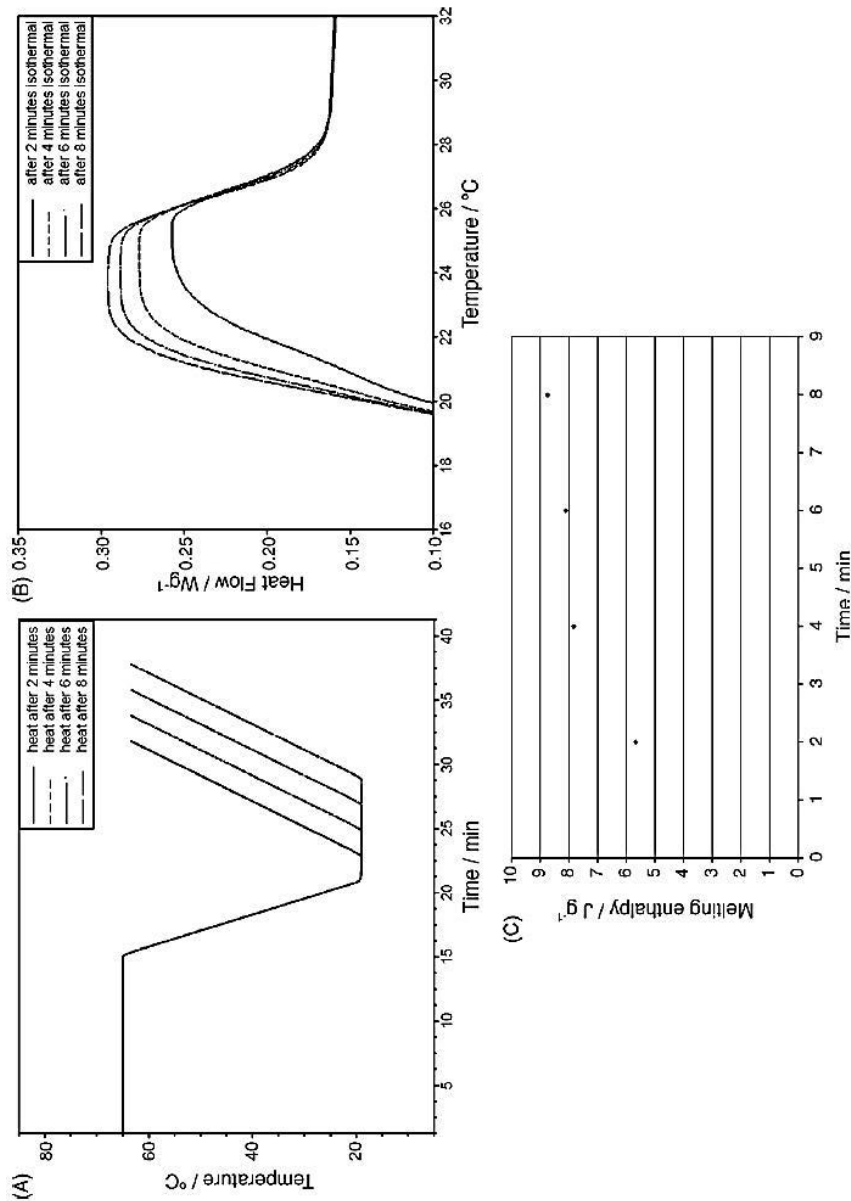
Figure 2.2 Basic principle of stop-and-return DSC technique: time-temperature program (A), melting profile (B) and crystallisation curve (C) at 2, 4, 6 and 8 minutes of isothermal crystallisation (Foubert et al., 2008)

2.3 Polarised Light Microscopy (PLM)

In crystallisation analysis, microscopy is known to be a very valuable tool as it has the advantage of not only allowing observation of crystallisation process visually, but also delivering additional quantitative information such as crystal size. In contrast to standard white light optical microscopy, the polarised light microscopy technique allows identification of crystalline material with enhanced detection of smaller crystal sizes between 1 and 3 µm (Hartel, 2001). Essentially, birefringent objects with highly ordered molecular structure like crystals have the ability to refract a single light ray into two corresponding rays. To perform polarised light microscopy, two polarising filter are needed (Figure 2.3).
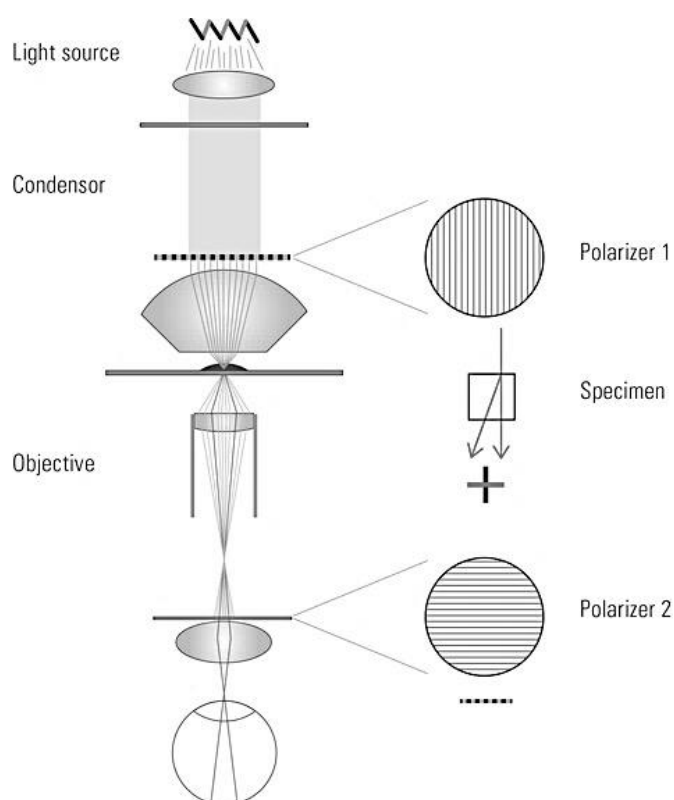
Figure 2.3 Principle of a polarising microscope (Ockenga, 2011)

The first filter is used to illuminate the object while the second filter is used to analyse the object by restricting the detected light to refracted light. Linearly polarised light is produced when light passes through the first filter and rotated in a specific manner by the presence of crystals and all light that is not rotated will be blocked by the second filter, enhancing visualisation of the appearance of crystals (Ockenga, 2011). In case of fat crystals, crystalline material will appear brighter than the surrounding fat (Hartel, 2001). Using a hot/cold stage, the effect of cooling rate on the crystallisation of fat crystals can also be studied with the optical microscope. Depending on the cooling rate, the temperature where the first nuclei become visible can be considered as the nucleation temperature while the time taken for appearance of the first crystals can be characterised as the induction time. Afterwards, crystal growth rate can be estimated by assessing the change in crystal size with time.

2.4 X-Ray Diffraction (XRD)

For the fats industry, polymorphism study has a practical significance in terms of textural properties that are caused by crystal fat transformation from β' to β polymorphs (deMan, 1992). Evaluation of the crystals or identification of any changes in crystallinity over time in a product can be done using X-ray spectroscopy. Since DSC cannot be used for straightforward identification of polymorphs present, the X-ray spectroscopy technique is needed to support the isothermal crystallisation study of the fat crystal network. This method is done by passing X-rays through crystalline material where a distinctive X-ray scattering profile (Figure 2.4) is obtained from the structural arrangement of molecules in the crystal network. The scattered X-ray spectrum, which is the variation in intensity of scattered X-rays with angle of observation, can then be used to identify the structure of the crystal network. In

the fat crystal network formation, the TAG molecules arrange themselves and create a three-dimensional structure that deflects the passing X-ray at a particular angle as illustrated in Figure 2.5, where d is the spacing between crystal planes and θ (theta) is the incident angle, which is equal to the diffracted angle. When the X-rays passed through the material, the angle between the undiffracted and diffracted beams was measured and known as the 2θ angle (deMan, 1992).
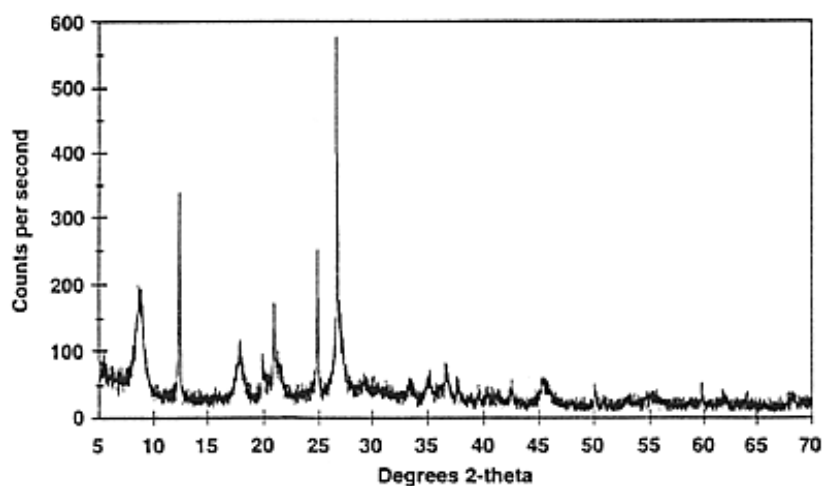
Figure 2.4 An example of X-ray powder diffractogram, where different peaks are seen as the X-rays were diffracted by the crystal lattice (Dutrow and Clark, 2007)
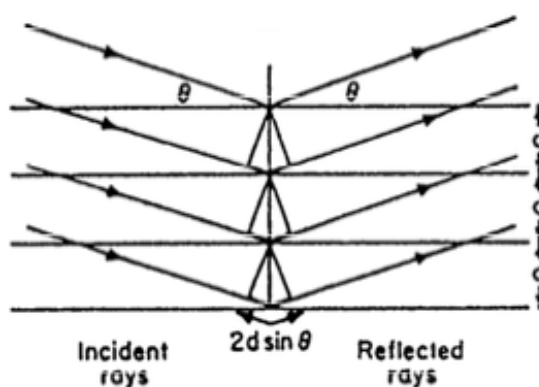
Figure 2.5 X-ray diffraction in a crystal network (deMan, 1992)

In general, the XRD analysis recognises two types of spacings namely long (observed in the 2θ range of 1-15°) and short (observed in the 2θ range of 16-25°) spacings. In the fat polymorphism study, however, the short spacing data is normally used to characterise the polymorphic forms of the fat (deMan, 1992) (see Section 1.3.3). It indicates the cross-sectional packing of the hydrocarbon chains and is independent of the chain length. Molecules in an ordered solid result in strong intensity and a concentrated X-ray spectrum compared to the ones in the liquid state. Ideally, a sufficiently large size of crystals is needed to determine the orientation of molecules in the network. Since it is difficult to grow large enough crystals and stabilise an unstable fat polymorph, a powder X-ray diffractometer that measures the scattering spectrum of randomly arranged fine crystals is best used in a fat crystallisation study (deMan, 1992; Hartel, 2001).

CHAPTER 3. MATERIALS AND METHODS

Two types of filling fat, Creamelt 601 and Creamelt 701, were provided by Loders Croklaan B.V. (Wormerveer, The Netherlands). Filling fats used in this study are particularly made by blending in some crude and fractionated materials before the refining process. Fatty acid and triacylglycerol compositions as well as SFC measurements were conducted by Loders Croklaan.

3.1 Materials

The raw starting materials are based on palm oil and shea butter fractions. The major ingredients in these filling fats are palm mid fractions and crude shea butter, while shea stearin (hard fat from fractionated shea butter) and crystallisation initiator (fully hydrogenated palm oil with melting point of 60°C) present in one of the filling fat formulation (Table 3.1). List of components present in the filling fat formulations is confidential and provided by Loders Croklaan B.V., The Netherlands. The required fractions were, then, blended and refined. This different formulation between the two filling fats, consequently gives different triacylglycerol and fatty acids compositions as presented in Table 3.2 and Table *3.3*.

Table 3.1 Filling fat formulation (Werleman, 2012)[*]

Components	Creamelt 601	Creamelt 701
Palm mid-fractions	✓	✓
Shea butter	✓	✓
Shea stearin	✓	✗
Crystallisation initiator	✓	✗

[*]confidential data provided by Loders Croklaan B.V., The Netherlands

Table 3.2 Triacylglycerol composition [%] of Creamelt 601 and Creamelt 701 (Werleman, 2012)[*]

Triacylglycerol		Creamelt 601	Creamelt 701
1,2,3-tripalmitoyl-glycerol	PPP	1.7	1.8
1,2-dipalmitoyl-3-stearoyl-glycerol	PPS	0.9	0.4
1,3-dipalmitoyl-2-oleoyl-glycerol	POP	41.2	54.9
1,3-dipalmitoyl-2-linoleoyl-glycerol	PLP	5.5	7.8
1-palmitoyl-2,3-distearoyl-glycerol	PSS	0.5	0.1
1-palmitoyl-2-oleoyl-3-stearoyl-glycerol	POS	9.6	10.4
1-palmitoyl-2,2-dioleoyl-glycerol	POO	6.2	6.8
1,2,3-tristearoyl-glycerol	SSS	0.5	0.1
1,3-distearoyl-2-oleoyl-glycerol	SOS	15.2	4.2
1-stearoyl-2,3-dioleoyl-glycerol	SOO	5.8	2.6
1,2,3-trioleoyl-glycerol	OOO	1.6	1.1

[*]confidential data provided by Loders Croklaan B.V., The Netherlands

Table 3.3 Fatty acid composition [%] of Creamelt 601 and Creamelt 701 (Werleman, 2012)[*]

Fatty acid	Scientific name	Creamelt 601	Creamelt 701
C12:0	Lauric	0.2	0.2
C16:0	Palmitic	36.2	49.0
C18:0	Stearic	19.3	8.5
C18:1 *cis*	Oleic	36.6	35.0
C18:2	Linoleic	5.4	5.3
trans	*trans*- fatty acids	0.2	0.1

[*]confidential data provided by Loders Croklaan B.V., The Netherlands

3.2 Differential Scanning Calorimetry (DSC)

Thermal analysis was performed with a Q2000 pan-type DSC (TA Instruments, Herts, UK). The temperature-time scheme of the stop-and-return technique is shown in Figure 3.1. It was first started by melting the filling fat at 70°C to erase any crystal memory. The filling fat was then cooled to the desired crystallisation temperature at $10°C.min^{-1}$ and held for different isothermal times before subsequent reheating at $5°C.min^{-1}$. In this study, two crystallisation temperatures of 20 and 15 °C were selected. At higher crystallisation temperature, the isothermal period ranged from 0 to 60 minutes (Figure 3.1A) while longer isothermal period (0 to 240 minutes) was applied at lower crystallisation temperature (Figure 3.1B). The system was run using 50 $ml.min^{-1}$ nitrogen flow rate. Sample (10-15 mg) was placed in an aluminium pan and an empty pan was used as reference. To maintain good reproducibility, the same reference pan was used for each test cycle. Melting profiles were plotted and the area under each curve was calculated to quantify the amount of solid fat present at that certain time (see Section 2.2). By constructing this data as a function of time, a crystallisation curve can be presented. The temperature peaks will indicate different polymorphs or mixed triacylglycerol crystals into which the fat has crystallised (Foubert *et al.*, 2008).

(A)

(B)
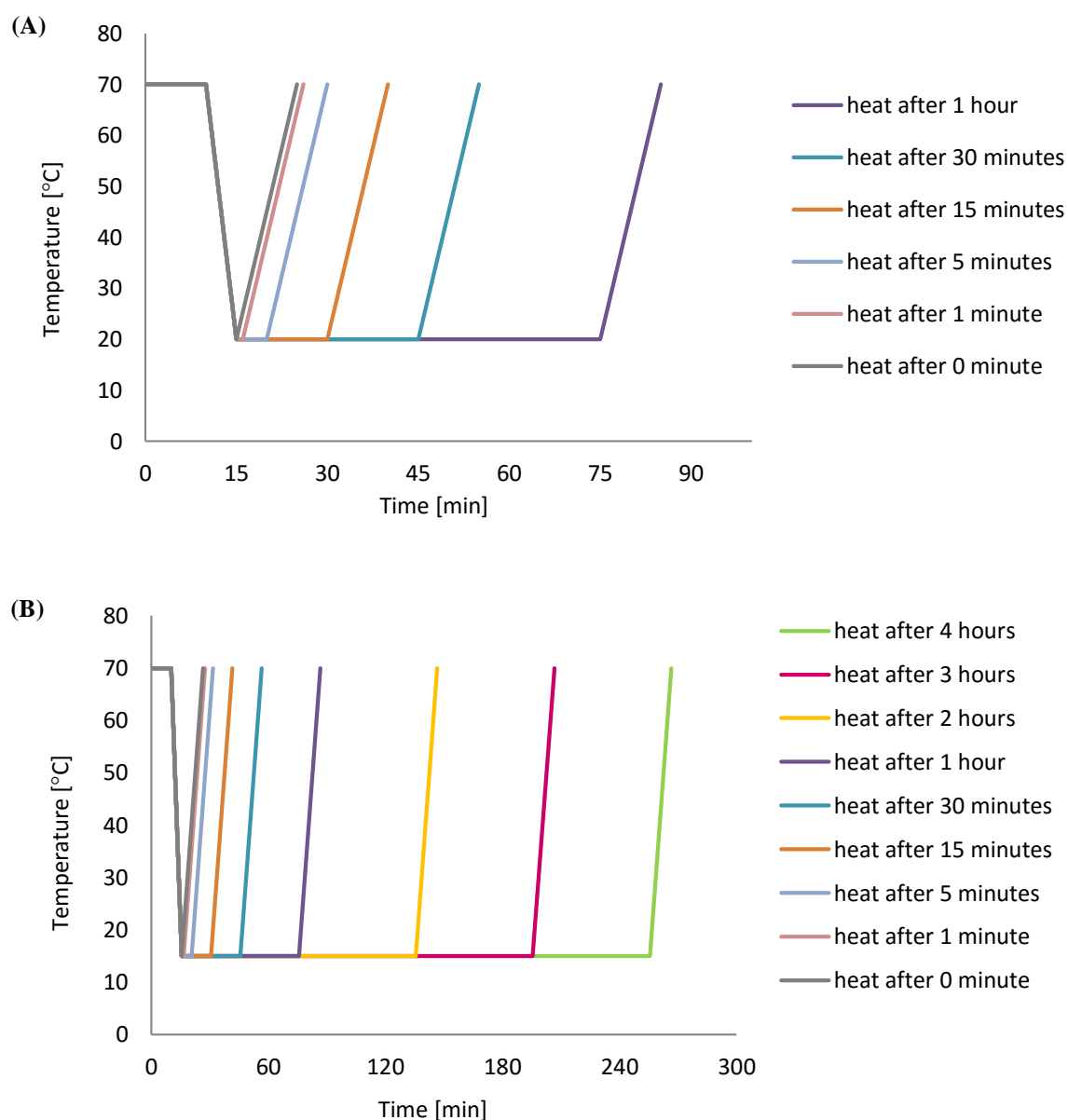
Figure 3.1 Stop-and-return time-temperature program of filling fat crystallisation at 20 (A) and 15 (B) °C

3.3 Polarised Light Microscopy (PLM)

Microstructure of the crystal network was observed with a Leica DM2500 M microscope (Leica Microsystem, Wetzlar, DE). An image was captured with the help of a built in digital camera and image archiving software. A molten sample was placed on a microscope slide

that was initially conditioned at 70°C using a Mettler Toledo hot/cold stage (Mettler-Toledo, Leicester, UK) as seen in Figure 3.2. Subsequently, a cover glass was placed on top of the sample to create a thin layer for better observation. To imitate the rheological test conditions, the sample was cooled at different cooling rates to 22°C. 20X magnification images were acquired at the appearance of the first crystals and periodically during the following 60 minutes and 24 hours crystallisation time.

Figure 3.2 Mettler Toledo hot stage (Mettler-Toledo, Leicester, UK)

3.4 X-Ray Diffraction (XRD)

The solid-state structure of the fat was characterised using a Bruker D8 Powder X-ray Diffractometer (Bruker Biosciences, Billerica, US). According to the rheological test condition, the fat sample was heated to 70°C and cooled to 15 and 20°C at 6°C.min^{-1} in a capillary tube for crystallisation. Signals between 12.5 and 27.5 2-theta (2θ) angle were recorded at different periods of isothermal times according to DSC thermal analysis. In addition, the fat sample was also cooled down at relatively slow rate of 0.2°C.min^{-1} from 70

to 19°C and kept isothermal for up to 24 hours. In this case, signals between 12.5 and 27.5° 2θ-angle were recorded every 30 minutes.

3.5 Oscillatory Rheology

The fat sample was prepared in the same manner for every rheological test. Initially, fat was melted in a 70°C water bath with continuous stirring for 30 minutes to remove any crystal memory (Figure 3.3A). As a consequence of the minimum limit of the IKA Labortechnik overhead stirrer, a value of 16 rpm was chosen to give the melted fat a gentle shear. Sample was then transferred to a Bohlin C-VOR 100D rotational rheometer (Malvern Instruments, Worcestershire, UK) (Figure 3.3B). Using this particular rheometer, oscillation test could be done while the upper geometry rotates.

(A)   (B)
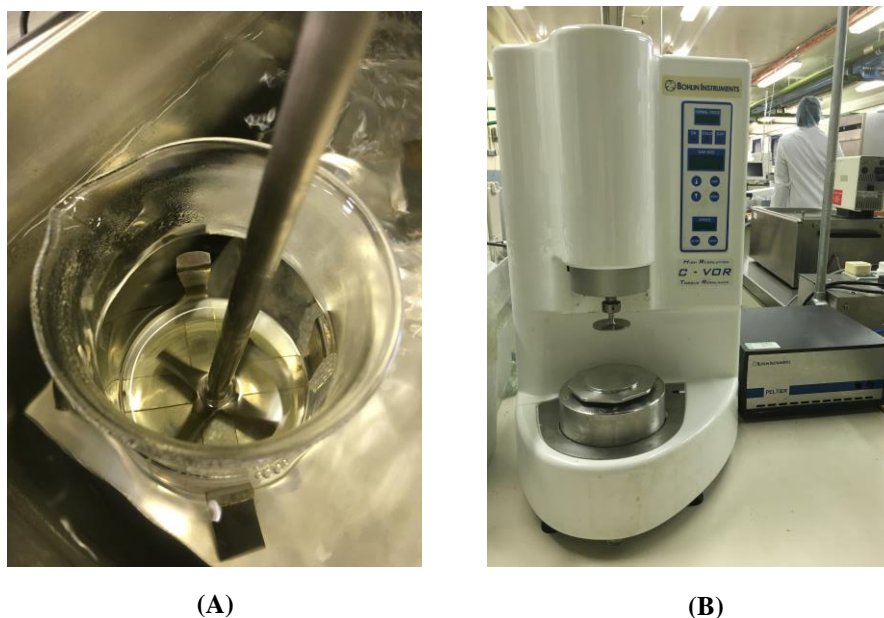
Figure 3.3 Sample preparation step (A); Bohlin C-VOR rheometer (B)

A 1° angle and 40 mm diameter cone-and-plate geometry (Figure 3.4) was chosen as it gives homogeneous shear condition across the entire gap. This particular geometry is also preferred

when dealing with homogenous fluids or fluid-like materials. To optimise the test condition, the bottom plate of the rheometer was conditioned at 70°C prior to sample placement. This was done to prevent any crystal formation due to a temperature difference between the molten fat and the rheometer plate. The sample was then allowed to crystallise during cooling to a specific temperature with a certain cooling rate with or without shear. Three repetitions were performed for each measurement category.
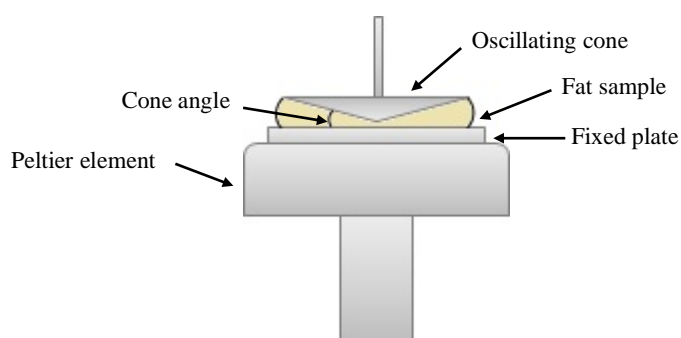
Figure 3.4 Illustration of the cone and plate geometry used in rheological measurements

3.5.1 Determination of Linear Viscoelastic Range (LVR)

To make sure that the crystallisation behaviour was observed appropriately, strain sweep tests were done in order to determine the LVR of the fats. First, the sample was naturally crystallised in the system to ambient temperature. Afterwards, an amplitude sweep test was done at a frequency of 1 Hz and within a strain range of 0.1 – 10%. The strain value within the LVR of the fats was, then, used to carry out the rheological measurements for this study.

3.5.2 Static and Slow Cooling Crystallisation Conditions

The behaviour of the crystallising fat was observed as the molten fat crystallised in the system during cooling to ambient temperature without shear. A water bath was used as the cooling system to control the natural crystallisation process. The natural crystallisation process of the fat sample is shown in Figure 3.5. The cooling rate of each test was then calculated and recorded.
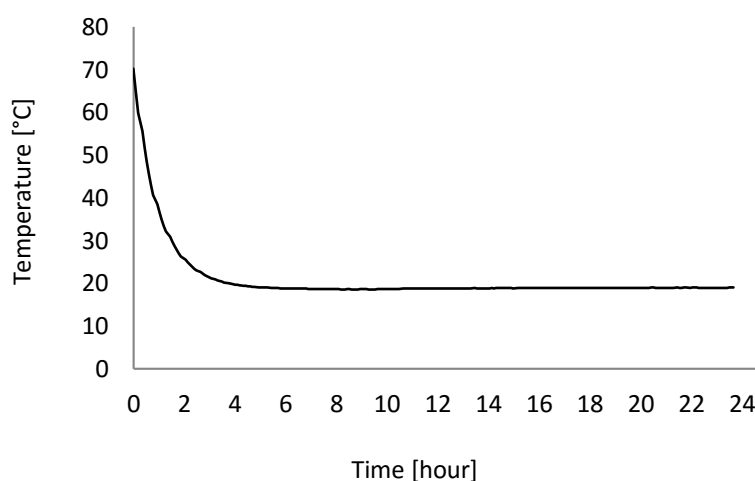
Figure 3.5 Temperature profile for naturally crystallising filling fat: filling fat sample was pre-melted at 70ºC for 10 mins and crystallised to room temperature at around 19ºC

3.5.3 Simulation of Processing Conditions

The molten fat sample was transferred to the preheated plate and subjected to a certain temperature, time and shear program as illustrated in Figure 3.6. This cooling program and selected parameter values were designed to imitate the processing conditions of these particular filling fats. Since the shear is only applied during the cooling period, a combination of three measurements was proposed i.e. completely static condition, sheared during both

cooling steps and sheared during the first cooling step alone. The program is divided into six different steps, representing pre-crystallisation, cooling tunnel and storage conditions. Accordingly, three crystallisation temperatures of 22, 10 and 20 °C were selected.

1. Holding at 70°C to erase the crystal memory

2. Cooling to 22°C at $10°C.min^{-1}$ with or without shear

3. Holding for 15 minutes

4. Cooling to 10°C at $10°C.min^{-1}$ with or without shear

5. Holding for 30 minutes

6. Heating at $10°C.min^{-1}$ to 20°C and holding for 2 hours

A medium shear rate value of $100 s^{-1}$ was chosen to demonstrate some processing conditions like mixing and pipe flow movements (Mezger, 2011) that are subjected to the filling fat during manufacturing process. Oscillation tests were done at 0.5% strain value and 1 Hz frequency. A solvent trap was also used for temperature control purposes. To achieve the desired cooling/heating rate, a Peltier system was used as a control system.
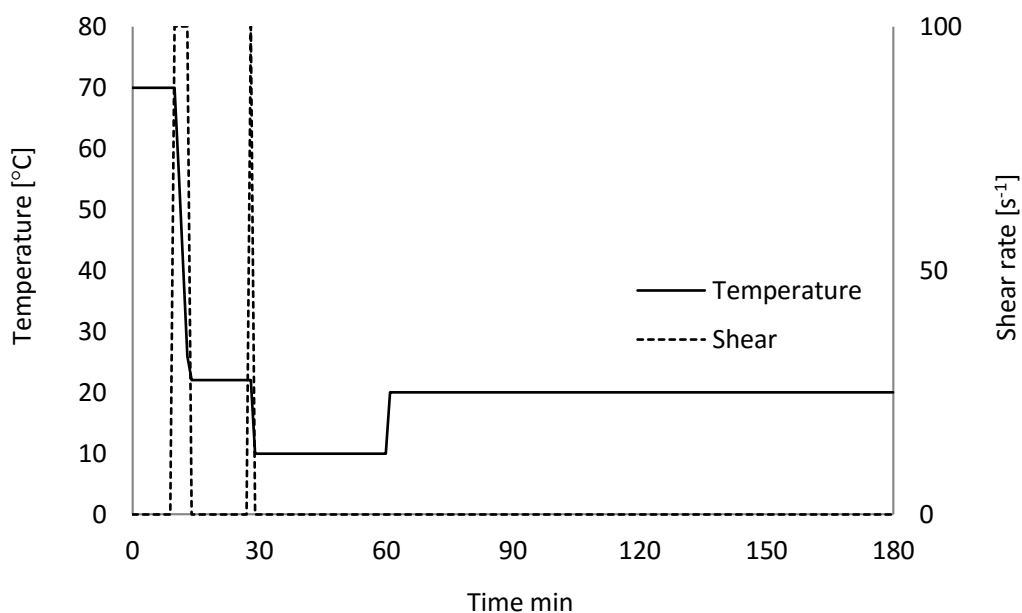
Figure 3.6 Time, temperature and shear program to imitate processing conditions of the filling fats

3.5.4 Oscillation Frequency Sweep

In order to further understand the crystal network structure, frequency sweep tests were done after each oscillation step of the simulated processing condition. Each frequency sweep was done at isothermal temperature according to the oscillation step condition. A frequency range of 0.1 to 10 Hz was selected to fully observe the crystal network structure of the filling fats at each processing step.

3.6 Solid Fat, Triacylglycerols and Fatty Acid Contents

Measurements for solid fat content, triacylglycerol and fatty acid compositions were done by Loders Croklaan B.V (Wormerveer, The Netherlands) according to their standard quality control and assurance procedures. Results for the analysis of both filling fats were provided by Loders Croklaan B.V (Wormerveer, The Netherlands). SFC is recorded at different chosen temperatures, ranging from 0 to 35 ^{o}C, using the pulsed nuclear magnetic resonance (pNMR) technique. Measured solid at each temperature was recorded after melting at 60^{o}C for 15 min, cooling to 0^{o}C and left isothermal for 90 min to initiate crystallisation process, stabilization at 20^{o}C for 24 or 40 hours, cooling to 0^{o}C and left isothermal for 90 min to allow formation of more stable crystals and 60 min at each of the following temperature: 20, 25, 30 and 35 ^{o}C (Werleman, 2013b). Both fatty acid and triacylglycerol composition profiles were determined using capillary gas chromatography (Werleman, 2013b).

3.7 Statistics

Data for each sample (n=3) were recorded as the mean and analysed by IBM SPCC Statistics 21 and Microsoft Excel Data Analysis. One-way analysis of variance (ANOVA) with Tukey's post-hoc test and single factor ANOVA were used to identify any significant differences between the samples. Significance was set at $P<0.05$ for both statistical methods.

CHAPTER 4. CRYSTALLISATION BEHAVIOUR OF FILLING FATS UNDER STATIC CONDITIONS

4.1 Introduction

In many confectionery products fat is present in a crystallised form and in significant amounts, giving a substantial contribution to both its physical properties and sensory attributes. An understanding of crystallisation characteristics and the interactions of the fats used in the products is, therefore, extremely important for both production and consumption (Talbot, 2008). The study of complex and multiphase fat systems provides useful information with practical implications for both confectionery manufacturers and confectionery fat suppliers.

The mechanical strength of a fat crystal network, which may subsequently determine the sensory qualities (like mouthfeel and texture) can be characterised by the macroscopic rheological properties (Narine and Marangoni, 1999a; Talbot, 1995). Development of the fat crystal network begins when certain polymorphs start to crystallise from the unstructured triacylglycerol mixture. These small crystals or nuclei subsequently grow larger after they are formed. Growth of such crystal "clusters" tends to occur with the initial crystal nucleus seeding crystallisation of the bulk fat. Voluminous aggregates of even larger microstructures will then form through agglomeration that continues until a three-dimensional network is achieved (Narine and Marangoni, 1999a; De Graef *et al.*, 2006). In order to understand this process, prior investigation of the crystallisation behaviour of the filling fats was conducted via small deformation oscillatory experiments (see Section 3.5.2).

Under the "static" conditions (non-stirred), Brownian motion and van der Waals attraction cause collisions and subsequent crystal "attachments" that lead to the agglomeration of fat crystals. Studying the crystallisation behaviour in stationary conditions will provide information on the structural characteristics of the crystal network (Kloek, 1998).

4.2 Linear Viscoelastic Range (LVR) of Filling Fats

Amplitude sweep tests were carried out for both filling fats to determine the LVR (see Section 2.1). Selecting a value within the LVR of the material is important to make sure that the crystallisation behaviour of the sample is observed appropriately. This type of measurement will define a range where the results are linear or independent of the applied strain. The LVR ended with a noticeable decrease in the overall moduli values when the response became non-linear (no longer independent of the applied strain). Prior to the measurements, the filling fat was melted in a 70°C water bath under continuous stirring for 30 minutes (see Section 3.5). It was, then transferred to the rheometer and allowed to cool naturally (around 0.2°C.min^{-1} cooling rate) and crystallise at ambient temperature (around 19°C). To avoid disruption of the crystallisation process, minimum strain allowed by the instrument and low frequency values of 0.000573% and 1 Hz were used. Figure 4.1 shows the similar LVR of the two filling fats, with slightly higher moduli and a somewhat larger region for Creamelt 601 when compared to the 701 both at ambient temperature. The higher moduli values are probably caused by the presence of the crystallisation initiator in Creamelt 601 that had already developed crystals at this stage. Based on this LVR profile, the strain value of 0.5% was found to be within the LVR of both filling fats and, therefore, was selected to be used in all further oscillation measurements.
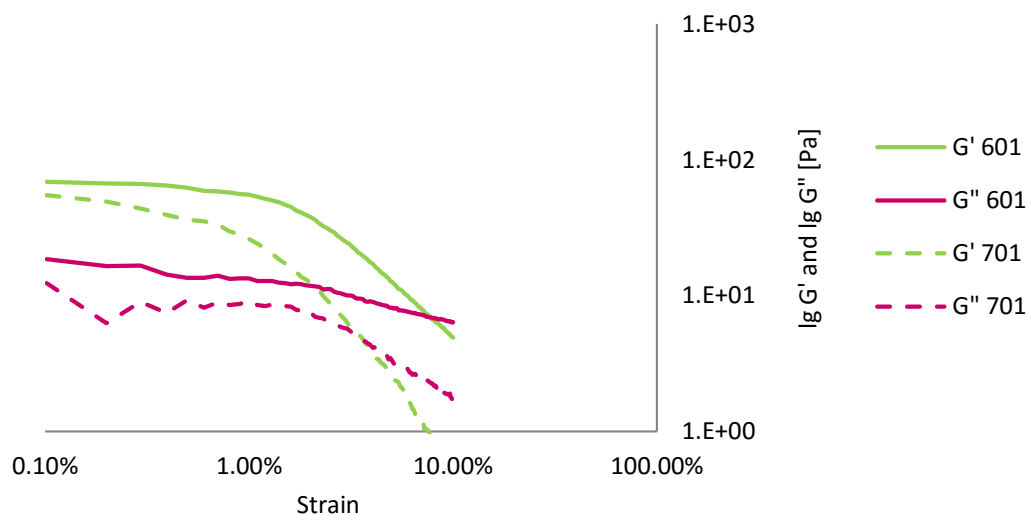
Figure 4.1 Typical linear viscoelastic region of Creamelt 601 and 701 after cooling at 0.2ºC.min⁻¹ to 19ºC

4.3 Rheological Behaviour

Prior to the test, the fat sample was prepared in the same manner to that described above and transferred to the rheometer to crystallise at temperature-time profile illustrated in Figure 3.5 ("cooling curve"). The melted fat sample (70ºC) was naturally crystallising at 0.2ºC.min⁻¹ cooling rate and reached the ambient temperature of around 19ºC in about 4 hours. The samples were left to crystallise further at this crystallisation temperature for up to 24 hours for Creamelt 601, and 15 hours for Creamelt 701. The crystallisation process, in both cases, was followed by plotting rheograms of both G' and G" moduli as a function of crystallisation time. These parameters were chosen as they reflect both the solid and liquid nature of the fat. G' is known to depend on the solid (elastic) characteristics of the system while G" reflects the liquid (or viscous) properties (Mezger, 2011). The rheograms were, then, compared with other crystallisation evidence from pNMR, XRD and microscopy (see Section 4.4 - 4.6).

The rheological profile gives a genuine indication of the crystallisation behaviour of the filling fats. The complex crystallisation mechanism of the fat molecules hinders the achievement of "noise-free" test results. Nonetheless, each measurement results in a consistent crystallisation pattern and the average values of three tests are presented. Although measurements were performed in triplicate, these graphs are representations of the typical curves obtained; these show similar pattern among the replicates (Figure 4.3 and Figure *4.4*). Since crystal growth occurred more gradually and the equilibrium was reached much later, Creamelt 601 was allowed to crystallise for a longer period. For Creamelt 701, measurements showed no change after a period of 15 hours so monitoring was restricted to this time period. Since fats are a mixture of different triacylglycerols, upon cooling of the fat sample the higher-melting triacylglycerols will tend to crystallise earlier from the liquid phase which will then be rich in the lower-melting ones (Chen *et al.*, 2002). As a consequence, different stages throughout the crystallisation process can be observed by changes in G' and G" moduli (Figure 4.2). Both filling fats showed three different regimes during the stationary crystallisation process with different behaviours. During the first 2 hours the moduli values of both fats were dominated by G", exhibiting mainly liquid characteristics. As nucleation took place, the first crystals developed from the melt, resulting in an increase of G' of both filling fats. At this point, onset of crystallisation of Creamelt 601 was observed to take place around 1 hour before Creamelt 701. This was caused by the high-melting TAGs (see Table 3.1 for composition) from the crystallisation initiator and also from the content of shea stearin components that crystallised earlier (PPP, PPS, PSS, SSS from the crystallisation initiator and SOS from the shea stearin). All of this promoting the crystallisation process and "seeding" the bulk fat.
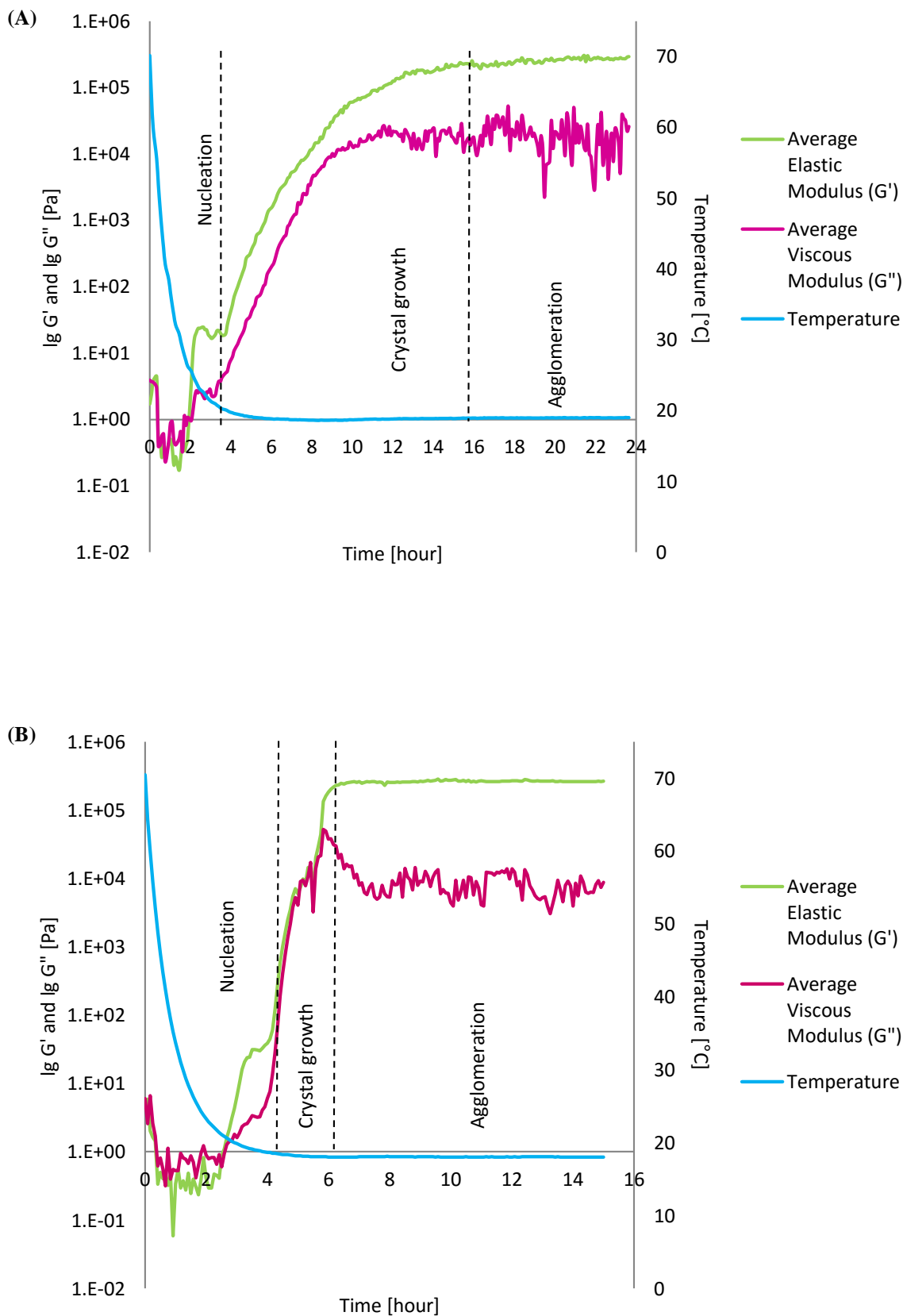
Figure 4.2 Average rheological crystallisation profiles of Creamelt 601 (A) and 701 (B) obtained from three replicates as shown in Figure 4.3 and Figure 4.4
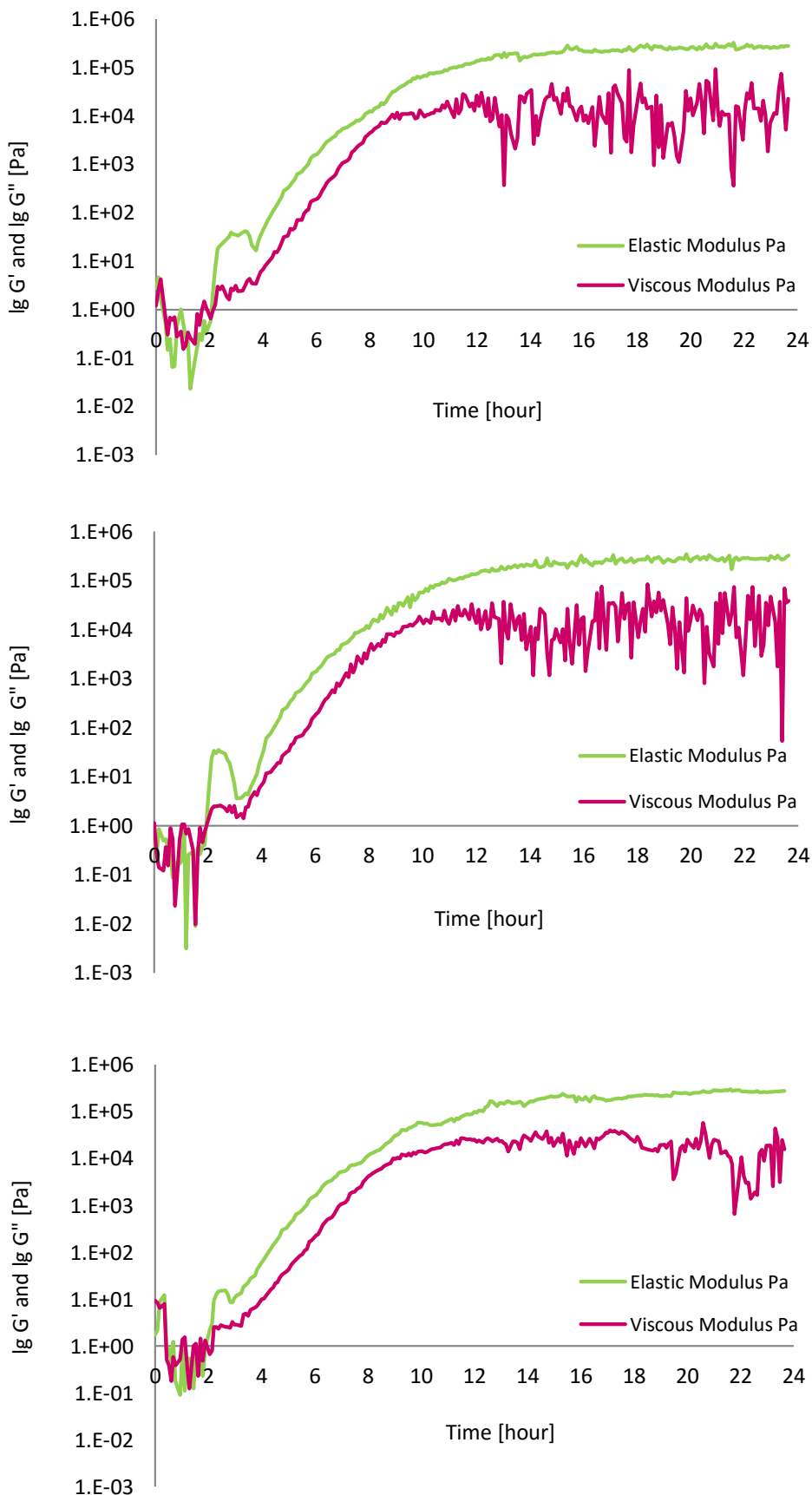
Figure 4.3 Typical rheological crystallisation profile of Creamelt 601, crystallising from 70°C to around 19°C at around 0.2°C.min⁻¹
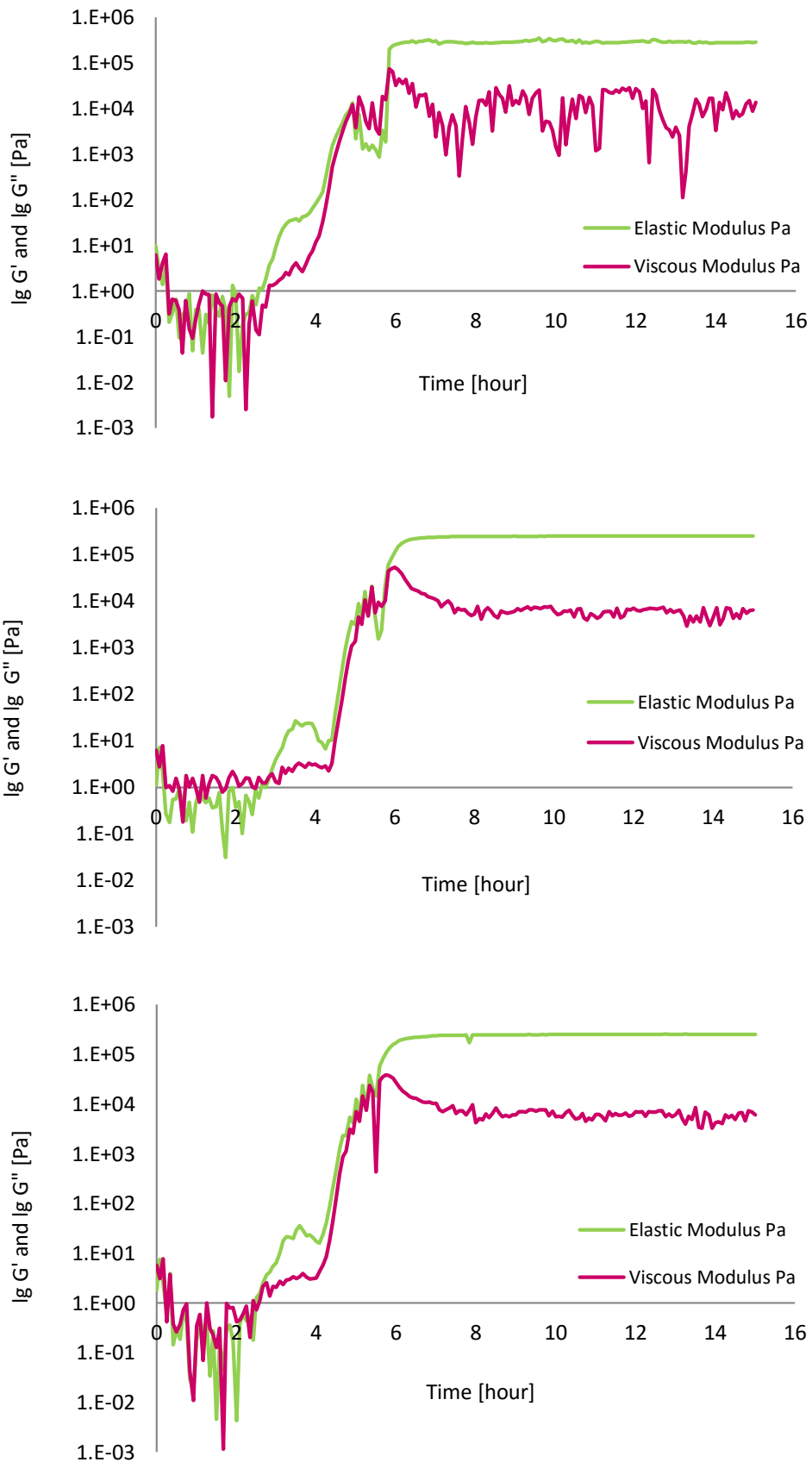
Figure 4.4 Typical rheological crystallisation profile of Creamelt 701, crystallising from 70°C to around 19°C at around 0.2°C.min⁻¹

With further decrease in temperature and increased solidification of the fat, G' became greater in value than G" as expected, indicating more energy stored in the solid during the deformation process (see Section 1.3.5). These results indicate that the samples exhibit a viscoelastic system in which the G' was eventually greater than G". It was observed that the G' of both filling fats started to rise at around 24ºC, suggesting that nucleation occurred before ambient temperature was reached. In the case of Creamelt 601, the moduli values were roughly stable for the period of 2 and 3 hours in the experiment suggesting that no additional crystallisation occurred (De Graef *et al.*, 2006). This does not, however, exclude the possibility that a secondary structure is developing at the expense of the former which may not be evident in the rheological results.

Different profiles for the two fats can also be seen after 3 hours of cooling i.e. Creamelt 601 exhibited a gradual moduli increase, while Creamelt 701 showed a much steeper rise in the values of the moduli. Thus, Creamelt 701 reached the equilibrium state at a much faster rate than Creamelt 601; specifically, Creamelt 701 entered the "equilibrium" state after about 6 hours, while Creamelt 601 reached the same stage after about 16 hours. Throughout the end period of the experiment, clear separation between G' and G" values was observed for both fat systems and their separation was maintained at a relatively constant value when both storage (elastic) and loss (viscous) moduli reached a plateau as maximum crystal formation was achieved. It is normal to expect the divergence between G' and G" values to increase with crystallisation time as the liquid phase is reduced due to the increase of solid phase formation. At the later stage of crystallisation, the aggregating crystals dissipate much less energy, causing much lower G" (De Graef *et al.*, 2006). The rheological crystallisation profiles of Creamelt 601 and Creamelt 701 show similarity in overall shape to that of the complex modulus of *trans*-free palm oil based confectionery fats observed by De Graef *et al.*

(2007), where the fats were rapidly crystallised at 10°C (Figure 4.5). Similar crystal growth behaviour i.e. gradual and fast modulus increases were also observed in the confectionery fat samples with different fatty acid and triacylglycerol composition. In this case, more gradual growth is seen in the fat sample with a lower ratio of saturated/unsaturated fatty acid content which is also the case for Creamelt 601 (see Table 4.2). Furthermore, unlike in Creamelt 601 and 701, a more rapid nucleation process was observed in both confectionery fat samples. This may be due to the more rapid cooling rate (5°C.min^{-1}) used in the crystallisation process, that resulted in a higher overall crystallisation rate including nucleation (Pérez-Martínez *et al.*, 2007). Neither of these studies analysed the G' and G" directly as a function of cooling, however, a brief consideration of the phase angle and the complex moduli data would suggest similar overall behaviour (Ferry, 1980).
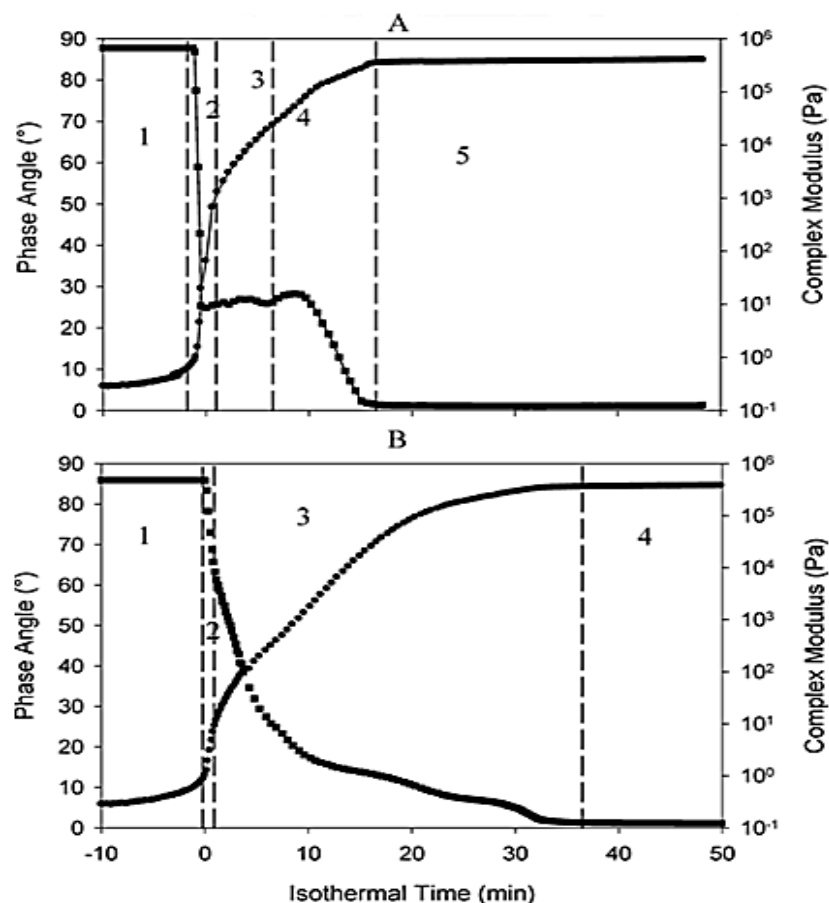
Figure 4.5 Phase angle (■) and complex modulus (•) of confectionery filling fats with higher (A) and lower (B) ratio of saturated/unsaturated fatty acid content as a function of isothermal time at 10°C obtained with oscillatory rheology. The evolution of phase angle and complex modulus can be divided into different sections indicating: fully melted sample (1), crystallisation in the α polymorph (2), transformation from α into β' polymorphs (3), aggregation and network formation (4) and completely crystallised sample (5) (De Graef et al., 2007)

4.4 Compositional Analysis

In this study, rheological analysis is performed as a mechanical testing tool to investigate the underlying crystal network. Since factors like triacylglycerol composition, polymorphism and microstructure are known to have a significant influence on the macroscopic mechanical strength of the fat crystal network (Narine and Marangoni, 1999a), investigation of the other structural properties of the filling fats in relation to their rheological behaviour was necessary.

Information about the composition of triacylglycerols, fatty acids and solid fat content were provided by Loders Croklaan B.V., The Netherlands and presented in Table 4.1 - Table 4.3. These measurements were done by the quality control analysts in Loders Croklaan B.V., The Netherlands using their standard method of analysis for this particular type of filling fats.

4.4.1 Triacylglycerol Composition

The variations between the two filling fats certainly depend on the components present in the formulations. PMF is known to have concentrated levels of POP and POS (Pease, 1985), while shea is an SOS-rich type of fat (Gunstone *et al.*, 2007). The POP, POS and SOS composition of PMF, shea butter and shea stearin are presented in Table 1.6 (Section 1.3.1). In addition, 26% SOO was also present in shea butter (Gunstone, 2011). In shea stearin, a low concentration of SOO (around 4%) and SSS (around 2%) was also evident (Ray *et al.*, 2013).

The triacylglycerols of Creamelt 601 and 701 are predominantly composed of POP, POS and SOS (Table 4.1). Small amounts of the high melting triacylglycerols such as PPP (1,2,3-tripalmitoyl-glycerol), PPS (1,2-dipalmitoyl-3-stearoyl-glycerol), PSS (1-palmitoyl-2,3-distearoyl-glycerol) and SSS (1,2,3-tristearoyl-glycerol) were also found in the compositional analysis. More of the high melting triacylglycerols were present in Creamelt 601 due to the presence of the crystallisation initiator. Higher SSS, SOS and SOO (1-stearoyl-2,3-dioleoyl-glycerol) content in Creamelt 601 are seen as a contribution from both shea butter and shea stearin present in the formulation, while the reduced content of these particular triacylglycerols in Creamelt 701 was mainly caused by the lack of shea stearin in the formulations. While, OOO (1,2,3-trioleoyl-glycerol) and PLP (1,3-dipalmitoyl-2-linoleoyl-glycerol) are present as characteristic of palm oil (Lipp and Anklam, 1998), POO (1-

palmitoyl-2,3-dioleoyl-glycerol) are typically contributed by the PMF (Pease, 1985). The higher concentration of PLP in Creamelt 701 indicates higher level of PMF in the formulation that results in higher solid fat content (see Section 4.4.3) and faster crystal growth as shown by the rheograms. As discussed by Bell *et al.* (2007), rheological changes during crystallisation of different fat blends were strongly influenced by the composition of the fat. At the onset of nucleation of palm oil crystallisation, Sulaiman *et al.* (1997) found high concentration of tri-saturated TAGs including PPP and PPS in the solid phase. Okawachi et al. (1985) determined the relation between addition of PPP and properties of fats and chocolate products, where PPP acts as a seed that accelerates the fat crystallisation process. It may, then, be deduced that small amounts of the high-melting TAGs like PPP, PPS, PSS and SSS, contributed by the crystallisation initiator and shea stearin, were responsible for decreasing the time for onset of crystallisation quite significantly. With the high concentration of SOS present, it is reasonable to expect Creamelt 601 to crystallise at a reasonably fast rate. However, in comparison to Creamelt 701 with a considerably lower concentration of SOS, Creamelt 601 exhibited contradictory behaviour. As explained by Danthine *et al.* (2014), this may be due to the high amount of SOO in the composition, that could interact with the SOS crystals surface, giving a poisoning effect that hinders crystal growth i.e. lower crystallisation rate.

Table 4.1 Triacylglycerol composition [%] of different confectionery fats

Triacylglycerol		Creamelt 601 (Werleman, 2012)[*]	Creamelt 701 (Werleman, 2012)[*]	*trans*-free palm-based filling fat (Vereecken et al., 2007)	Cocoa butter (Lipp et al., 2001)	CBE (Lipp et al., 2001)
1,2,3-tripalmitoyl-glycerol	PPP	1.7	1.8	2.4	n/a	n/a
1,2-dipalmitoyl-3-stearoyl-glycerol	PPS	0.9	0.4	n/a	n/a	n/a
1,3-dipalmitoyl-2-oleoyl-glycerol	POP	41.2	54.9	47.3	18.27	41.58
1,3-dipalmitoyl-2-linoleoyl-glycerol	PLP	5.5	7.8	9.3	1.82	3.43
1-palmitoyl-2,3-distearoyl-glycerol	PSS	0.5	0.1	n/a	n/a	n/a
1-palmitoyl-2-oleoyl-3-stearoylglycerol	POS	9.6	10.4	9.2	42.08	18.41
1-palmitoyl-2,3-dioleoyl-glycerol	POO	6.2	6.8	12.7	5.58	6.80
1,2,3-tristearoyl-glycerol	SSS	0.5	0.1	n/a	n/a	n/a
1,3-distearin-2-oleoyl-glycerol	SOS	15.2	4.2	n/a	26.39	24.80
1-stearoyl-2,3-dioleoyl-glycerol	SOO	5.8	2.6	1.6	4.64	3.47
1,2,3-trioleoyl-glycerol	OOO	1.6	1.1	2.1	1.23	1.52
Saturated-Unsaturated-Saturated	S-U-S	66	69.5	56.5	86.74	84.79

[*] data provided by Loders Croklaan B.V., The Netherlands

In general, the TAG compositions of Creamelt 601 and 701 are comparable to the TAG composition of the filling fats used by Vereecken *et al.* (2007) in which the palm oil nature is clearly indicated and it would appear the differences seen are due to the crystallisation initiator and shea components present in the filling fat formulations. In addition, compositional differences between the filling fats, cocoa butter and CBE can also be seen in the sum of symmetrical (saturated, unsaturated, saturated or S-U-S) TAGs namely POP, POS and SOS. The high concentration of S-U-S TAGs (sum of POP, POS and SOS) is known to give cocoa butter the desired functionality to be used in confectionery applications i.e. mouth feel, snap and gloss (Lipp *et al.*, 2001). As a cocoa butter alternative, relatively high S-U-S TAG concentration is commonly found in CBEs to obtain similar physical and sensory properties to cocoa butter. Compared to cocoa butter and CBEs, the S-U-S TAG concentration in filling fats is commonly much lower. However, it can be seen that higher S-U-S TAG concentration is achieved by using palm oil and shea fractions.

4.4.2 Fatty Acids Profile

Since it is evident that the use of lauric fat in the presence of cocoa butter in confectionery applications results in fat bloom formation (Traitler and Dieffenbacher, 1985), the absence of lauric acid (C12:0) in this type of filling fat makes them fully compatible for use with cocoa butter (Table 4.2). The filling fats used in this study were mainly comprised of palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids with a major difference in the concentration of palmitic and stearic acids (Table 4.2) residues. For palm mid fractions, 55.7% palmitic, 6% stearic, 32.4% oleic and 3.7% linoleic acids were found (Md.Ali and Dimick, 1994), while shea mainly contains 4-8% palmitic, 36-42% stearic, 45-50% oleic and

4-8% linoleic acids (Gunstone, 2011). Lower oleic and higher stearic concentrations of 32.8 and 62.3%, respectively were found in shea stearin (Kang *et al.*, 2013). Thus, it can be deduced that high amounts of palmitic and oleic acids in Creamelt 601 and 701 are mostly contributed by the palm mid fractions and the shea butter. A considerable amount of stearic acid was also found due to the shea stearin and shea butter used in the filling fats formulation. The lack of shea stearin in the Creamelt 701 formulation resulted in less stearic acid and to maintain the characteristics of a filling fat, the proportion of the palmitic acid was increased by adding more of the palm fractions into the formulation that resulted in the higher concentrations of palmitic acid. Palm oil is responsible for the contribution of palmitic acid, which represented about 36.2 and 49 % of the total fatty acids in the mixture of Creamelt 601 and 701 respectively. By taking into account that palmitic acid in the palm oil mid fraction is typically 55.7% (Md.Ali and Dimick, 1994), it is reasonable to conclude that the Creamelt 601 and 701 contained no less than 65% and 88% of palm mid fractions respectively. The fact that the increase of G' at the beginning of the crystallisation process occurred sooner in the case of Creamelt 601 is consistent with the higher concentration of stearic acid since the longer fatty acid chains would tend to cause faster crystallisation due to the presence of higher melting triacylglycerols. Moreover, higher content of saturated fatty acids in Creamelt 701 contributed to the steeper rise of moduli values during the crystal growth stage as they have higher melting points and expected to crystallise earlier. Since compositional differences between different filling fats can be explained by the ratio of saturated/unsaturated fatty acids (De Graef *et al.*, 2007), the rapid crystal growth in Creamelt 701 also reflected the higher ratio of saturated to unsaturated fatty acids.

Table 4.2 Fatty acid composition [%] of different confectionery fats

Fatty acid	Scientific name	Creamelt 601 (Werleman, 2012)[*]	Creamelt 701 (Werleman, 2012)[*]	*trans*-free palm-based filling fat (Vereecken *et al.*, 2007)	Cocoa butter (Pease, 1985)	Commercial CBE (Kyu Kang, 2013)
C12:0	Lauric	0.2	0.2	0.2	trace	n/a
C16:0	Palmitic	36.2	49.0	50.3	25.2	26.2
C18:0	Stearic	19.3	8.5	5.0	35.5	36.8
C18:1 cis	Oleic	36.6	35.0	30.2	35.2	33.0
C18:2	Linoleic	5.4	5.3	7.4	3.2	2.1
trans	*trans*- fatty acids	0.2	0.1	0.2	n/a	n/a
sat./unsat	saturated/unsaturated	1.33	1.43	1.48	1.58	1.79

[*] data provided by Loders Croklaan B.V., The Netherlands

4.4.3 Solid Fat Content (SFC)

Given that this particular type of filling fat is designed as a chocolate filling, there is a specific melting profile required; this can be represented by plotting the solid fat content of the fat as a function of different practical and reasonable temperatures i.e. ambient to near mouth temperatures. The melting profile can be a useful indicator in determining the physical characteristics of the fat. Based on the interpretation of SFC curve (Figure 1.4B) in Section 1.2.1, these filling fats melted quickly over a narrow range of temperatures, giving a cool-melting sensation with good flavour release upon consumption (Figure 4.6). It was found that there was about 5% solid at 30°C and almost no solid at 35°C to ensure an excellent clean-melting profile (Table 4.3). Furthermore, the presence of around 43% and 67% solid fat at 25 and 20 °C respectively demonstrated both the required hardness and the heat resistance of the fat.

Based on the solid fat content measurement, 1% of solid from the Creamelt 601 melt was observed at 35°C. This 1% of solid fat correlates with the amount of tri-saturated high-melting TAG and, hence, can be linked to earlier nucleation. This could also account for the higher G' of Creamelt 601 during the first crystallisation period. Since the mechanical behaviour of a fat is partly influenced by the solid fat content (DeMan *et al.*, 1976), the rapid increase in rheological parameters of Creamelt 701 is consistent with the higher percentage of solids at 25 and 20 °C. Also, after between 4 and 5 hours crystallisation time for Creamelt 701, the divergence between G' and G" was reduced. This may also be caused by the relatively fast crystallisation process that does not allow clear separation between the solid and liquid phase of the material (rapidly crystallising material "entrapping" the liquid phase).
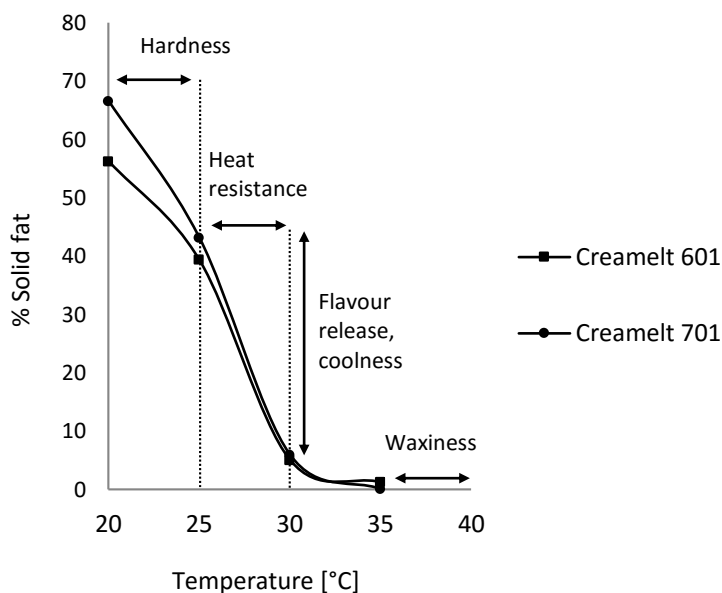
Figure 4.6 Traditional interpretation (see Figure 1.4) of the SFC curve of Creamelt 601 and 701

Table 4.3 Solid fat content measurement of Creamelt 601 and 701 by pNMR (Werleman, 2012)[*]

Temperature [°C]	Creamelt 601 [% solid]	Creamelt 701 [% solid]
20	56.3	66.6
25	39.4	43.2
30	5.1	6.0
35	1.3	0.1

[*]data provided by Loders Croklaan B.V., The Netherlands

Figure 4.7 presents the SFC curves of a CBE, polymorphic type of filling fat (Talbot, 2008), palm-based trans-containing and *trans*-free filling fats (Vereecken *et al.*, 2007). Both filling fats used in this study are polymorphic filling fats that need tempering for application purposes. Although the solid fat content of the filling fats is mostly lower than that of a CBE, they exhibit the same sharp melting profile that enriches the coolness sensation. In comparison to the general polymorphic type of filling fat, the filling fat samples show higher solid fat percentages at 20 and 25°C. At this temperature, solid fat content of the filling fat

samples are more similar to that of the CBE that often contains the same components i.e. PMF and shea (Figure 4.7A). Moreover, a less sharp melting profile is seen from the *trans-free* (TF) filling fat (Figure 4.7B) that is based on fractionated palm oil. Evidently, a sharper melting profile that is similar to that of cocoa butter and CBE can be achieved by blending PMF and shea fractions. PMF, which often used as the main component in a CBE, exhibits a short melting range similar to that of cocoa butter and typically has a steeper SFC curve compared to palm oil (Deffense, 1985).
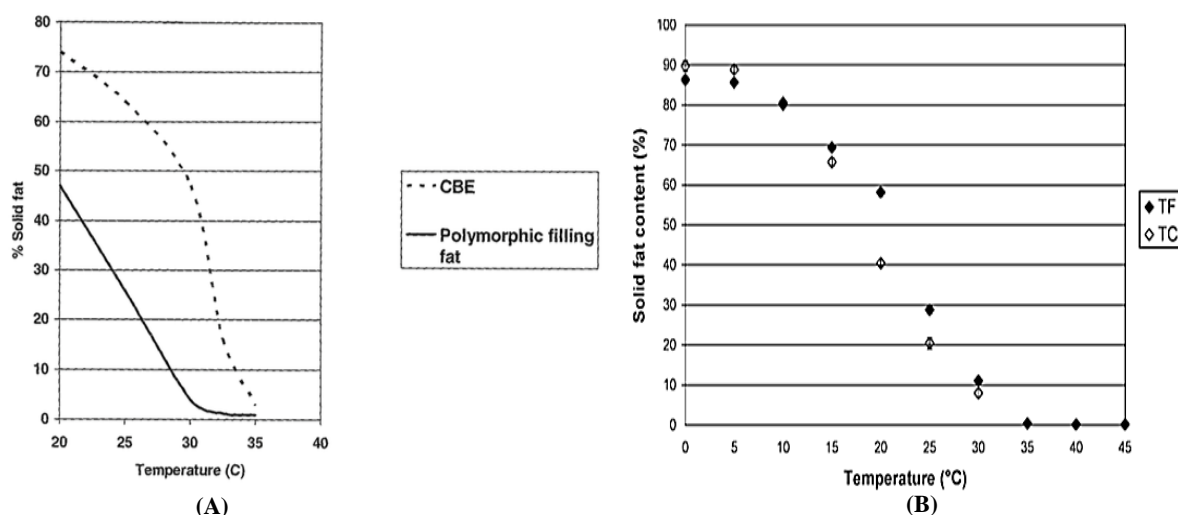
Figure 4.7 SFC curves of CBE and polymorphic filling fat (A) (Talbot, 2008); trans-containing (TC) and trans-free (TF) filling fats (B) (Vereecken et al., 2007) at different temperatures

4.5 Polymorph Identification

By applying a similar theory of using values of the phase angle to indicate different polymorphic forms as used for the crystallisation of cocoa butter (Toro-Vazquez *et al.*, 2004) and palm oil (De Graef *et al.*, 2006) to filling fats, the first increase in G' can be associated with the early crystal formation of high-melting triacylglycerols or the formation of a less

stable polymorph as the samples approach the crystallisation temperature. Moreover, the subsequent increase during the second time period can be linked to polymorphic transitions resulting in the formation of more stable polymorphs and crystallisation of those more stable forms directly from the melt. To confirm this hypothesis, the polymorphic behaviour of the filling fats was studied using powder X-ray diffraction analysis. The same temperature-time program as used for the rheological tests was applied and the diffraction pattern at various isothermal times in relation to the rheological measurements was measured. The filling fats were crystallised at 0.2°C.min^{-1} from 70 to 19°C. They were left to further crystallise at this temperature for 24 and 12 hours for Creamelt 601 and 701, respectively. Once the crystallisation temperature was reached, signals between 12.5 and 27.5° 2-theta (2θ) angle were recorded every 30 minutes. The patterns obtained are presented in Figure 4.8 and Figure *4.9*. The overall d-spacing values of Creamelt 601 and 701 are presented in Table 4.4. Using the short spacing data in Table 1.6 (Section 1.3.3), the polymorphic behaviour of the filling fats was determined. Unlike Creamelt 701, a weak signal at 4.16 Å was noticed in the Creamelt 601 sample as soon as the crystallisation temperature was reached. This peak disappeared after 2.5 hours isothermal treatment as weak β' peaks started to appear, suggesting early formation of α polymorph by the crystallisation initiator and transformation of the α crystals into β'. By 12 hours of isothermal time, significantly stronger β' peaks were seen at 3.95 Å together with a broad peak consisting of medium and weak signals at 4.40, 4.29 and 4.23 Å that also indicate the presence of the β' polymorph. Furthermore, these signals grew stronger in intensity as crystallisation progressed but remained with β' characteristics. With the absence of the α polymorph signal in the Creamelt 701 sample, the overall XRD pattern indicates direct development of the β' polymorph from the melt. This was identified by prominent β' peaks between 3.93 and 4.42 Å.
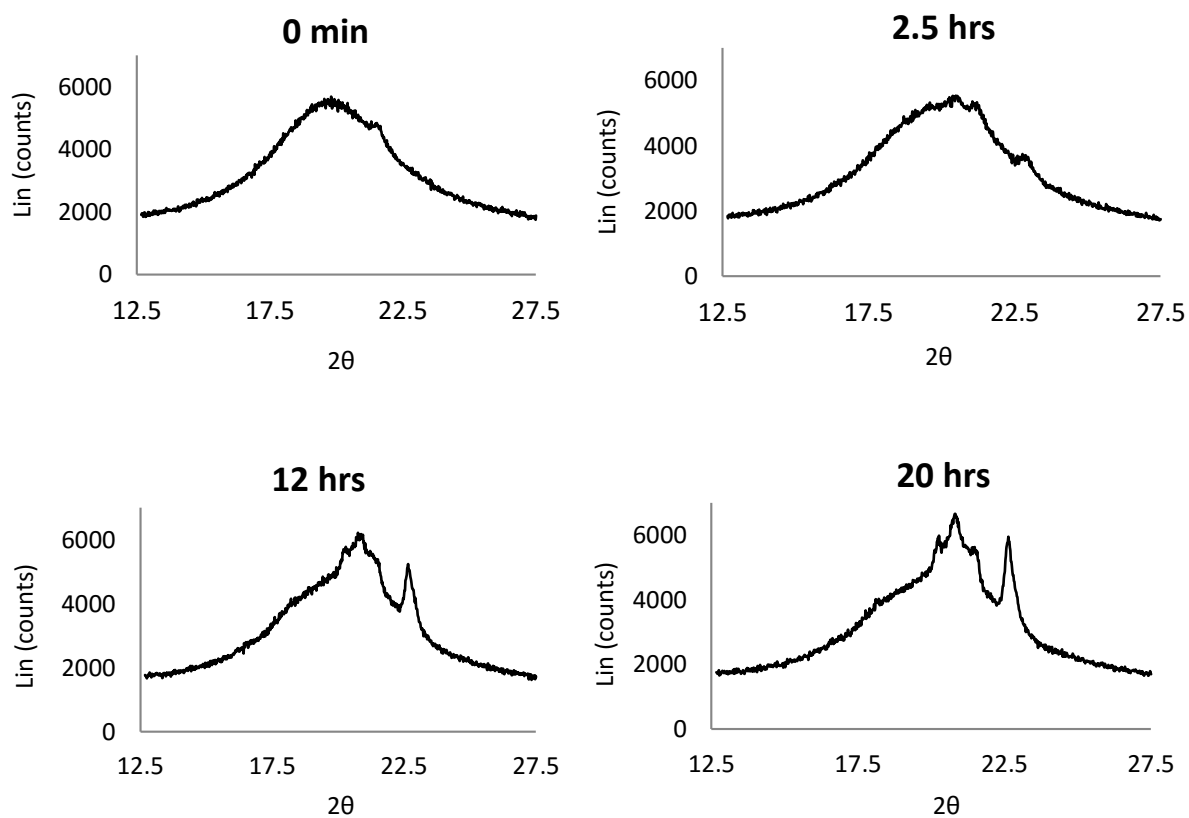
Figure 4.8 Powder XRD pattern of Creamelt 601 crystallised for different isothermal periods at 19ºC
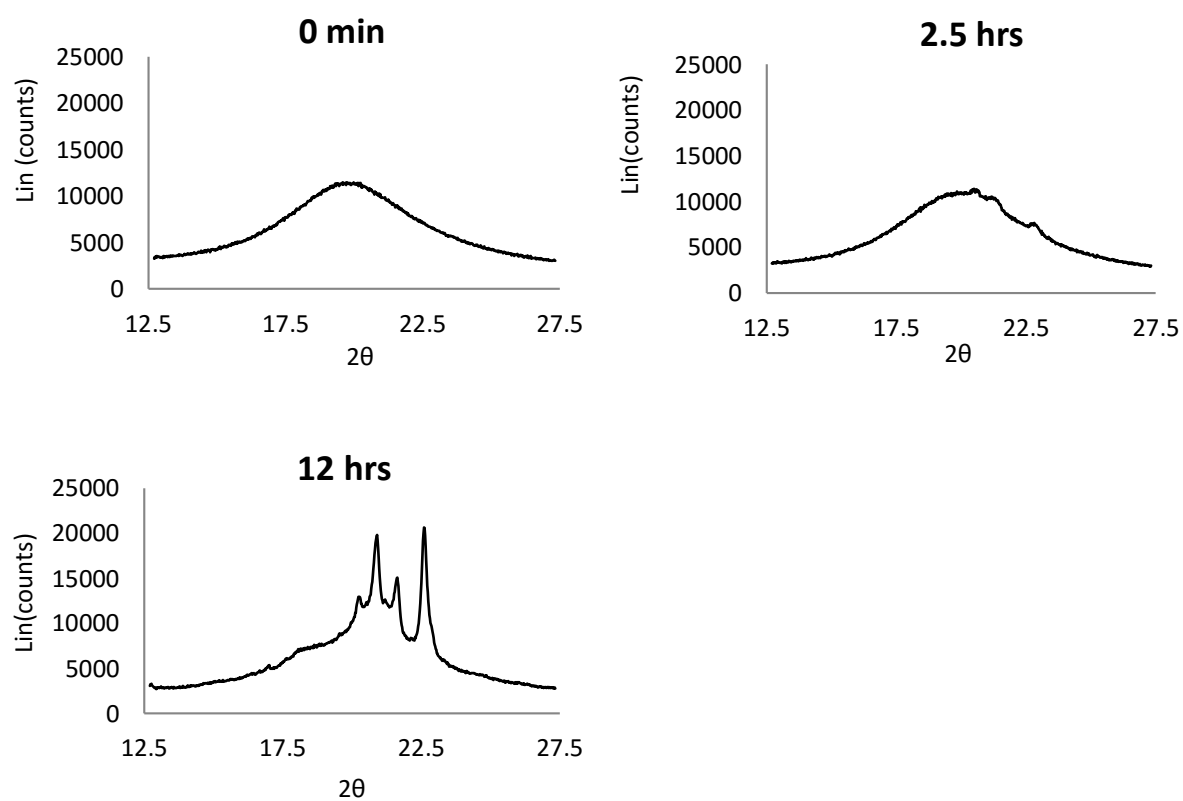
Figure 4.9 Powder XRD pattern of Creamelt 701 crystallised for different isothermal periods at 19ºC

Table 4.4 Short spacing data of Creamelt 601 and 701 at different isothermal periods

Filling fats	Isothermal time	Short spacing (Å)[*]	Polymorph
	0 min	4.16w	α
Creamelt 601	2.5 hrs	4.37w, 4.23w, 3.93w	β'
	12 hrs	4.40w, 4.29m, 4.23w, 3.95s	β'
	20 hrs	4.42m, 4.30s, 4.20w, 3.96s	β'
	0 min	-	
Creamelt 701	2.5 hrs	4.35w, 4.23w, 3.93w	β'
	12 hrs	4.42w, 4.29s, 4.14m, 3.96s	β'

[*]w: weak; m: medium; s: strong

The short spacing data demonstrate that the β' polymorph was dominant throughout the crystallisation process for both filling fats. Unlike cocoa butter which is known to be stable in the β polymorph (D'Souza, 1990), Yap *et al.* (1989) reported that palm oil and palm oil products are stable in the β' polymorph (see Section 1.3.3). POP, which is normally found in palm oil, is known to be stable in β polymorph (Sato *et al.*, 1989). Due to its high POP content, PMF would essentially be stable in β polymorph. However, PMF is highly meta-stable in the β' polymorph partly due to PPO (1,2-dipalmitoyl-3-oleoyl-glycerol) (Smith, 2016) that will consistently be found in the TAGs mixture of PMF (Bhaggan, 2016). Due to the deodorisation process at high temperature (see Table 1.1), some randomisation of TAG occurred and as a result, a low level of PPO will be found after fractionation process i.e. 5 - 10 % of POP level in the PMF is PPO (Bhaggan, 2016). Unlike POP, PPO is known to be stable in the β' polymorph (Minato *et al.*, 1997). Other than PPO, Ali *et al.* (2001) also found that POO and PPP from PMF resulted in β'-stable polymorphic behaviour. Since the main component in the two filling fat formulations is PMF, it is reasonable to expect the filling fats to be β'-stable. Fat polymorphism is greatly influenced by the crystallisation conditions including cooling rate (Sato, 2001). A slowly cooled liquid fat generally produces a more stable crystal form due to the slow crystallisation kinetics, allowing sufficient time for the TAG molecules to become orientated and to co-crystallise with each other (Campos *et al.*,

2002). Rapid cooling rate, however, commonly results in the formation of a less-stable polymorph that quickly transforms into a more stable one (Metin and Hartel, 2005). That being said, this study found a small amount of α crystals and their transformation to β' early in the crystallisation process of Creamelt 601. Similarly, formation of the α polymorph under rapid cooling conditions was also seen in the crystallisation study of milk fat (Ten Grotenhuis *et al.*, 1999) and palm oil-based shortening (Litwinenko *et al.*, 2002). Short spacing length and polymorphic forms of natural and commercial solid fats, including palm oil and shea were reviewed by D'Souza (1990) and are presented in Table 4.5. The short spacing data of Creamelt 601 and 701 are similar to those exhibited by palm oil and shea. Based on the short spacing data of POP, POS and SOS recorded by Sato *et al.* (1989), it can be seen that the polymorphs are typical for mixtures of POP, POS and SOS. The reported short spacing signal of α polymorph formed by PPP and SSS at 4.15 Å by Ishikawa *et al.* (1997) supports the suggested formation of α crystals by the high-melting TAGs in Creamelt 601. Although shea is known to be β-stable, the addition of palm oil in fat blends is known to inhibit the polymorphic transition into β crystals (Berger, 2001). With higher proportion of palm oil in the formulations, it is expected to find the filling fats to be stable in the β' polymorph. In a study of crystallisation behaviour of filling fats, Vereecken *et al.* (2007) hypothesised α crystal formation and transformation into β' as well as development of β' crystals from the melt. However, no XRD analysis was performed to confirm this hypothesis in that report.

Table 4.5 Short spacing length and polymorphic form of palm oil, shea, POP, SOS and POS

Compound/product	Short spacing (Å)	Polymorphic forms
Palm oil (D'Souza, 1990)	4.35m, 4.20s, 3.87s	β'
Shea (D'Souza, 1990)	4.55s, 4.03m, 3.97m, 3.73m, 3.68m	β
POP (Sato *et al.*, 1989)	4.17	α
	5.22, 4.74, 4.47, 3.88, 3.58, 4.32, 4.11	β'
SOS (Sato *et al.*, 1989)	4.19	α
	4.59, 4.40, 4.21, 4.07, 3.76	β'
POS (Arishima *et al.*, 1991)	4.23	α
	4.30, 4.13, 3.85	β'

4.6 Microstructure Observation

To understand the structural features of the filling fats, the changes in microstructure were also observed under the same time-temperature program as the rheological measurement in Section 4.3. The molten fat sample (70°C) was subjected to crystallisation at 19°C under controlled conditions using a hot stage (see Section 3.3) and viewed under the polarised light microscope. A slow cooling rate of $0.2°C.min^{-1}$ was used and 20x magnification images are presented in Figure 4.10. As the crystallisation temperature was reached, several spherulitic crystals were initially formed from the melt of both filling fat samples (Figure 4.10A,D). The appearance of crystals from Creamelt 701 at the early stage of crystallisation process did not support the identification of the polymorph determined by XRD (Figure 4.10D). Normally, sufficient amount of properly oriented crystals are present to diffract the incident rays (deMan, 1992). In this case, the amount of crystallised material orienting in a proper manner may not be sufficient to diffract the incident rays and produce any peak. This different results between XRD and PLM was also observed by De Graef *et al.* (2008) during the

crystallisation study of palm oil at 18°C under 10 s^{-1}. The study suggested that this particular occurrence can be due to different instruments sensitivity to detect the onset of crystallisation (De Graef *et al.*, 2008). Early in the isothermal crystallisation process, a much larger crystal size was noticeable in the Creamelt 601 sample, for which the crystallisation initiator may be responsible (Figure 4.10A). It is assumed that once the nuclei are formed, they will act as seeds that quickly drive further crystal formation through secondary nucleation and growth in the liquid phase. However, crystal development was found to be relatively slow for Creamelt 601. There are two factors involved in the observed differences. Firstly, the amount of high-melting TAG that was at higher level in the Creamelt 601 and, hence, earlier crystallisation was initiated. Since crystallisation occurred at a higher temperature initially, fewer and larger crystals were formed than for Creamelt 701. Secondly, the overall amount of crystallisable TAG (both high- and medium-melting) will influence the driving force for crystallisation. Since the SFC at 20°C was higher for Creamelt 701, the supersaturation, and hence crystallisation driving force, is greater. Thus, once nucleated, Creamelt 701 crystallised more rapidly. Lack of agitation might be partly responsible for the relatively slow change in microstructure, but in this case the fat composition caused crystals in the Creamelt 701 sample to aggregate already after 60 minutes (Figure 4.10E). Aggregation is thought to be facilitated by the lower medium-melting TAG, of which there was more in the Creamelt 701 compared to the Creamelt 601. As the crystallisation process continued, particle clusters were formed as a result of the aggregation process. According to the rheological data, the crystallisation state of both filling fats had reached equilibrium after 6 and 16 hours for Creamelt 701 and 601 respectively. Nonetheless, the microscopic image of Creamelt 601 showed a great portion of liquid phase present even after 24 hours of isothermal crystallisation (Figure 4.10C). At the end of the crystallisation process, Creamelt 601 developed a needle-like structure on the surface of the aggregates, leaving much of liquid

phase trapped, while Creamelt 701 created a much more firmly arranged structure with very small liquid gaps (Figure 4.10F). Based on the overall visualisation, a different microstructure of the two filling fats was observed. Throughout the crystallisation process, Creamelt 701 created a crystal network containing grain-like crystals whereas Creamelt 601 formed spherulites with needle-like crystals on the surface. Such different crystal morphologies were also observed by Chen *et al.* (2002) i.e. spherical and needle-like shaped crystals during the isothermal crystallisation study of refined palm oil at around 18°C. In that study, the spherical and needle-like crystals were shown as α and β' crystals, respectively. Since crystal size and shape are known to relate to the polymorphic state of the fat (D'Souza, 1990), the different morphologies of Creamelt 601 and 701 are consistent with their different polymorphic behaviour. Since the XRD short spacing data indicate formation of α polymorph from Creamelt 601 (Table 4.4) the spherical shaped crystals at the initial crystallisation stage of Creamelt 601 correspond to α crystals (Figure 4.10A). As the isothermal time increased, the presence of the β' polymorph with needle-like shaped was observed on the surface of the α crystals (Figure 4.10C), perhaps after these latter had transformed into β'. For Creamelt 701, direct development of the β' polymorph from the melt was shown as numerous smaller grain-like crystals at the initial stage of crystallisation (Figure 4.10D). The β' crystals continued to grow rapidly and aggregate with each other into irregular shaped clusters (Figure 4.10E) that eventually created a firm crystal network (Figure 4.10F). This finding of different crystal morphologies was also reported by Litwinenko *et al.* (2002) in a crystallisation study of palm- and soybean-based shortenings at 22°C under a slow cooling rate, where two different networks of smaller, more numerous, and needle-like crystals were observed.
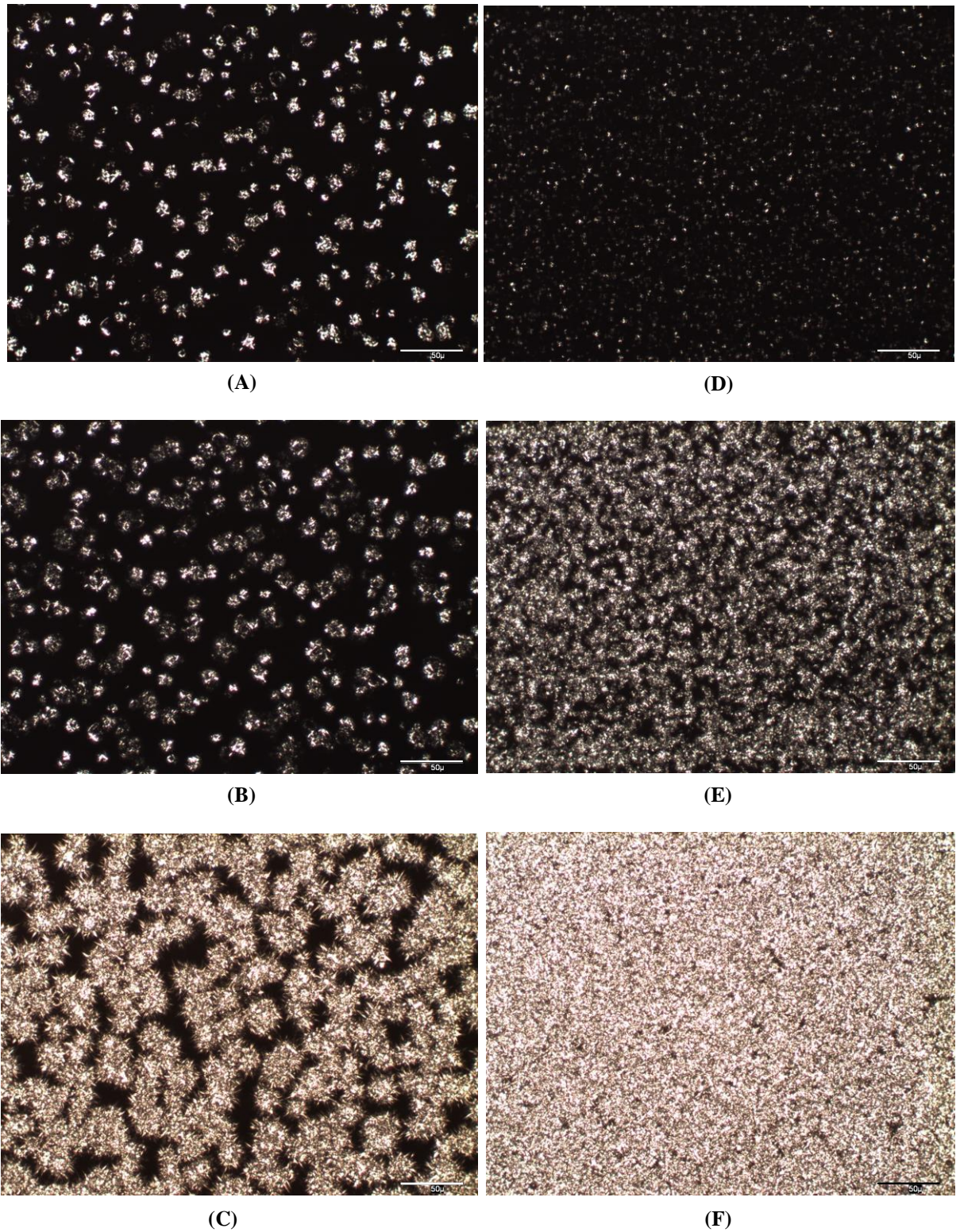
Figure 4.10 20X magnification typical images of isothermal crystallisation of filling fats at 19°C at different time intervals: Creamelt 601 initial stage of crystallisation (A), after 60 mins (B) and after 24 hrs (C); Creamelt 701 initial stage of crystallisation (D), after 60 mins (E) and after 24 hrs (F). The scale bar represents 50µ

4.7 Summary of Findings

A similar LVR between 0.1 to 1 % was found for both filling fats and 0.5% strain value was chosen to be used in further oscillation measurements (Figure 4.1). The crystallisation behaviour was studied with slow cooling without agitation. As the temperature decreased and the degree of solidification increased, viscoelastic properties of both filling fats were evident. Three different regimes that indicated nucleation, crystal growth and agglomeration were seen in both filling fats (Figure 4.2). Reasonably different behaviour was noticed during nucleation and crystal growth. At the beginning of the measurement, the crystallisation initiator present in the formulation of Creamelt 601 contributed to faster nucleation. The oscillation tests were able to recognise the onset of nucleation that occurred before the fats reached the crystallisation temperature. Furthermore, higher solid fat and saturated fatty acid contents in Creamelt 701 caused a more rapid crystal growth compared to Creamelt 601. The rheological crystallisation behaviour of the filling fats used in this study is similar to the rheological crystallisation profiles of trans-free palm oil based confectionery fats observed by De Graef *et al.* (2007), although in this study the complex moduli have been further resolved into their G' and G'' components.

Table 4.1 shows that the triacylglycerol compositions of the two filling fats are mainly composed of POP, POS and SOS, indicating the nature of palm oil. The compositional differences between the two filling fats were seen due to the crystallisation initiator and shea components present in the filling fat formulations. The main fatty acids in the filling fats are known to be palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids with a major difference in the concentration of palmitic and stearic acids (Table 4.2) residues. The differences were seen due to the different amounts of palm mid fractions and shea components in the formulations. Interpretation of SFC curve (Figure 4.6) shows that these

filling fats meet the required melting profile of a chocolate filling i.e. quickly melt over a narrow range of temperatures, producing a cool-melting sensation with good flavour release upon consumption. Around 1% solid fat was already observed in Creamelt 601 at 35°C, while Creamelt 701 indicated almost 0% solid fat at the same temperature. This small amount of solid fat was due to the tri-saturated high-melting TAGs in Creamelt 601 and can be linked to earlier nucleation shown by oscillatory rheology.

Unlike cocoa butter that is stable in the β polymorph, the filling fats were found to be β'-stable (Table 4.4). This polymorphic stability is expected as the major component in the filling fat formulations was PMF, which is known to be meta-stable in the β' polymorph. The presence of the α polymorph and the transformation into the β' form was evident in Creamelt 601 whereas the more commonly observed development of β' crystals directly from the melt was observed within Creamelt 701. Further differences between the two filling fats were seen in the crystal microstructure. Similar to microscopic observation during crystallisation of shortening by Litwinenko *et al.* (2002), two different networks were observed in the filling fats i.e. spherulites with needle-like crystals on the surface in Creamelt 601 and grain-like texture in Creamelt 701 (Figure 4.10). This different crystal morphology may be associated with the different polymorphs formed by the filling fats.

It was demonstrated that oscillatory rheology can be used to follow the crystallisation process of the filling fats under static conditions and study the crystallisation behaviour using rheological parameters (in this case the elastic and viscous moduli). In addition to the other static crystallisation analysis like XRD and microscopy, oscillatory rheology was able to provide more information in a single analysis including primary crystallisation, phase transition and microstructure development that will in turn determine the quality of the end product. Therefore, oscillatory rheology is believed to add great benefit as an alternative or

additional technique of analysis that can be used to evaluate the static crystallisation behaviour of filling fats.

CHAPTER 5. CRYSTALLISATION BEHAVIOUR OF FILLING FATS UNDER PROCESSING CONDITIONS

5.1 Introduction

As well as being dependent on the lipid composition, the physical properties of the fat crystal network are affected by the processing conditions such as cooling rate, crystallisation temperature and shear rate. Adjustment of the processing parameters will result in different structures and rheological behaviour of the fat (Marangoni and Litwinenko, 2005). In manufacturing of confectionery products like chocolate, tempering is a good example of how processing conditions can influence crystallisation of the fat component, which in turn determines the structure of the end products (Stapley *et al.*, 1999). In recent decades, the effects of processing parameters on the crystallisation behaviour of various fat-based materials have been studied by many scientists (Tarabukina *et al.*, 2009; Pérez-Martínez *et al.*, 2007; Sonwai and Mackley, 2006; Dhonsi and Stapley, 2006; Campos *et al.*, 2002; Herrera and Hartel, 2000a). In the food industry, shear flow is used to improve heat transfer and to encourage the development of textural characteristics. When crystallised under shear, crystallisation kinetics and the arrangement of the solid-liquid phases are greatly affected by the shear applied (Mazzanti *et al.*, 2011). Shear is known to influence the fat crystallisation process in different ways. Commonly it leads to the destruction of crystal networks, but it can also induce secondary nucleation or rearrangement of particles as the fat crystallises (De Graef *et al.*, 2008). In this study, oscillatory rheology is used to assess the crystallisation properties of filling fats under different processing conditions. To characterise the crystallisation behaviour, G' and G" were monitored as a function of crystallisation time. The

results were then compared with other crystallisation data obtained from DSC, XRD and PLM.

5.2 Rheological Behaviour

A rheological method using the oscillatory technique was developed by De Graef *et al.* (2008) to monitor crystallisation of palm oil under shear. It was suggested that oscillatory rheology can be used to study the crystallisation behaviour of fats in terms of rheological parameters. Toro-Vazquez *et al.* (2004) also demonstrated that oscillatory rheology was able to monitor the development of the crystal network during crystallisation of cocoa butter. In this study, oscillatory rheology was used to simulate the manufacturing of chocolate filled products where the filling fats were used (see Section 3.5.3). Based on the processing conditions, three different test combinations that primarily differ in their shear profile were designed to study not only the effect of shear rate on the crystallisation behaviour of the filling fats, but also the influence of applying shear in different stages. The shear profile during processing steps where the three test combinations differed is illustrated in Figure 5.1. Prior to the measurements, the filling fat sample was melted in a 70°C water bath with mild agitation (see Section 3.5). Each test combination was studied by initially melting the sample that was then kept isothermal at 70°C for 10 minutes. The shear cooling conditions applied represent those used in the manufacturing of confectionery products in which the filling fat is used. In the first test combination, shear was applied in both the first (from 70 to 22 °C) and second (from 22 to 10 °C) cooling steps (Figure 5.1B). Static cooling conditions were applied to study the effect of the shear application (Figure 5.1A). To study the influence of different shear conditions, sectional-shear cooling was applied i.e. the shear was only applied during the first cooling step (Figure 5.1C). Six separate results determined at different measurement

points were obtained from each test combination. The G' and G" at those points were monitored as a function of isothermal time and the mean value of three replicates is presented.
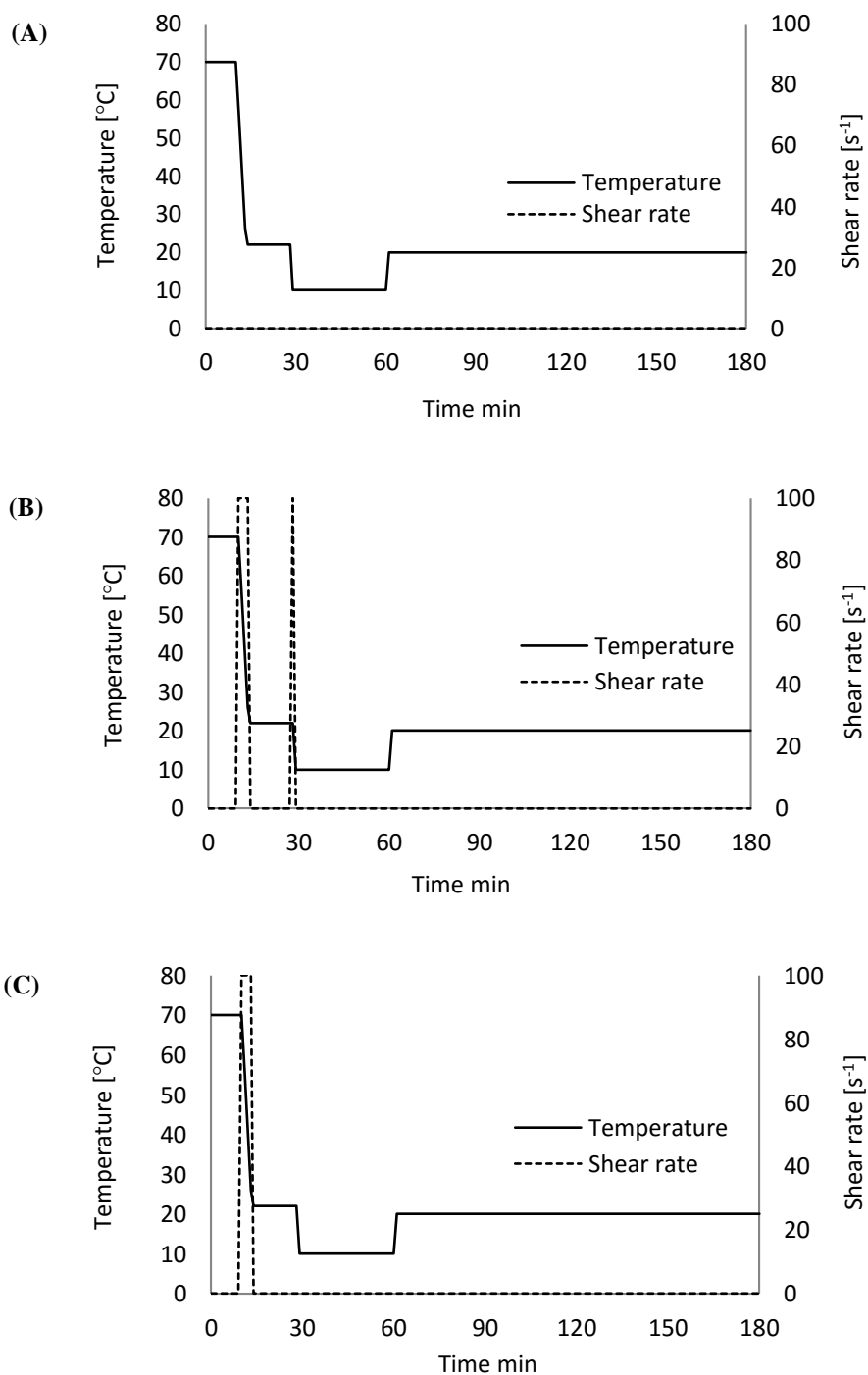
Figure 5.1 Schematic diagrams of the three different test conditions: static cooling (A), shear-cooling (B) and sectional-shear cooling (C)

5.2.1 Static Cooling

Rheological changes of the filling fats under the static crystallisation conditions are summarised in Table 5.1. At the beginning of the static crystallisation process the G" value was higher than G' in both filling fats, suggesting mainly liquid characteristics. This behaviour continued until the samples reached the first crystallisation temperature. During 15 minutes of isothermal crystallisation at 22°C, the viscoelastic properties of the filling fat samples were demonstrated as G' became higher than G". In the static isothermal crystallisation of palm oil at 18°C, De Graef *et al.* (2006) detected the onset of primary crystallisation i.e. formation of α crystals from the melt by a sigmoidal increase of the complex modulus (see Section 1.3.5.1). However, the study also found that the elastic modulus, G', showed the same pattern as the complex modulus, G*. Thus, the slight increase in the elastic modulus of the filling fats during crystallisation at 22°C can also be attributed to the onset of primary crystallisation.

Table 5.1 Rheological changes of Creamelt 601 and 701 during crystallisation under static cooling conditions

Processing steps	Creamelt 601	Creamelt 701
Isothermal at 70°C for 10 min	Average G': 2.26E-01 Pa Average G": 3.57E-01 Pa	Average G': 3.01E-01 Pa Average G": 4.04E-01 Pa
Cooling to 22°C at 10°Cmin^{-1}	Average G': 3.42E-01 Pa Average G": 4.96E-01 Pa	Average G': 3.11E-01 Pa Average G": 4.25E-01 Pa
Isothermal at 22°C for 15 min	G' slight increased to 1.70E+01 Pa G" slight increased to 7.93E+00 Pa	G' slight increased to 3.30E+01 Pa G" slight increased to 1.26E+01 Pa
Cooling to 10°C at 10°Cmin^{-1}	G' increased to 9.46E+04 Pa G" increased to 4.68E+04 Pa	G' increased to 1.46E+04 Pa G" increased to 5.76E+03 Pa
Isothermal at 10°C for 30 min	G' further increased to 1.52E+05 Pa G" further increased to 1.50E+04 Pa	G' further increased to 2.27E+05 Pa G" further increased to 4.13E+04 Pa
Heating to 20°C at 10°Cmin^{-1} and holding for 2 hours	Average G': 1.64E+05 Pa Average G": 1.10E+04 Pa	Average G': 2.20E+05 Pa Average G": 8.17E+03 Pa

With further decrease in temperature, a major increase of G' and G'' was seen (Figure 5.2), indicating more solid phase formation from the bulk fat and crystal growth of the existing crystals into crystal clusters. Similarly, a strong increase of G* was also seen in the static crystallisation study of palm oil at 18°C and this was interpreted as being due to the polymorphic transition from α to β' and direct development of β' polymorph from the melt (De Graef *et al.*, 2006).
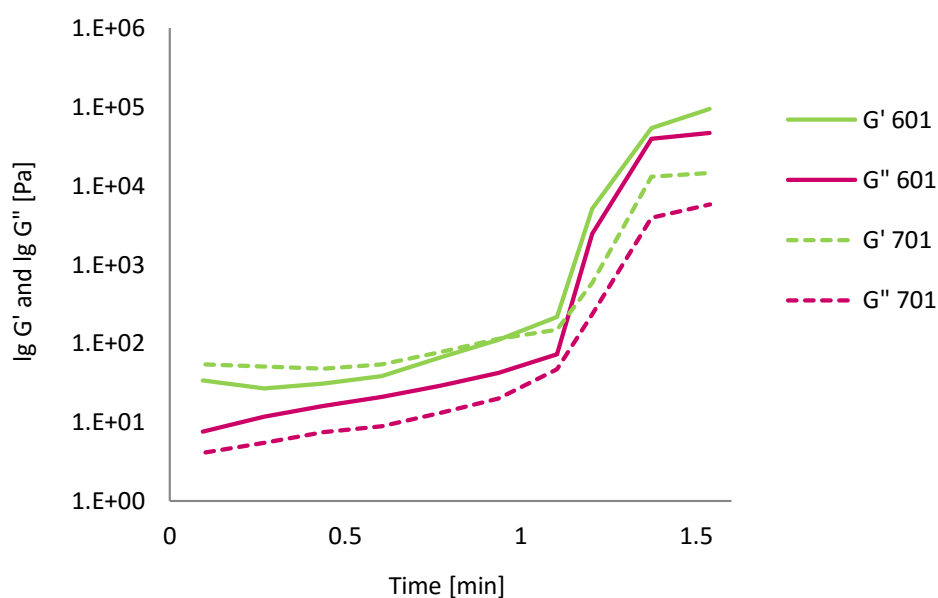
Figure 5.2 Average (n=3) rheological properties of Creamelt 601 and 701 during static crystallisation to 10°C

Further increase in G' occurred during 30 minutes of isothermal crystallisation at the lower crystallisation temperature, which can be attributed to the aggregation process. In the static crystallisation study of palm oil at 18°C, a further increase of G* was also seen and interpreted as being due to strengthening of the developing crystal network and aggregation (De Graef *et al.*, 2006). The moduli value of the two filling fats remained broadly constant even after the sample was heated to 20°C and kept at this temperature for 2 hours. Similar

behaviour was observed for the two fats at the end of the crystallisation process (see Appendix A).

5.2.2 Shear Cooling

Rheological changes during the crystallisation of the filling fats under shear were examined and are summarised in Table 5.2. As observed under static cooling conditions, liquid characteristics were dominant in both fat samples until the first crystallisation temperature was reached. Following the first cooling step under shear, slightly higher moduli values of Creamelt 601 were seen relative to the static cooling conditions. This may suggest enhancement of the crystallisation process by either seeding, formation of more crystals by secondary nucleation, or internal rearrangement of the crystal network that may result in more compact crystal packing. Furthermore, crystal aggregation may also be enhanced by shear application during the crystallisation process as it increases the frequency of collision of crystals (Kloek *et al.*, 2005). This is consistent with the findings of a faster crystallisation process at increasing shear rates reported by Toro-Vazquez *et al.* (2004), Dhonsi and Stapley (2006) and De Graef *et al.* (2008).

Table 5.2 Rheological changes of Creamelt 601 and 701 during crystallisation under shear cooling conditions

Processing steps	Creamelt 601	Creamelt 701
Isothermal at 70°C for 10 min	Average G': 3.64E-01 Pa Average G'': 5.36E-01 Pa	Average G': 2.73E-01 Pa Average G'': 3.08E-01 Pa
Cooling to 22°C at 10°Cmin^{-1}	Average G': 9.17E+00 Pa Average G'': 3.38E+01 Pa	Average G': 6.39E+00 Pa Average G'': 8.55E+00 Pa
Isothermal at 22°C for 15 min	G' slight increased to 5.96E+01 Pa G'' slight increased to 1.74E+01 Pa	G' slight increased to 2.43E+01 Pa G'' remained at 8.47E+00 Pa
Cooling to 10°C at 10°Cmin^{-1}	G' increased to 6.19E+02 Pa G'' increased to 5.87E+02 Pa	G' increased to 1.58E+02 Pa G'' increased to 1.41E+02 Pa
Isothermal at 10°C for 30 min	G' further increased to 2.31E+05 Pa G'' further increased to 8.98E+03 Pa	G' further increased to 2.08E+05 Pa G'' further increased to 1.23E+04 Pa
Heating to 20°C at 10°Cmin^{-1} and holding for 2 hours	Average G': 1.96E+05 Pa Average G'': 1.73E+04 Pa	Average G': 2.08E+05 Pa Average G'': 1.08E+04 Pa

Figure 5.3 presents the rheograms during the second cooling step of Creamelt 601 (A) and Creamelt 701 (B), respectively. At this stage, the effect of shear application was noticed. When cooled from 22 to 10 °C under shear, significantly lower moduli values were observed compared to the static cooling conditions as an indication that the force acting upon the fat crystals breaks down the crystal aggregates. When a dense-packing crystal network has been formed by the aggregates, the applied shear force leads to destruction of the network and internal rearrangement of the crystal fractions (Kloek *et al.*, 2005).

(A)

(B)
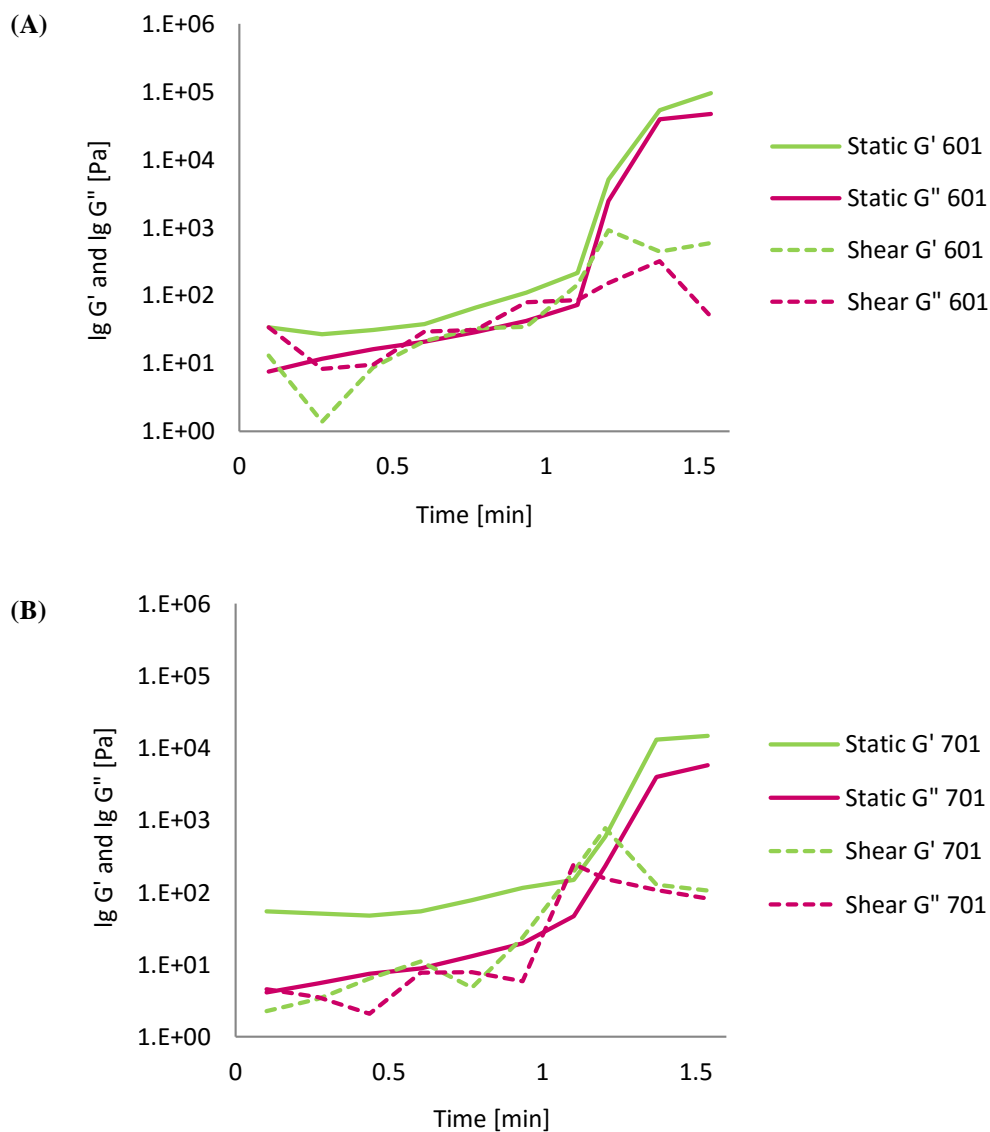
Figure 5.3 The effect of shear on the average (n=3) rheological properties of Creamelt 601 (A) and 701 (B) during cooling to 10°C

Furthermore, clearer separation between the solid and liquid characteristics of the viscoelastic system was achieved when cooling under static conditions. However, rheological properties for both the static and shearing conditions were found to be similar at the end of the crystallisation stages (see Appendix B).

5.2.3 Sectional-shear Cooling

For over a decade the effect of shear rate on crystallisation of fat materials has been studied (Tarabukina *et al.*, 2009; De Graef *et al.*, 2008; Sonwai and Mackley, 2006; Dhonsi and Stapley, 2006; Toro-Vazquez *et al.*, 2004; Stapley *et al.*, 1999). However, these studies often focus on the different magnitude and/or the duration of shear application. In this study, the effect of shear applied at different stages of processing was explored. This was done by observing the rheological behaviour of the filling fats under two different shearing conditions i.e. when shear was applied during both cooling steps and during the first cooling step alone. Table 5.3 outlines the rheological changes of Creamelt 601 and 701 when cooled from 70 to 22 °C at 100 s^{-1} shear rate, leaving the second cooling step from 22 to 10 °C without shear. Compared to the shear conditions described previously, sectional shear application caused a clear difference in the rheological behaviour during the second cooling stage. The absence of shear during cooling to 10°C resulted in higher moduli values (Figure 5.4), suggesting no damage to the crystal network during the second cooling stage as seen in the shear cooling conditions. This shows different effects of shear at different points of application during the crystallisation process. Shear application during cooling at a higher crystallisation temperature enhanced the crystallisation process, while shear application during cooling at a low crystallisation temperature resulted in crystal network destruction. At the lower crystallisation temperature, a firmer crystal network was formed, so there were no crystal collisions and rearrangement of crystals for the shear to enhance. Therefore, applying shear at this stage would only lead to damage of the network. Similarly, Dhonsi and Stapley (2006) found that the induction time for crystallisation of cocoa butter was reduced by a higher shear rate application at higher crystallisation temperatures of 20 and 23 °C that was not noticeable at lower crystallisation temperatures (13 and 17 °C). This finding is also consistent with the previous evidence that the formed aggregates were destroyed by the shear applied during the

second cooling step. However, no clear effect on the final rheological properties of the filling

fats was seen (see Appendix C).

Table 5.3 Rheological changes of Creamelt 601 and 701 during crystallisation under sectional-shear cooling conditions

Processing steps	Creamelt 601	Creamelt 701
Isothermal at 70°C for 10 min	Average G': 2.59E+00 Pa Average G": 2.84E+00 Pa	Average G': 3.26E-01 Pa Average G": 4.10E-01 Pa
Cooling to 22°C at 10°Cmin^{-1}	Average G': 9.65E+00 Pa Average G": 1.74E+01 Pa	Average G': 6.96E+00 Pa Average G": 7.33E+00 Pa
Isothermal at 22°C for 15 min	G' slight increased to 5.22E+01 Pa G" slight increased to 1.68E+01 Pa	G' slight increased to 3.02E+01 Pa G" slight decreased to 6.24E+00 Pa
Cooling to 10°C at 10°Cmin^{-1}	G' increased to 7.01E+04 Pa G" increased to 3.31E+04 Pa	G' increased to 1.57E+05 Pa G" increased to 2.14E+04 Pa
Isothermal at 10°C for 30 min	G' further increased to 2.30E+05 Pa G" further increased to 9.20E+03 Pa	G' further increased to 2.34E+05 Pa G" further increased to 8.46E+03 Pa
Heating to 20°C at 10°Cmin^{-1} and holding for 2 hours	Average G': 1.60E+05 Pa Average G": 2.15E+04 Pa	Average G': 2.28E+05 Pa Average G": 7.98E+03 Pa

(A)

(B)
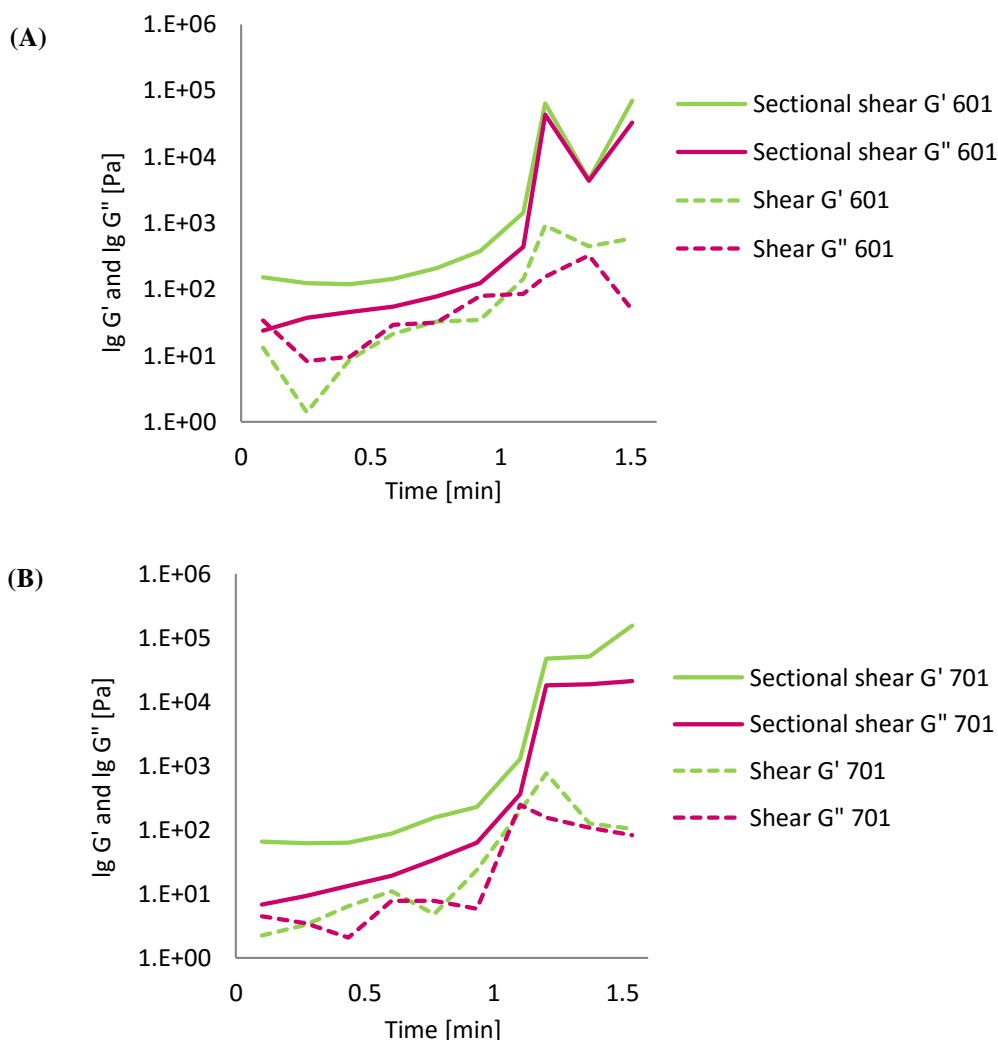
Figure 5.4 The effect of different shear conditions on the average (n=3) rheological properties of Creamelt 601(A) and 701 (B) during cooling to 10°C

5.3 Isothermal Crystallisation Kinetics

More information on the isothermal crystallisation behaviour of the filling fats was obtained using the stop-and-return DSC technique (see Section 3.2) at two different crystallisation temperatures, namely 20 and 15 °C. At the end of this stop-and-return analysis, melting profiles were obtained by measuring the heat flow as a function of temperature as seen in Figure 5.5 and Figure 5.6. From these melting curves, the melting peak and melting enthalpy

data were analysed and presented in Table 5.4 and Table *5.5*. While the melting peak data can be used to indicate which fraction or polymorph the fat has crystallised into, the melting enthalpy information was related to the amount of crystallised fat present during the different isothermal periods (Foubert *et al.*, 2008).
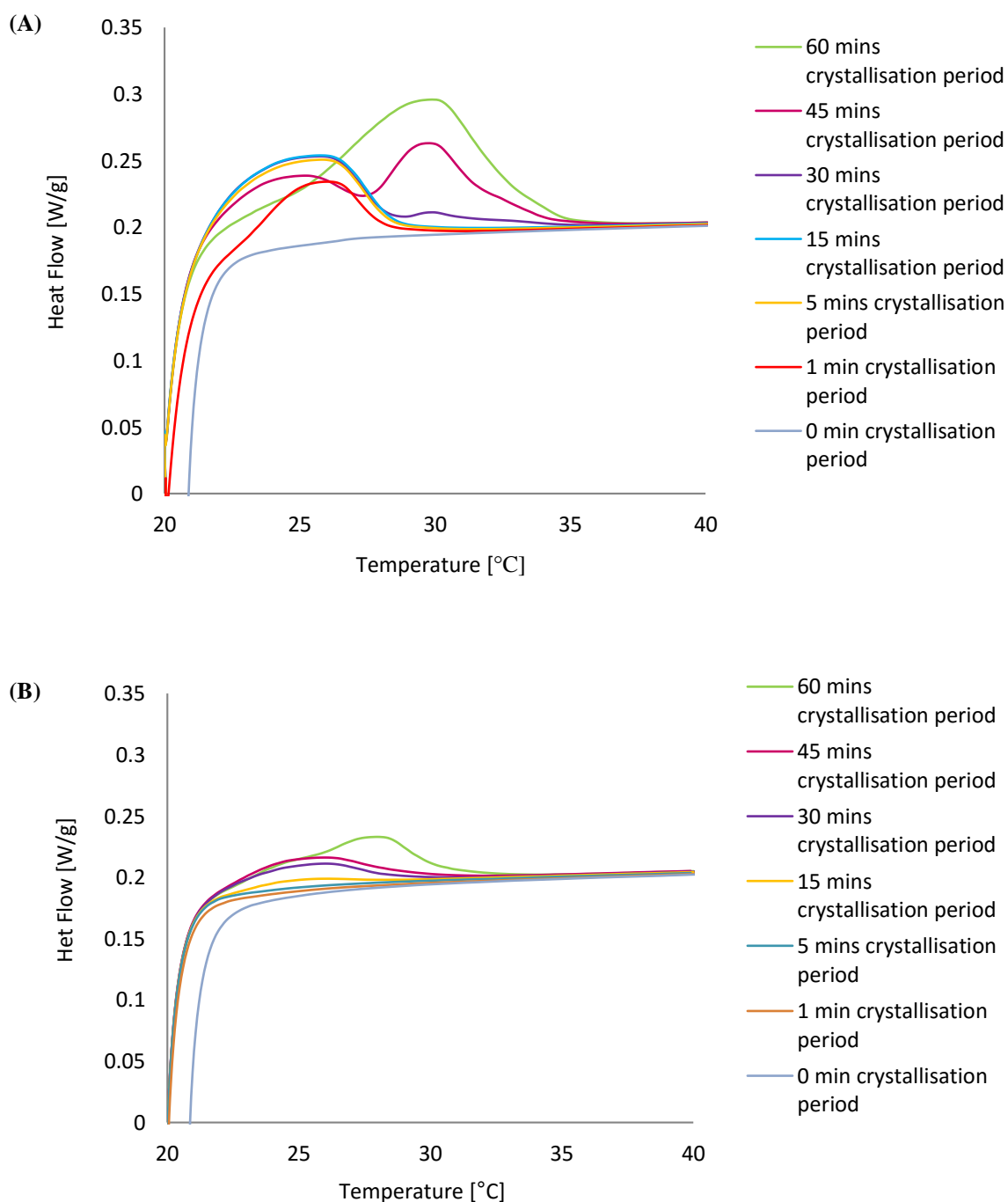
Figure 5.5 Typical DSC melting profile of Creamelt 601 (A) and 701 (B) crystallising at 10°C.min^{-1} to 20°C for different crystallisation periods

Table 5.4 Melting properties of Creamelt 601 and 701 at 20°C[*]

Isothermal time [min]	Creamelt 601		Creamelt 701	
	Melting peak [°C]	Melting enthalpy [J/g]	Melting peak [°C]	Melting enthalpy [J/g]
0	-	-	-	-
1	26.08 ± 0.29^a	1.440 ± 0.31^a	-	-
5	25.90 ± 0.18^a	2.810 ± 0.08^b	-	-
15	25.84 ± 0.32^a	3.006 ± 0.13^b	-	-
30	25.84 ± 0.24^a 25.79 ± 0.33^a	3.219 ± 0.07^b	26.05 ± 0.17^a	0.450 ± 0.07^a
45	25.35 ± 0.43^a 29.78 ± 0.09^b	4.357 ± 0.15^c	25.99 ± 0.24^a	0.695 ± 0.05^b
60	29.97 ± 0.17^b	6.566 ± 0.19^d	28.03 ± 0.11^b	1.535 ± 0.11^c

[*]values with different superscript letter in a column are significantly different

At 20°C, the early formation of crystals giving medium-melting peaks around 26°C is seen in Creamelt 601. These are observed already after 1 minute of isothermal crystallisation (Figure 5.5A), while the same peaks were only found in Creamelt 701 after 30 and 45 minutes (Figure 5.5B). This is due to the high-melting TAGs from the crystallisation initiator and SOS from the shea stearin present in the formulation of Creamelt 601 that would start to crystallise earlier at a higher temperature. For Creamelt 601, a small number of crystals providing a higher-melting peak around 30°C started to develop at 30 minutes. With the increase in isothermal time, the area of the medium-melting peak decreased (at 45 minutes) and it eventually disappeared (at 60 minutes), leaving the developed higher-melting peak. This behaviour illustrates a transition stage from a less-stable polymorph into a more stable one. The medium-melting peaks can be associated with the formation of α crystals that transformed into β' at longer isothermal periods. Consequently, two melting peaks are seen at

30 and 45 minutes as the α polymorph transformed into the β' polymorph. For Creamelt 701, the polymorphic transition was not as apparent as in the 601.The appearance of two melting peaks as seen in Creamelt 601 was also not reported for another type of filling fat based on fractionated palm oil when crystallised isothermally at 20°C (Vereecken *et al.*, 2007). Using the stop-and-return technique, the reported study only showed one developing melting peak (around 38°C) as the crystallisation time increased. This is consistent with the differences in the behaviour of Creamelt 601 and 701 since the latter is known to have a greater amount of PMF and no crystallisation initiator or shea stearin (the higher-melting TAGs) in the formulation.

When Creamelt 601 was cooled to the lower temperature of 15°C and maintained isothermally for up to 1 hour, the melt crystallised into a single melting crystallisation bulk with the peak melting temperature around 24°C (Figure 5.6A). This provides evidence that the first formed crystals are richer in the medium-melting TAGs, which include POP, POS and SOS. At longer isothermal periods, separation between fractions and growth of the α crystals followed by β' crystals can clearly be seen. The high-melting peak at around 33°C provides evidence of crystals containing the small amount of the trisaturated TAGs from the crystallisation initiator. The composition of the crystallisation bulk will mostly comprise a mixture of low- and medium-melting TAGs melting at around 22 and 26°C. The medium-melting peak was a sign of α polymorph that contains both trisaturated and SOS TAGs, while the low-melting one showed a growing α polymorph as well as their transformation into β' polymorph. The small difference in the values of the melting points of the peak implies that the majority of crystals that developed during 2, 3 and 4 hours were similar in composition. Thus, it can be deduced that beyond 2 hours, the isothermal period has a minimal effect on the crystals composition.

(A)

(B)
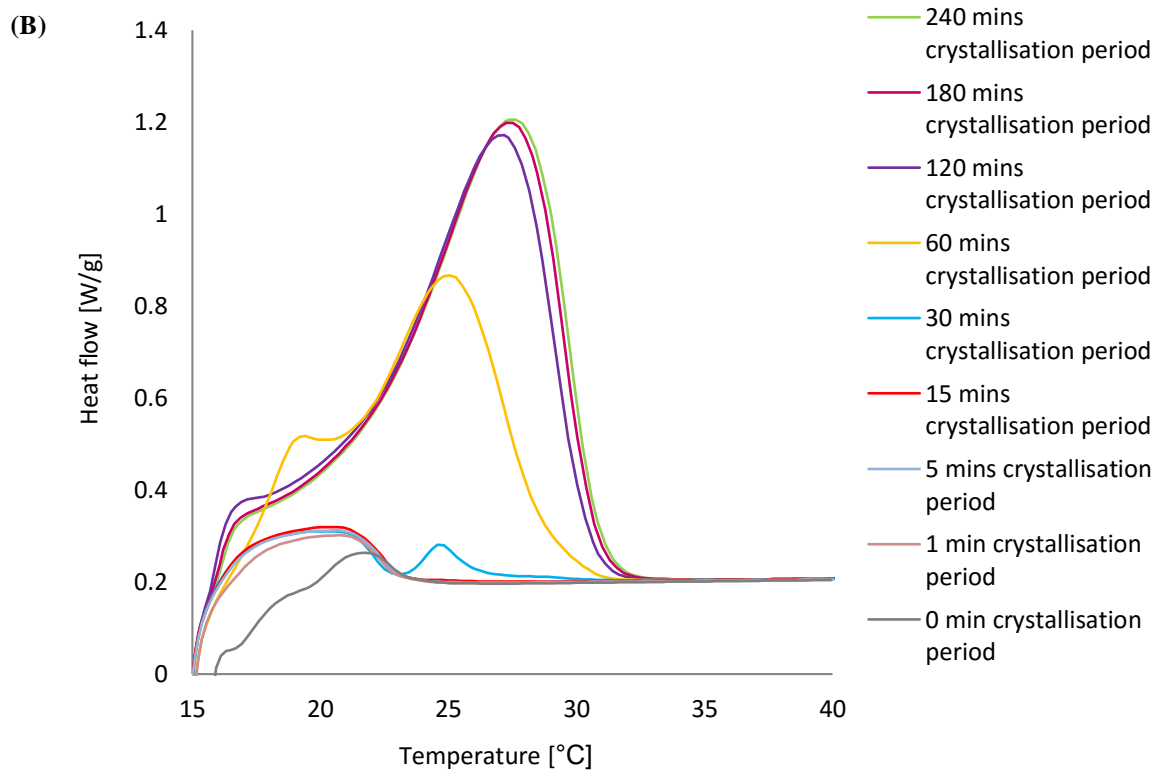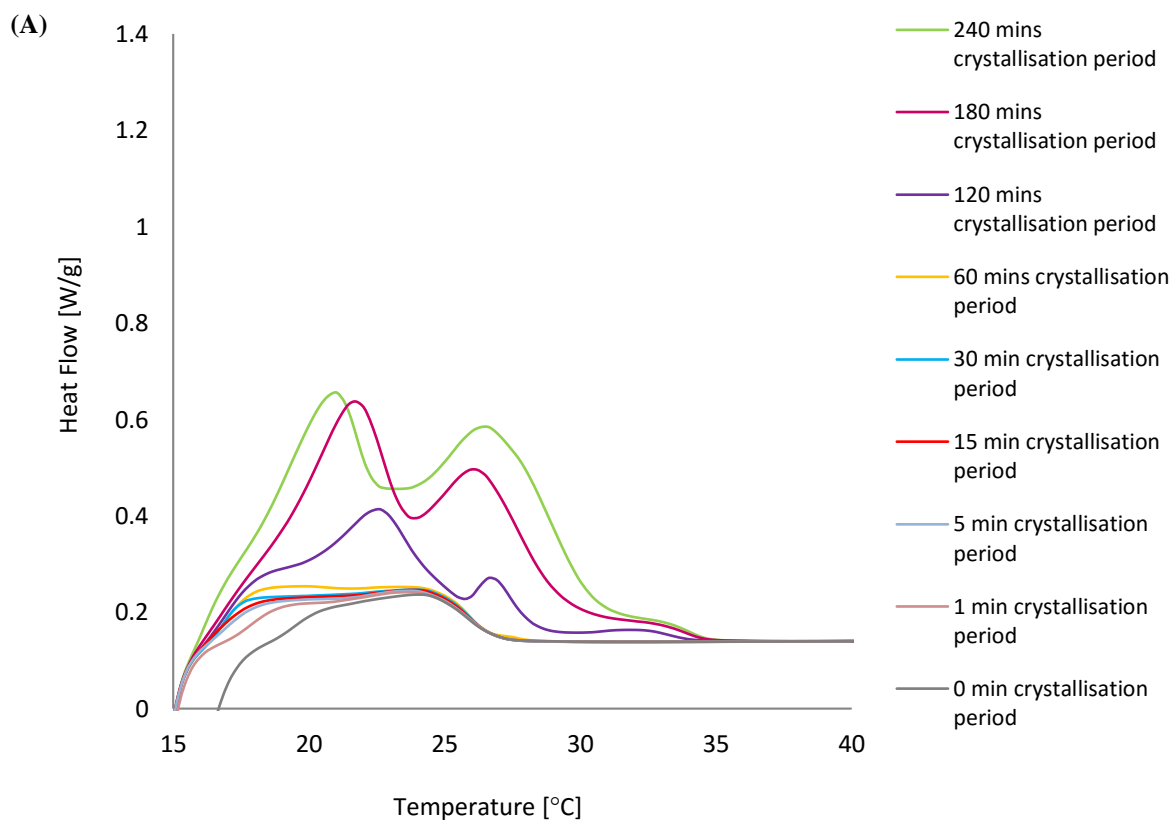
Figure 5.6 Typical DSC melting profile of Creamelt 601 (A) and 701 (B) crystallising at 10°C.min^{-1} to 15°C for different crystallisation periods

Table 5.5 Melting properties of Creamelt 601 and 701 at 15°C[*]

Isothermal time [min]	Creamelt 601		Creamelt 701	
	Melting peak [°C]	Melting enthalpy [J/g]	Melting peak [°C]	Melting enthalpy [J/g]
0	24.27 ± 0.14^a	2.125 ± 0.07^a	21.55 ± 0.04^a	0.136 ± 0.01^a
1	23.97 ± 0.09^a	2.606 ± 0.01^b	20.11 ± 0.11^a	1.315 ± 0.01^b
5	24.00 ± 0.11^a	2.815 ± 0.03^b	19.98 ± 0.37^a	1.465 ± 0.07^b
15	24.17 ± 0.20^a	2.918 ± 0.00^b	20.58 ± 0.80^a	1.564 ± 0.07^b
30	24.20 ± 0.23^a	2.825 ± 0.03^b	20.55 ± 0.82^a 24.62 ± 0.04^b	1.585 ± 0.02^b
60	24.05 ± 0.27^a	2.953 ± 0.07^b	25.05 ± 0.07^b	5.266 ± 0.02^c
120	22.62 ± 0.05^b 26.76 ± 0.11^c 32.33 ± 0.15^d	3.616 ± 0.18^c	27.19 ± 0.03^c	7.872 ± 0.02^d
180	21.71 ± 0.23^b 26.12 ± 0.25^c 33.07 ± 0.13^d	4.522 ± 0.08^d	27.58 ± 0.08^c	$8.178 \pm 0.20^{d,e}$
240	21.03 ± 0.21^b 26.45 ± 0.20^c 33.16 ± 0.08^d	5.358 ± 0.01^e	27.67 ± 0.04^c	8.336 ± 0.12^e

[*]values with different superscript letter in a column are significantly different

For Creamelt 701 (Figure 5.6B), a lower melting peak was found at the shorter isothermal periods. This may be due to the lower amounts of high-melting TAGs in the formulation. Development of a medium-melting peak that started to develop at 30 minutes as the area of the lower-melting peak decreased suggested formation of the α polymorph by the low-melting crystals at around 20°C for the first 15 minutes and later transformation into β' at around 25°C. These β' crystals continued to grow as shown by the slightly higher area of the melting peaks and after 1 hour, additional crystallisation was no longer significant. In the crystallisation study of fractionated palm based filling fat at lower crystallisation temperatures (between 12 and 17.5 °C), two melting peaks (around 25 and 31 °C) were also

observed by Vereecken *et al.* (2007) at a short crystallisation period of which the low-melting one disappeared when the fat crystallised for a longer period. This melting behaviour was similar to the melting profile of Creamelt 701 but was not seen with Creamelt 601. This also suggests that the difference in melting profile between the two filling fats was due to the differences in the filling fat formulations.

Figure 5.7 shows the crystallisation curves obtained by using melting enthalpy as a measure of crystallinity at different isothermal periods for the two filling fats. The measured enthalpy is the sum of those polymorphs and TAG present in the solid phase. Higher melting TAG tends to have higher enthalpies and more stable polymorphs have higher enthalpies than less stable ones. Hence, the sum of the enthalpies can only be used as rough estimate of the crystallisation that has occurred. At 20°C, Creamelt 601 showed much faster crystallisation than Creamelt 701 presumably due to the presence of high-melting TAGs from the crystallisation initiator and shea stearin that crystallises earlier, producing more solid fat at a higher crystallisation temperature particularly at 45 and 60 minutes. At 15°C, a higher amount of crystallised fat was present in Creamelt 701 after 30 minutes of isothermal crystallisation time. This agrees with the SFC measurement by pNMR that shows more solid fat in Creamelt 701 at the lower crystallisation temperature (see Section 4.4.3). The recorded melting enthalpy for crystallisation at 20°C for shorter isothermal periods (1, 5 and 15 minutes) was similar to that of the fractionated palm oil based trans-free filling fat measured by Vereecken *et al.* (2007). At longer periods (30, 45 and 60 minutes), however, a much higher degree of crystallinity was shown by the higher melting enthalpy of the fractionated palm oil based *trans*-free filling fat (up to around 11 J/g melting enthalpy) compared to the Creamelt 601 and 701. This may be due by the higher amount of unsaturated fatty acids (C18:1 *cis*) present in the two filling fats (35% and 36.6% for Creamelt 601 and 701, while

the *trans*-free filling fat contained only 30.2%) which leads to lower crystallinity. In a study

of the thermal behaviour of palm oil, palm oil based products and coconut oil, Tan and Man

(2002) found generally lower melting enthalpy values in the oil samples with higher degree

of unsaturation because less energy is required to melt the samples.

(A)

(B)
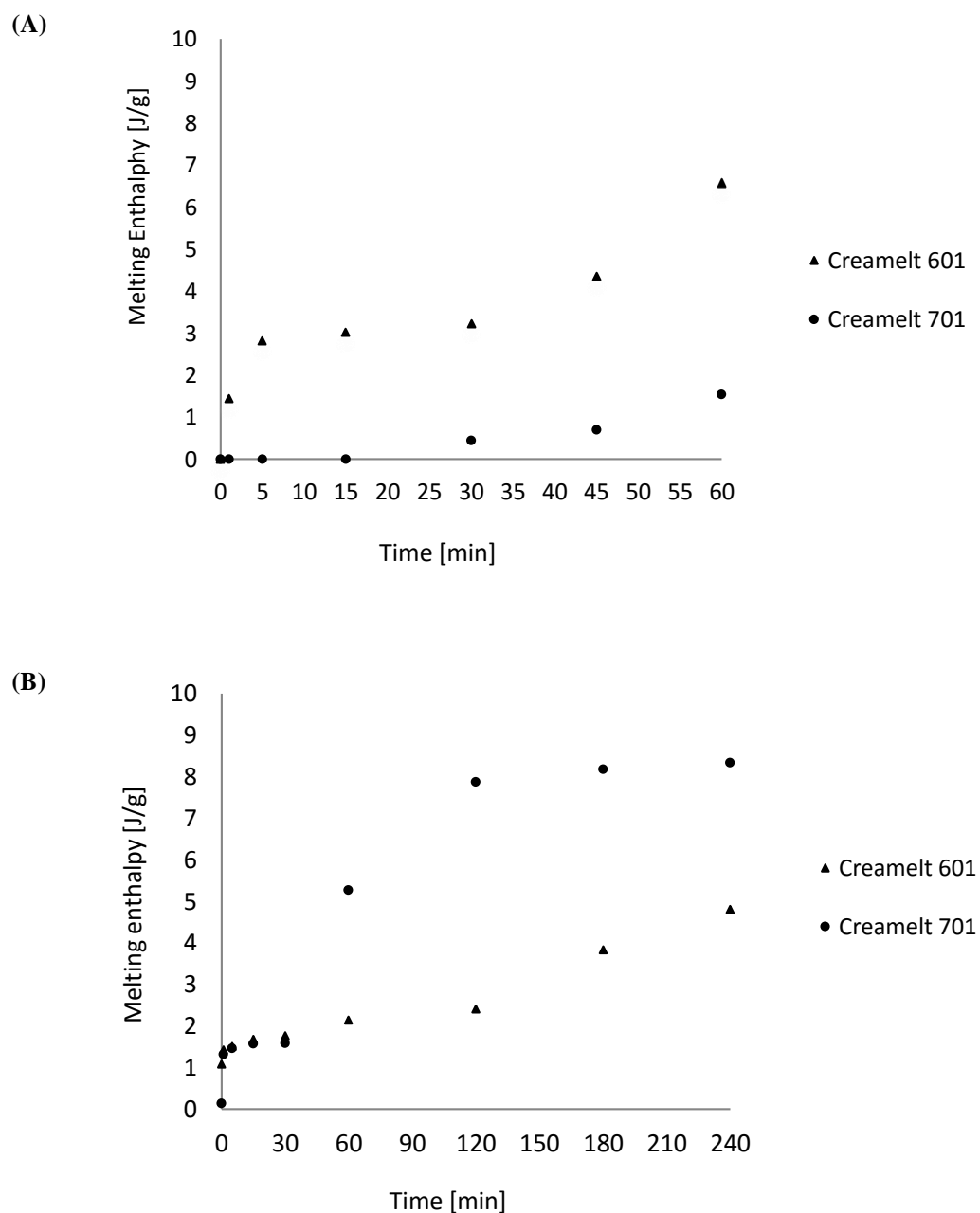
Figure 5.7 DSC crystallisation curve of Creamelt 601 and 701 at 20 (A) and 15 (B) °C

At a lower crystallisation temperature, the melting enthalpy of Creamelt 601 was mostly lower than that of the fractionated palm oil based filling fat even at a shorter isothermal period. At 10 and 15 minutes of the crystallisation period, the melting enthalpy value of the fractionated palm oil based filling fat was similar to that of Creamelt 601 and 701 after 4 hours of crystallisation. At 30, 45 and 60 minutes, the melting enthalpy value of the fractionated palm oil filling fat was in the range of 30 to 70 J/g (Vereecken *et al.*, 2007). Besides the different degree of unsaturation, differences in the melting enthalpy values may also be due to the slower cooling rate (5°C.min^{-1}) used in crystallising the fractionated palm oil based filling fat, resulting in the formation of a more stable crystal form that is higher in enthalpies.

5.4 Polymorph Identification

Determined by the molecular structure, polymorphism is regarded as an internal factor that strongly influences the macroscopic properties and crystallisation behaviour of triacylglycerols (Sato, 2001). The characteristics of the crystal network formed will, in turn, dictate the performance of the end product (Campos *et al.*, 2002). Since processing parameters like crystallisation temperature and cooling rate directly affect polymorphism of the fat system, investigations into the polymorphic behaviour of the filling fat were conducted accordingly. On the basis of the processing parameters used in the rheology study, short-spacing data from the rapid crystallisation (6°C.min^{-1}) at 20 and 15 °C were recorded using powder XRD. Figure 5.8 shows the XRD pattern obtained from both filling fat samples at the beginning and 60 minutes of isothermal crystallisation at 20°C. While rheology and DSC shows the evidence of fat crystallisation, the short-spacing data of the filling fats show a

broad liquid spectrum with indications of amorphous material and no polymorphic form was visible. Since crystals formation was confirmed by rheology and DSC, the number of crystallising materials during XRD analysis was probably too small to diffract the incident rays and produce any peak. According to deMan (1992), sufficient amount of properly oriented crystals are needed to diffract the incident rays.
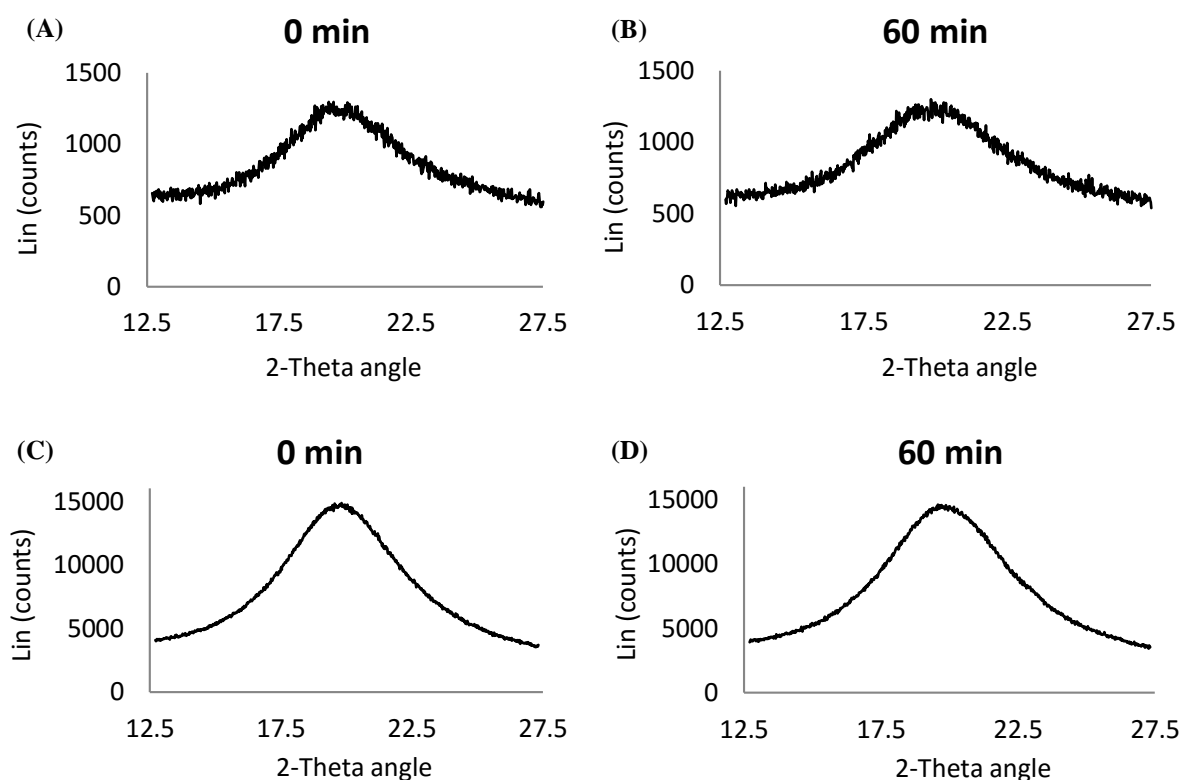
Figure 5.8 XRD pattern of Creamelt 601 (A, B) and Creamelt 701 (C, D) crystallising at 6°C.min⁻¹ to 20°C

At the lower crystallisation temperature, the appearance of the α polymorph in both filling fats was observed as soon as the samples reached 15°C. This is indicated by the medium-intensity peak at 4.17 Å (Table 5.6). Under rapid cooling conditions, the formation of α crystals at the onset of crystallisation is commonly found as the nucleation rate of the less stable form is higher (Koyano and Sato, 2002). The peak, then, decreased as the isothermal time increased to 60 minutes as seen in Figure 5.9 and Figure *5.10*, suggesting the

transformation of α to β' polymorph. In Creamelt 701, however, earlier formation of β' crystals was shown by one strong peak at 3.96 Å. The appearance of another broad peak, which consisted of a single strong peak at 4.27 Å and two weak peaks at 4.39 and 4.18 Å, indicated a mixture of mostly β' crystals (with possibly a small quantity of α crystals). Whilst retaining the same characteristics, the intensity of these peaks increased as the isothermal crystallisation period increased. Although still rather weak, β' signals started to appear in Creamelt 601 after 2 hours with a single peak at 3.93 Å and additional broad peaks at 4.38 and 4.22 Å. At this point, the α polymorph peak was no longer evident.

Table 5.6 Short-spacing data of crystallised Creamelt 601 and 701 at 15°C[*]

Filling Fats	Isothermal time (min)	Short spacing (Å)
Creamelt 601	0	4.17m
	60	4.17w
	120	4.38w, 4.22w, 3.93w
	240	4.34s, 4.20s, 3.94s
Creamelt 701	0	4.17s
	60	4.39w, 4.27s, 4.18w, 3.96s
	120	4.39w, 4.27s, 4.16w, 3.96s
	240	4.39w, 4.28s, 4.18w, 3.96s

*w: weak; m: medium; s: strong

The same β' peaks with higher intensity were found after 4 hours of isothermal crystallisation. Similar behaviour was also found in the polymorphism study of palm oil by Ishikawa *et al.* (1997), where a mixture of α and β' crystals were found under rapid cooling conditions. XRD allows both determination of the polymorph type and the amount of crystalline materials by the peak intensity (De Graef *et al.*, 2008), the greater amount of crystals is indicated by the higher peak intensity of Creamelt 701 from 60 minutes onwards. This is consistent with the crystallisation curve data obtained by DSC at the same crystallisation temperature.
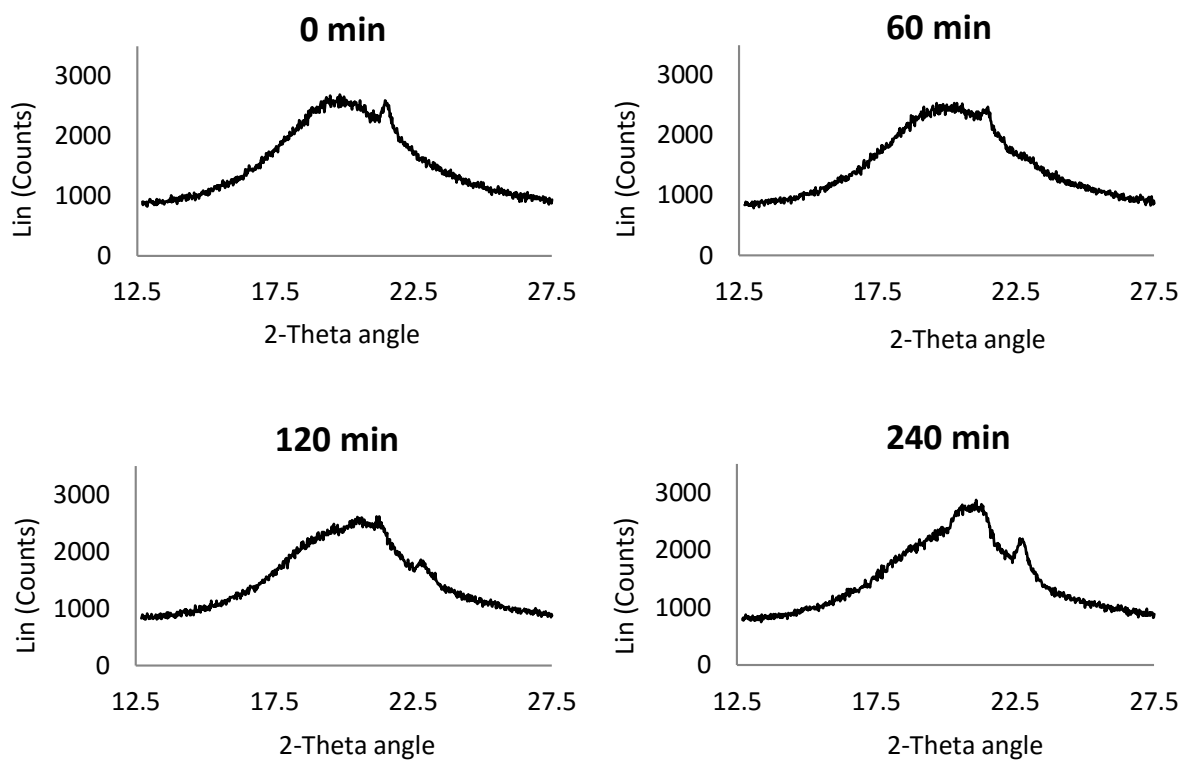
Figure 5.9 XRD pattern of Creamelt 601 crystallising at 6°C.min⁻¹ to 15°C
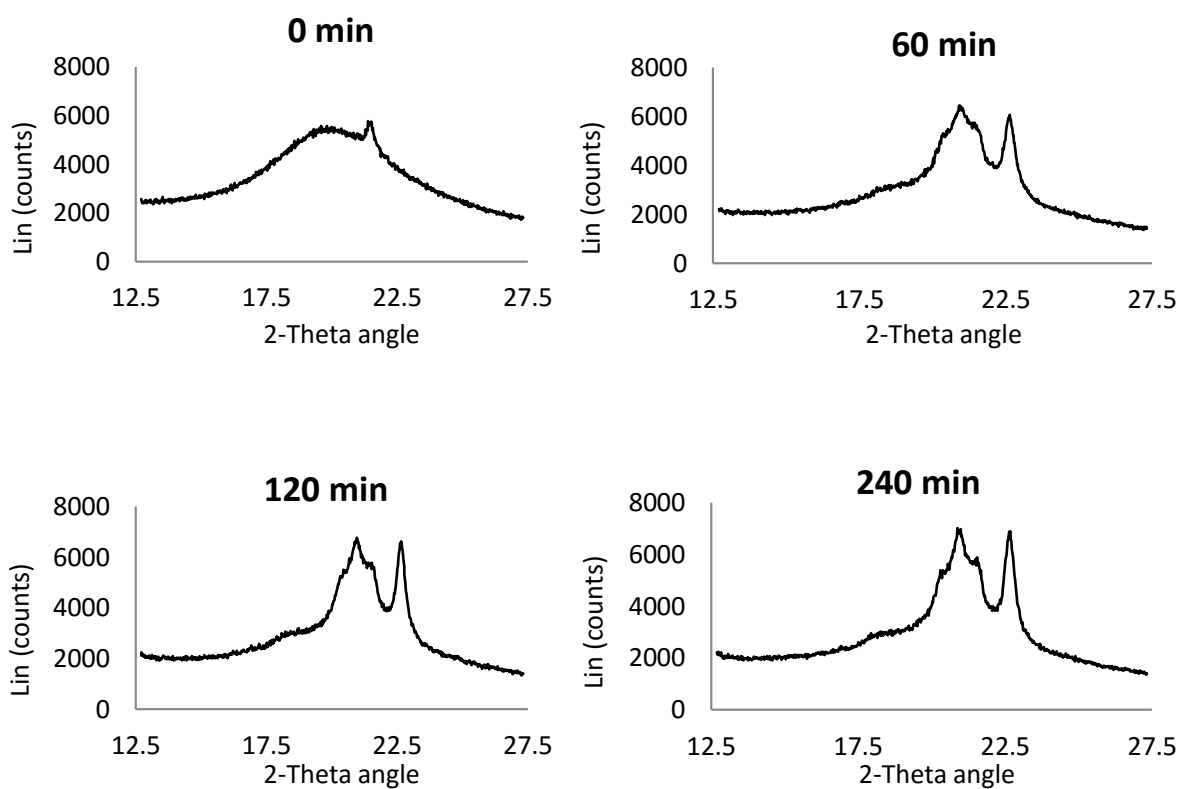
Figure 5.10 XRD pattern of Creamelt 701 crystallising at 6°C.min⁻¹ to 15°C

5.5 Summary of Findings

The manufacturing of confectionery products in which the filling fats are used requires specific processing steps. A test procedure based on these particular processing conditions, which involve different crystallisation temperatures and shear applications, can be demonstrated using oscillatory rheology. Moreover, oscillatory rheology can be used to study the effect of shear and different shear conditions on the crystallisation behaviour of the filling fats. For all three test conditions studied, the G' and G" values as a function of time showed the same pattern i.e. a slight increase followed by a larger increase with the parameters eventually reaching an equilibrium state (Table 5.1 - Table 5.3). The resulting G' values following the first cooling step were slightly higher when shear was applied than observed under static cooling conditions (Figure 5.3). However, the shear applied during the second cooling step caused damage to the crystal network (Figure 5.4). From these findings, different effects of shear can be seen depending on the manner of application; application of shear at higher temperatures (i.e. during the initial cooling) indicated an enhancement of the crystal network development, while at low temperatures (i.e. during the final cooling) damage to the crystal network was implied. Nonetheless, no clear effect on the final rheological properties of the filling fats was observed.

The isothermal crystallisation behaviour of the filling fats was studied using stop-and-return DSC at 20 and 15 °C under rapid cooling conditions. At the higher crystallisation temperature, the presence of the α polymorph and the transformation into β' were indicated by development of a medium-melting peak at around 26°C and a higher-melting one at around 30°C at longer treatment times (Figure 5.5). This behaviour occurred at a much faster rate for Creamelt 601 because of the high-melting TAGs from the crystallisation initiator and shea stearin that promoted formation of solid fat at the higher crystallisation temperature. The

growth of α crystals followed by transformation into β' in both filling fats was also seen at the lower crystallisation temperature (Figure 5.6). When the filling fats were crystallised at 15°C, a shorter isothermal time resulted in a single melting peak at around 25°C for Creamelt 601 and 20°C for Creamelt 701. This difference in peak temperature was due to lower amount of high-melting TAGs in the composition. Different fractions of TAGs were seen in Creamelt 601 at prolonged isothermal periods. The trisaturated TAGs from the crystallisation initiator and SOS from shea stearin were evident at 33°C, while the rest of the crystallisation bulk was mostly composed of a different mixture of low- and medium-melting TAGs at around 22 and 26°C. For Creamelt 701, a longer isothermal period produced further growth of the medium-melting TAGs at around 25°C. In addition, crystallisation curves of the two filling fats were obtained using the melting enthalpy data as a measure of crystallinity at different isothermal periods. At 20°C, Creamelt 601 showed much faster crystallisation than Creamelt 701 presumably due to the presence of high-melting TAGs from the crystallisation initiator and shea stearin that crystallises earlier, producing more solid fat at a higher crystallisation temperature. At 15°C, crystallisation rate was higher for Creamelt 701 and this agrees with the SFC measurement by pNMR that shows more solid fat in Creamelt 701 at the lower crystallisation temperature (see Section 4.4.3).

The XRD analysis could not detect any solid formation at the higher crystallisation temperature (20°C) even after 60 minutes of isothermal crystallisation (Figure 5.8). This perhaps was due to insufficient number of orienting crystals that were needed to diffract the incident rays. Once the crystallisation temperature was decreased to 15°C, the presence of the α polymorph was observed. As the isothermal period increased, transformation of α to β' and development of β' crystals from the melt were shown. A stronger and earlier development of β' crystals as well as higher peak intensity of Creamelt 701 after 60 minutes onwards were consistent with the melting and crystallisation curves obtained by DSC. According to Narine

and Marangoni (1999b), the β' polymorph is often the most functional crystal form for fat-based products. The DSC and XRD results showed that crystallisation temperature between 15 to 20 °C can be used to crystallise the filling fat into β' polymorph, allowing the β' crystals to seed the crystallisation bulk in β'-stable.

CHAPTER 6. STRUCTURAL CHANGES OF FILLING FATS UNDER VARIOUS PROCESSING CONDITIONS

6.1 Introduction

Material properties of fats are fundamentally defined by their elastic and viscous components. The different distribution of mechanical energy between these two structural "elements" on exposure to shear application separates essentially solid materials from the more fluid one. Solid materials exhibit elastic behaviour due to the mechanical energy stored on deformation, while fluid materials are viscous because of the dissipated (as heat) energy. Nonetheless, some materials are viscoelastic, exhibiting both of these mechanical responses with relative proportions depending on the frequency and the strain used (Mason and Weitz, 1995). Essentially, the viscoelastic response of a material is established by the tendency to movement under deformation (shear). Performing oscillation tests over a wide range of frequencies/strain will, therefore, characterise the viscoelastic properties of the material. Oscillation frequency sweep tests have been used as tools to study the structural characteristics of food materials such as dough (Weipert, 1990), yogurt (Hassan *et al.*, 2003), shortenings (Jirasubkunakorn, 2009) and milk (Harbourne *et al.*, 2011). In this study, the structural characteristics of the filling fats are further investigated using frequency sweep tests at the end of each processing step to assess the rheology/structure. This provides more information on the effect of the processing conditions on the structure of the fat crystal network at each processing stage. The oscillation frequency sweep was performed at the same frequencies and relatively small strain for all three test combinations with the temperature specified according to the different processing stages. Additionally, the visual microstructure was analysed using polarised light microscopy.

6.2 Rheological Behaviour

Further insight into the structural changes of filling fats through the processing steps was obtained by monitoring the frequency-dependent properties at frequencies ranging from 0.01 to 10 Hz. A small strain value of 0.5% and different temperatures, depending on the processing step, were also applied. The elastic and viscous moduli were recorded as a function of frequency. The frequency sweep test was done following each processing step mentioned in Section 3.5.3. Prior to the crystallisation, the molten filling fat sample was kept at a 70°C "isothermal soak" for 10 minutes to ensure removal of any crystal memory in the fat material. At this stage, similar values between G' and G" as well as strong frequency-dependent behaviour were found for each test combination of both filling fats (Figure 6.1). According to Ferry (1980), frequency-dependent behaviour of the moduli can be related to materials showing essentially weak viscous liquid behaviour. This behaviour is similar to the one exhibited by a fully melted palm fractions based shortenings during the rheological study i.e. indication of a weak viscoelastic liquid structure with higher G" than G' throughout the measured frequency range (0.1 – 10 Hz) (Jirasubkunakorn, 2009).
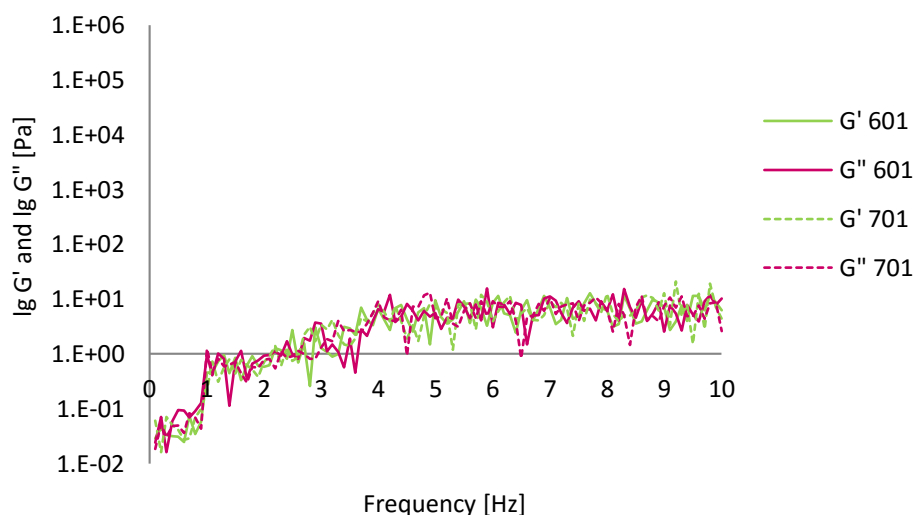
Figure 6.1 Average (n=3) frequency sweep of Creamelt 601 and 701 after melting at 70°C for 10 mins

Under both sets of cooling conditions (outlined in Section 5.2), slightly less frequency-dependent behaviour was exhibited by the two filling fats after cooling to 22°C at 10°C.min^{-1} (Figure 6.2). This shows the characteristics of a weak viscoelastic gel system (Ferry, 1980). When the first crystallisation temperature was reached, higher moduli values were exhibited by Creamelt 601 compared to Creamelt 701 due to high-melting TAGs of the crystallisation initiator component that crystallised earlier, resulting in a more solid fat formation at this crystallisation stage.
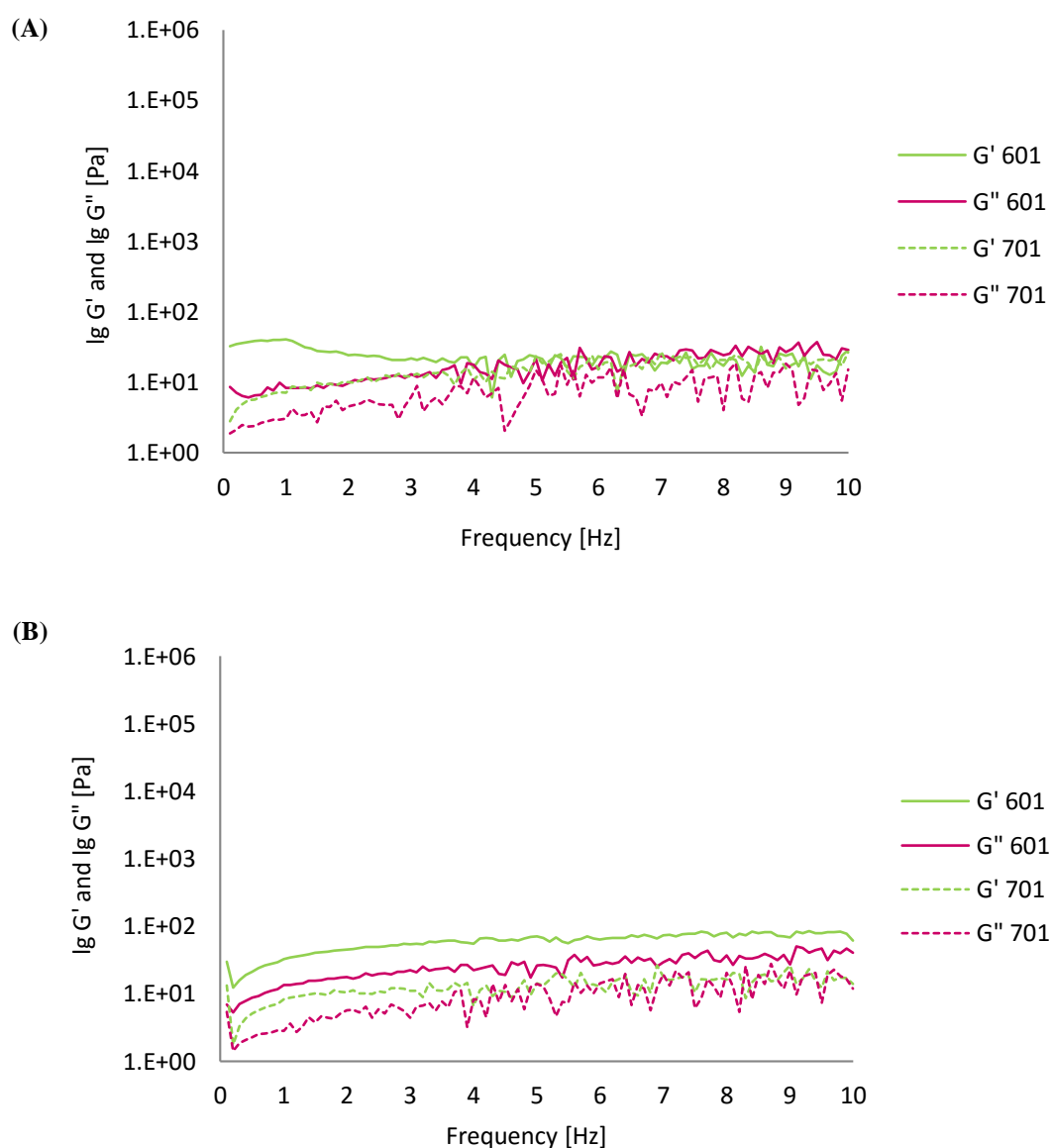
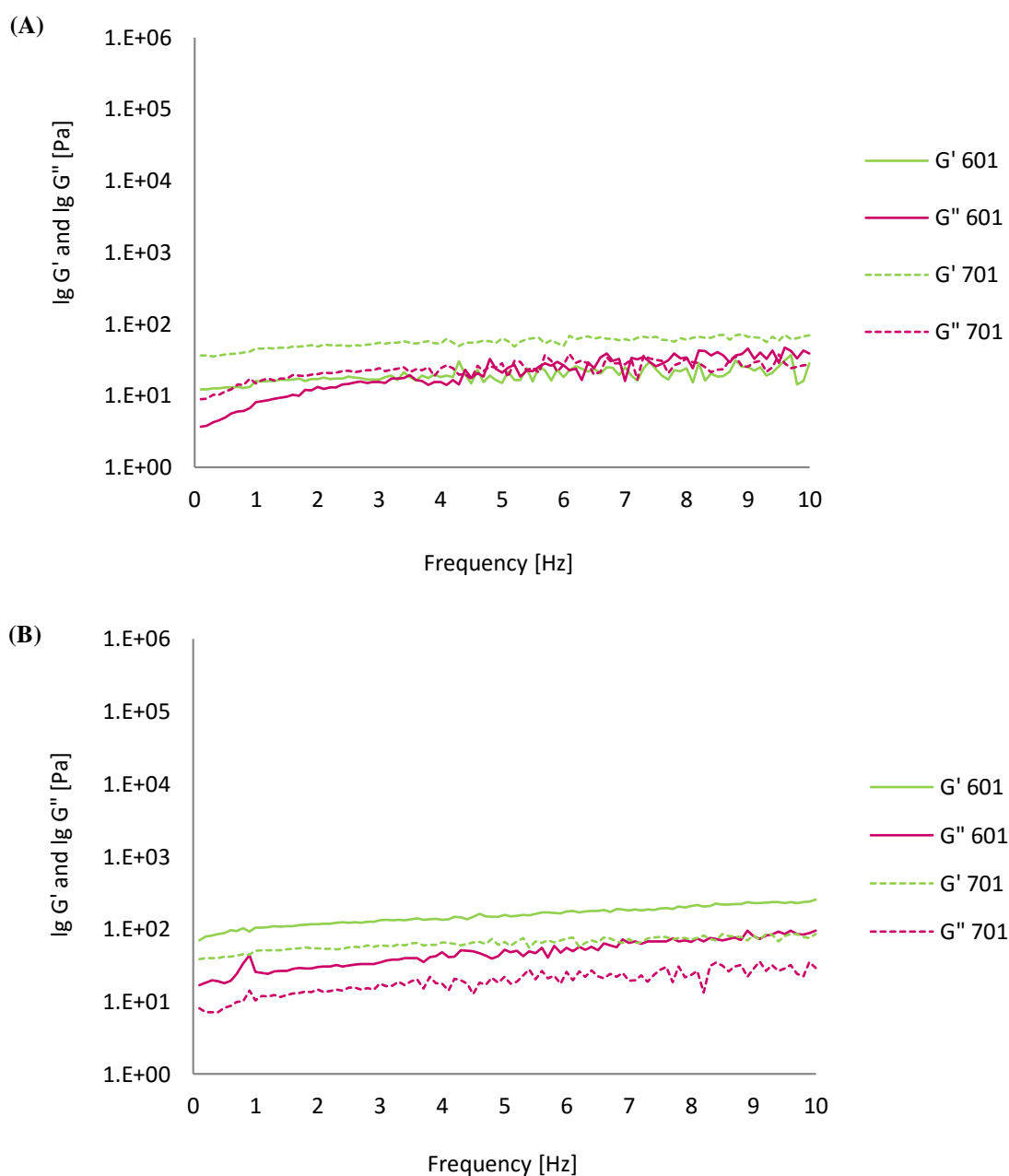**Figure 6.2 Average (n=3) frequency sweep of Creamelt 601 and 701 after cooling to 22°C at 10°C.min^{-1} cooling rate under static (A) and shear (B) cooling conditions**

However, under static cooling conditions, the G' value of Creamelt 601 decreased over the frequency range as the G" value increased (Figure 6.2A). As a result, a liquid-like system (G" > G') was indicated at higher frequency range. This may be due to liquid slip between the crystals caused by the application of higher frequencies that did not occur in Creamelt 701 system perhaps due to smaller number of crystals in the system. Under shear cooling conditions, the divergence between solid (G') and liquid (G") components with the solid component dominating (G' > G") over the frequency range was maintained (Figure 6.2B). This indicated a more structured crystal network development due to shear application during cooling at higher temperatures.

After 15 minutes of isothermal crystallisation at 22°C, both filling fat samples that were sheared during cooling from 70°C showed a higher increase of moduli and clearer separation between moduli values (Figure 6.3B). This behaviour confirmed the suggestion of an enhanced crystallisation through both secondary nucleation and crystal network rearrangement. After 15 minutes of isothermal crystallisation at 22°C under static cooling conditions, a decrease in moduli values of Creamelt 601 was observed (Figure 6.3A). This indicated some destruction of the crystal network by the applied strain. In contrast, the developed crystal network of Cremelt 701 was able to retain the structure despite the applied strain and frequencies. This was shown by the increase in the moduli values with solid characteristic remaining higher. Also, similar frequency behaviour to the one after cooling to 22°C (Figure 6.2A) was seen i.e. cross-over moduli occurrence in Creamelt 601 that resulted in a liquid-like behaviour at higher frequency range. At this point, the liquid-like behaviour that was exhibited by Creamelt 601 under static cooling conditions indicated lower crystallisation rate. If the crystallisation rate is too low, the required solid fat content may not be achieved in time. Having the appropriate amount of solid fat at the right time is important especially for filled chocolate. When the required fat crystals were not formed in time, the

following manufacturing steps would be affected. For instance, when longer crystallisation time of the filling fat was needed, softer filling may be produced by the time the filling was coated with chocolate shell. If the filling was too soft at this point, the chocolate shell may sink into the filling, giving both unappealing appearance and possibility of fat migration into the surrounding coating.

Figure 6.3 Average (n=3) frequency sweep of Creamelt 601 and 701 after 15 mins of isothermal crystallisation at 22°C; static cooling (A) and shear cooling (B)

As a result of further crystallisation on cooling to 10°C, major increase was seen in the moduli values of both filling fats (Figure 6.4). Despite the different cooling conditions, frequency sweep behaviour of the filling fats were independent of the frequency. Since solid behaviour is less dependent on frequency (Ferry, 1980), this shows that a solid-like system was established by the two filling fats. When crystallised under shear cooling conditions, slightly decreasing G' value and less degree of separation between the G' and G" were observed in both filling fats (Figure 6.4B). Slightly lower moduli values were also seen in Creamelt 601 when crystallised under static cooling conditions (Figure 6.4A). These behaviours, however, were not exhibited by the filling fats when sectional-shear cooling conditions were applied (Figure 6.4C). Under sectional-shear cooling conditions, the decrease in the solid characteristic was not apparent and slightly better separation between the solid and liquid characteristics was seen. This suggested an increased arrangement of crystals by the applied shear at higher temperatures. More importantly, this shows the effect of shear application at lower temperatures. Shear is commonly found in the industrial processes like mixing and pumping. However, minimum shear application should be kept when low temperatures were used as this may lead to disruption of the fat crystal network and produce a filling with too low solid fat content that can enhance fat migration, giving undesirable effects into the final product (Vereecken *et al.*, 2007).

(A)

(B)

(C)
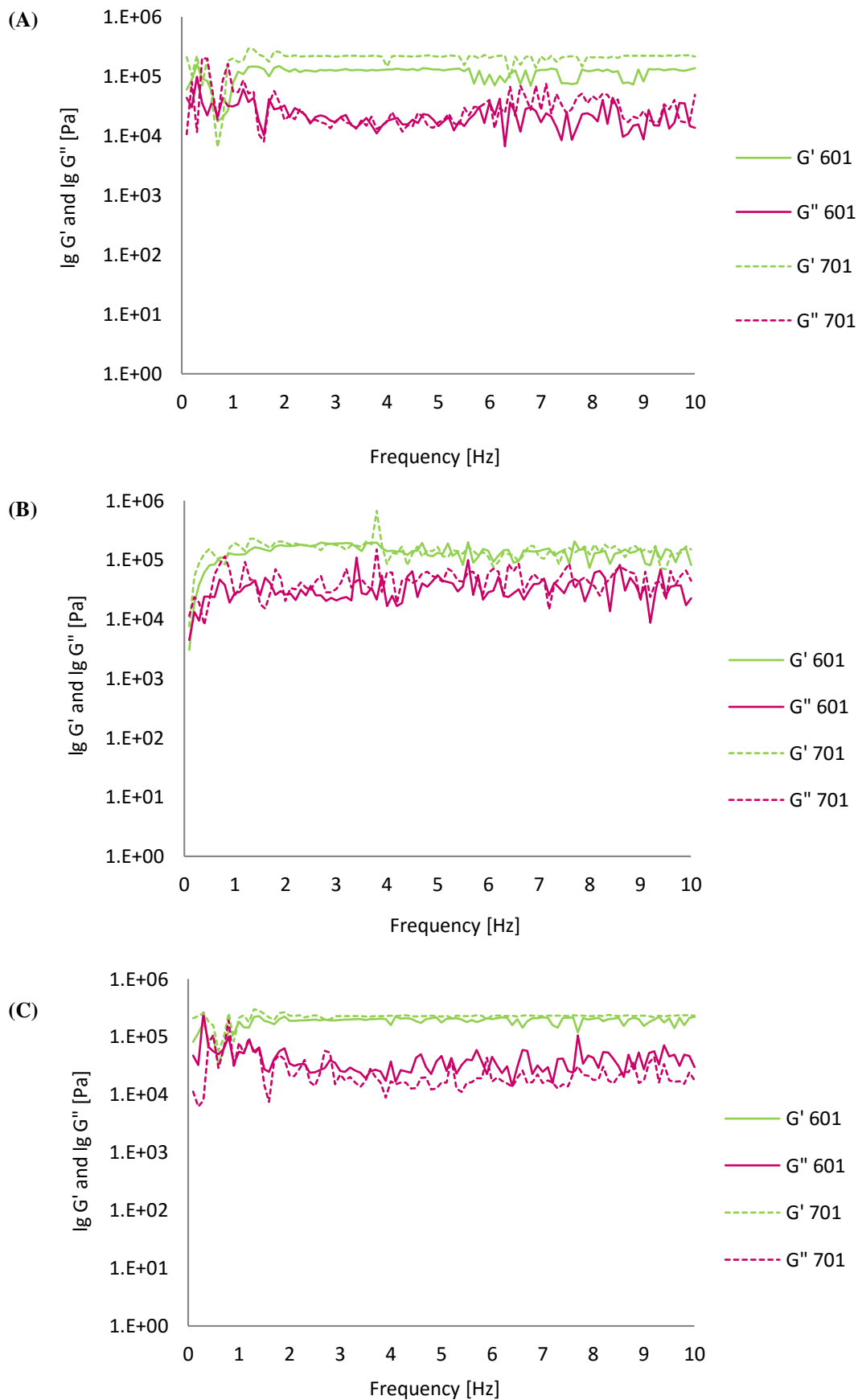
Figure 6.4 Average (n=3) frequency sweep of Creamelt 601 and 701 after cooling to 10°C at 10°C.min^{-1}; static cooling (A), shear cooling (B) and sectional-shear cooling (C)

Following the second isothermal crystallisation period (30 minutes) at 10°C, different frequency sweep behaviours were evident between each test combination (Figure 6.5). When crystallised under static conditions, declining G' value was seen in Creamelt 601 as the frequency increased (Figure 6.5A). The absence of shear at higher crystallisation temperatures that was proven to enhance crystallisation has the consequence that the crystallisation under completely static crystallisation conditions requires more time for the fat crystals to rearrange and create a network. As a result, a less-structured network may have been formed under these crystallisation conditions compared to the shear or sectional-shear conditions. Higher frequency application can, therefore, result in destruction of the fat crystal network. When crystallised under shear cooling conditions, the G' and G" values of the two filling fats were slightly decreased and increased, respectively, as the frequency increased (Figure 6.5B). The slightly increased G" value was also noticed in the filling fats crystallised under sectional-shear cooling conditions. However, G' values of both filling fats were maintained at higher level. The same patterns of each crystallisation conditions continue until the last isothermal crystallisation period (2 hours) at 20°C was established (Figure 6.6).

Figure 6.5 Average (n=3) frequency sweep of Creamelt 601 and 701 after 30 mins of isothermal crystallisation at 10°C; static cooling (A), shear cooling (B) and sectional-shear cooling (C) conditions
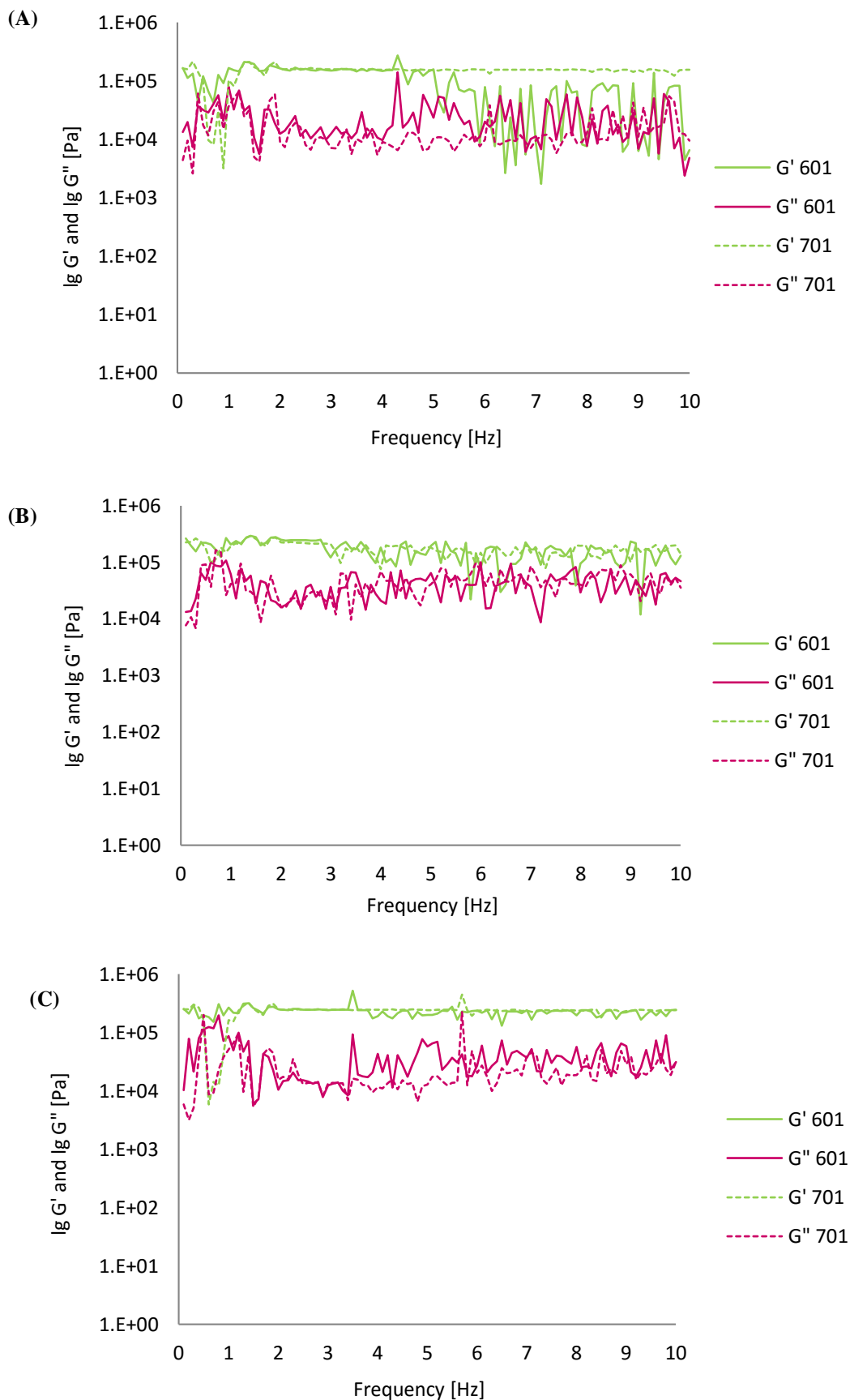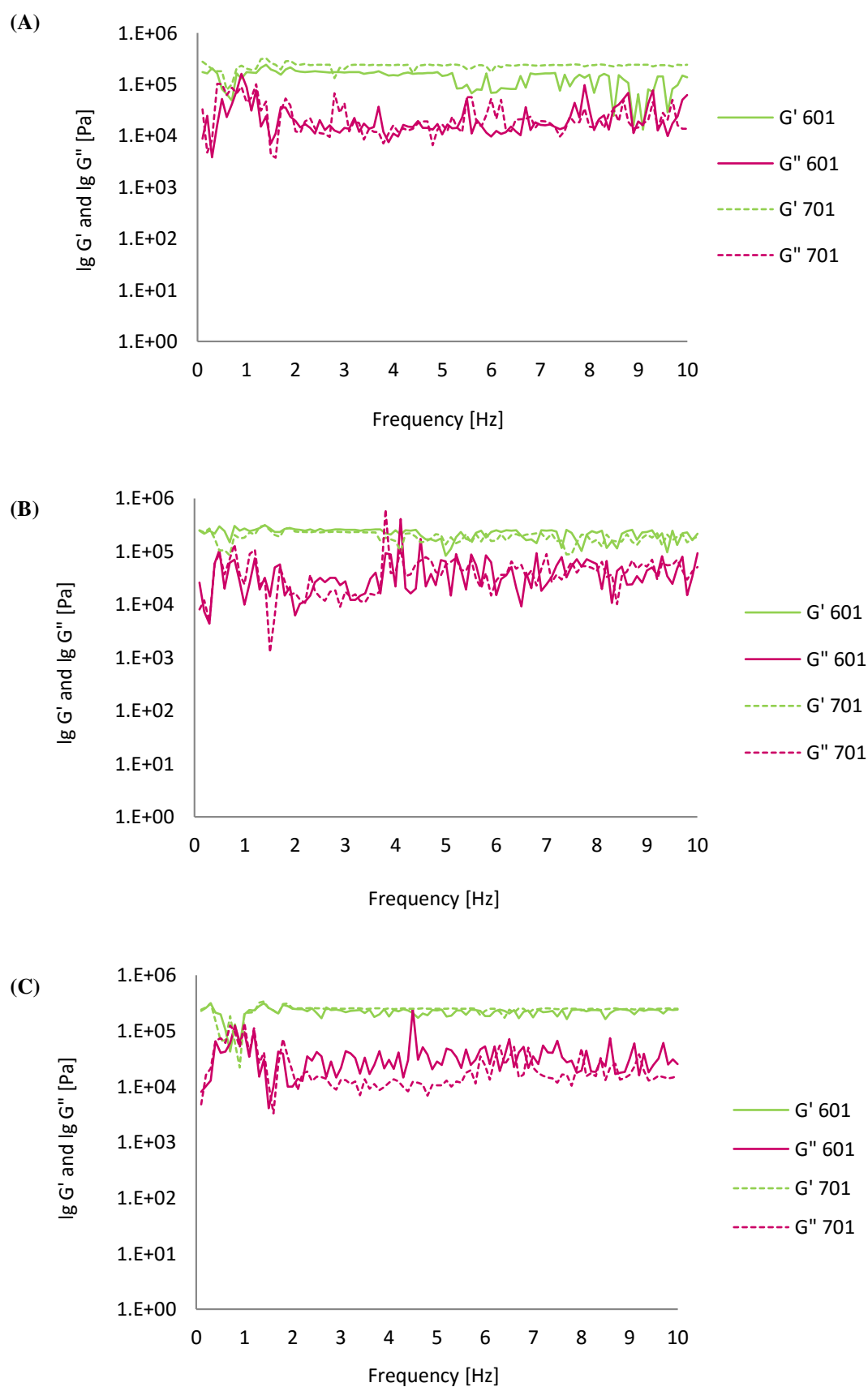
(A)

(B)

(C)

Figure 6.6 Average (n=3) frequency sweep of Creamelt 601 and 701 after 2 hrs of isothermal crystallisation at 20°C; static cooling (A), shear cooling (B) and sectional-shear cooling (C) conditions

In general, the frequency sweep results supported the suggestion from oscillatory rheology measurements i.e. application of shear at higher crystallisation temperatures would enhance the crystallisation process by both secondary nucleation and crystal network rearrangement, while shearing at low crystallisation temperatures may result in a crystal network destruction that will lead to undesirable properties such as too low solid fat content. Compared to the completely static cooling conditions, shear application at higher crystallisation temperatures resulted in the development of a more structured crystal network. The effect of shear application on the crystallisation behaviour of palm based shortenings was studied by Jirasubkunakorn (2009) under stirring conditions of 30 and 60 rpm at 31°C. Using frequency sweep tests, a weak viscoelastic gel system was observed during the first 15 minutes of stirring (G' > G" with less frequency-dependent behaviour). However, reverted change in behaviour (G" > G' with increasing frequency-dependent behaviour) was seen after 30 minutes of stirring. When shorter period of shear was applied, the effect of shear on the rheological properties of the shortenings was similar to that seen in the filling fats. The sudden change of rheological behaviour, however, was a result of liquid slip between the increasing numbers of smaller crystals in the viscoelastic liquid system and weaker interactions between the fat crystals caused by longer period of stirring (Jirasubkunakorn, 2009). Although the effect of shear application at lower temperature was not studied, the effect of different shearing periods can clearly be seen. Evidently, even at high temperature the shear may weaken the fat crystals interactions when longer shearing period was applied. In addition to the industrial importance of shear application at higher temperatures, care must also be taken in terms of the shearing duration.

6.3 Microstructure Observation

Microstructural observation of the underlying fat crystal network is very important as the network determines the macroscopic properties of an end product. Modification of the processing conditions is known to have significant impact on the crystal network properties, including the size, shape and crystal arrangement (Maleky *et al.*, 2011). The microstructure of the filling fat samples was studied using polarised light microscopy. In order to monitor the effect of the designed processing conditions on the fat crystal network, the fat samples were rapidly crystallised according to the processing parameters used in the rheological analysis. The fat samples were, therefore, rapidly crystallised by cooling at 10°C.min^{-1} to 22°C and kept isothermal for up to 24 hours. Micrographs of this crystallisation process are shown in Figure 6.7. Processing conditions are considered to be key factors that influence the fat crystallisation kinetics (Herrera and Hartel, 2000a). Moreover, cooling rate is considered to be one of the most influential factors in the development of crystalline microstructure (Metin and Hartel, 2005).

Using a milk fat model system, Herrera and Hartel (2000b) studied the effect of processing conditions including cooling rate. In that study, noticeably smaller and more uniform crystals were found under a rapid cooling rate (5.5°C.min^{-1}) compared to the slow cooling one (0.2°C.min^{-1}). This is because higher rate of nucleation was promoted by the rapid crystallisation process and this results in the formation of numerous small crystals (Herrera and Hartel, 2000b). In addition to milk fat, Campos *et al.* (2002) also used lard to study the effect of cooling rate on the fat structure. Similarly, the study also found that under rapid cooling conditions, the faster crystallisation kinetics resulted in a larger number of and smaller, crystals. Furthermore, Pérez-Martínez *et al.* (2007) investigated cooling rate effect on the microstructure of blends of cocoa butter in canola and soybean oils. At around 10°C,

larger number of numerous small crystals was formed in a more rapid crystallisation process ($5°C.min^{-1}$) than in the slower one ($1°C.min^{-1}$).

Unlike the slow cooling rate that produced two different crystal morphologies of spherulites with needle-like crystals on the surface (for Creamelt 601) and grain-like texture (for Creamelt 701) (Figure 4.10), crystallisation of the two filling fats under rapid cooling rate resulted in a similar grain-like crystal network. The finding of numerous grain-like crystals under rapid cooling rate agrees with the evidence provided by previous studies mentioned above. When crystallised under slow cooling rate, smaller number of larger crystals were formed by the two filling fats (see Section 4.6). The difference in fat microstructures between slow and fast crystallisation was also observed by Campos *et al.* (2002) using anhydrous milk fat and lard samples. When rapid cooling rate was applied, crystallisation of the fat system occurred in a short period of time accompanied by instantaneous formation of a high number of nuclei and rapid viscosity increase in viscosity that limit molecular diffusion and crystal growth, resulting in a smaller crystal size (Campos *et al.*, 2002). Relative to its volume fraction of solid, number of nuclei formed during crystallisation determine the crystal size i.e the more nuclei formed, the greater the surface area of the solid phase and the smaller diameter of the resulting crystals (DeMan, 1964). Compared to Creamelt 701 (Figure 6.7D) bigger crystals were formed from Creamelt 601 shortly after the sample reached the crystallisation temperature (Figure 6.7A). Presumably, this was also caused by the earlier crystallisation of high-melting triacylglycerols from the crystallisation initiator. However, after 60 minutes of isothermal crystallisation, the crystals image of Creamelt 601 (Figure 6.7B) was less enhanced compared to Creamelt 701 (Figure 6.7E), showing less-structured network. Since the solid characteristic is also represented by the elastic modulus, this is consistent with the higher G' value of Creamelt 701 during the third step of the oscillation test and the lower G' values of the Creamelt 601 during the subsequent oscillation frequency

sweeps. Notwithstanding this, no significant difference was seen in the microscopic images of either filling fat after 24 hours (Figure 6.7C and F).

(A)

(D)

(B)

(E)

(C)

(F)
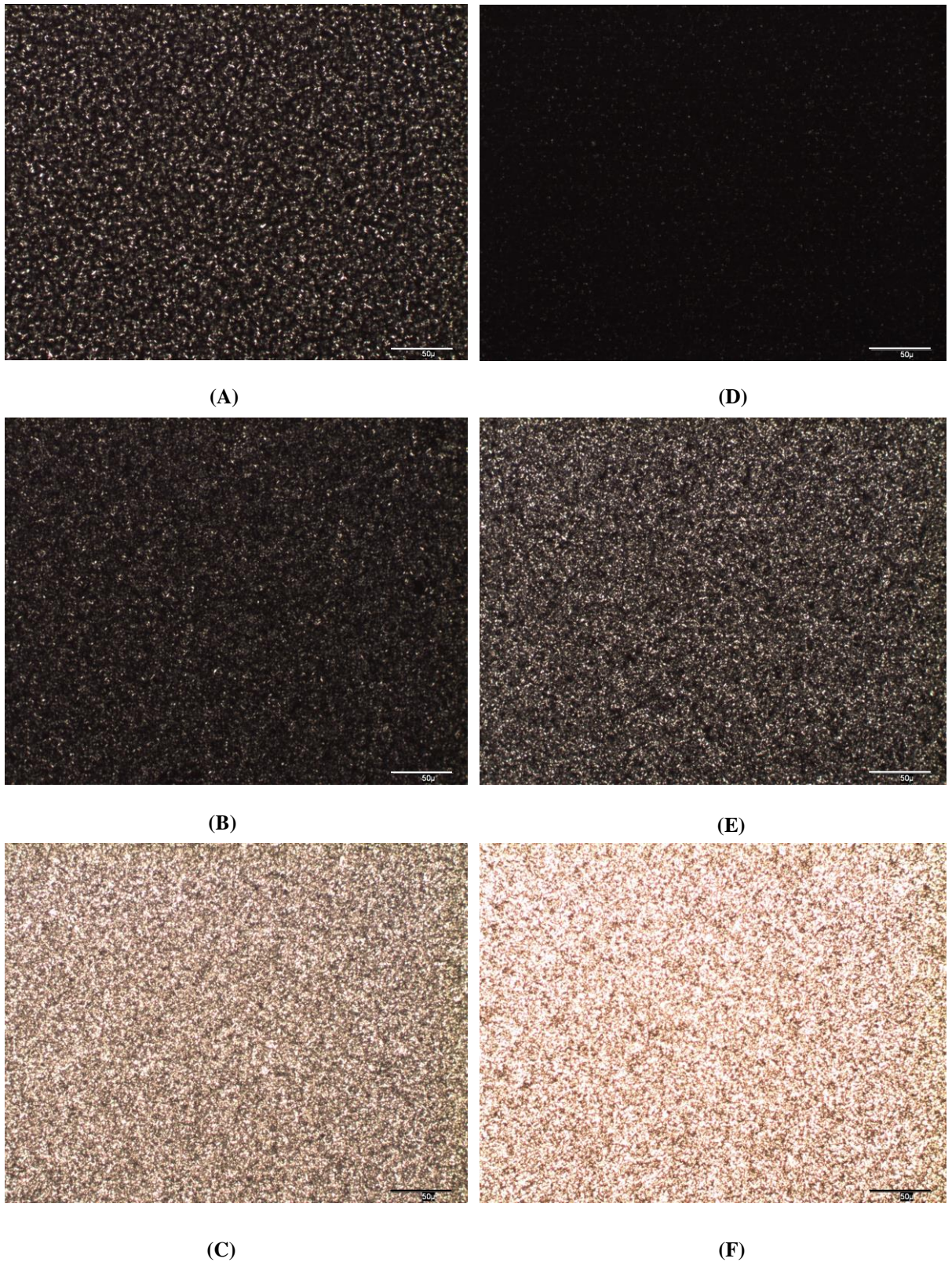
Figure 6.7 20X magnification typical images of rapid isothermal crystallisation of filling fats at 22°C at different time intervals: Creamelt 601 initial stage of crystallisation (A), after 60 mins (B), after 24 hrs (C); Creamelt 701 initial stage of crystallisation (D), after 60 mins (E), after 24 hrs (F). The scale bar represents 50μ

6.4 Summary of Findings

The viscoelastic properties of the filling fats were studied by performing an oscillation frequency sweep after each of the developed processing steps. Increasing frequency-dependent behaviour at the start of the processing procedure demonstrated strong liquid characteristics of the molten fats (Figure 6.1). Slightly less frequency-dependent behaviour was seen as the temperature decreased to 22°C, indicating development of a weak viscoelastic gel system (Figure 6.2). When the filling fats were sheared during cooling at higher temperatures, a more structured crystal network was developed compared to those formed using the static cooling conditions. After 15 minutes isothermal period at 22°C, both filling fat samples that were sheared during cooling from 70°C showed a higher increase of moduli and clearer separation between the moduli, G' and G" respectively, suggesting an enhanced crystallisation through both secondary nucleation and crystal network rearrangement (Figure 6.3B). This will ensure that the filling fat will crystallise in time and prevent the formation of undesirable filling for the filled chocolate.

Following the second cooling stage to 10°C, the frequency-independent behaviour of the two filling fats indicated the establishment of a solid-like system (Figure 6.4). When crystallised under shear and static cooling conditions, however, a decrease in the elastic component and less degree of separation between the G' and G" were observed in both filling fats (Figure 6.4A,B). On the contrary, the decrease in the solid characteristic was not apparent and slightly better separation between the solid and liquid characteristics was seen when sectional-shear cooling conditions was applied (Figure 6.4C). This suggested an increased arrangement of crystals by the applied shear at higher temperatures. After the second isothermal crystallisation period at 10°C for 30 minutes, different frequency sweep behaviours were evident between each test combination (Figure 6.5). The findings confirmed

that the application of shear at higher crystallisation temperatures enhanced the crystallisation process by secondary nucleation and rearrangement of the crystal network, while shear application at low temperatures may result in disruption of the crystal network and structure loss. Similar structure of each crystallisation condition was maintained until the last isothermal crystallisation period (2 hours) at 20°C was reached (Figure 6.6).

It was demonstrated that frequency sweep rheology could be used to study the structural changes during crystallisation of filling fats under different processing conditions. A more structured network was developed when shear was applied at higher temperatures, but structure loss was evident when shear was also applied at low temperatures. The structure loss and less-structured network seen under shear and static cooling conditions, respectively, would give undesirable properties i.e. too low solid fat content and hinder the achievement of good quality products during filled chocolate manufacturing (Vereecken *et al.*, 2007).

The effect of a rapid cooling process onto the microstructure of both filling fats was studied at 22°C. When crystallised rapidly, the same grain-like texture was observed for both filling fats. This agrees with the evidence provided by previous studies (Herrera and Hartel, 2000b; Campos *et al.*, 2002; Pérez-Martínez *et al.*, 2007). Due to the crystallisation initiator, Creamelt 601 formed larger crystals at the beginning of the isothermal period (Figure 6.7A). The less enhanced crystalline image after 60 minutes was consistent with the slightly lower G' values observed during isothermal crystallisation at 22°C under static condition (see Table 5.1 - Table 5.3 in Section 5.2 for values) and the subsequent liquid-like systems shown by the observing oscillation frequency sweep (Figure 6.3A). However, after 24 hours the microscopic images of both filling fats were similar (Figure 6.7C and F). Sensory attributes of a fat-based food product like chocolate are influenced by the mechanical strength of the underlying fat crystal network (Narine and Marangoni, 1999b). To ensure clean melting

sensation during consumption, small crystal size is desirable. This can clearly be achieved through rapid crystallisation process. Therefore, it is important to maintain the crystallisation at a high rate in order to achieve the most functional microstructure.

CHAPTER 7. GENERAL CONCLUSION AND FUTURE WORK

7.1 General Conclusion

Using oscillatory rheology, viscoelastic properties of the filling fats under static and slow cooling conditions were demonstrated. Three different regimes with reasonably different behaviour that were dominated by nucleation, crystal growth and agglomeration mechanisms were observed for both filling fats. The differences were especially noticeable at the nucleation and crystal growth stages. The high-melting TAGs from the crystallisation initiator present in Creamelt 601 resulted in faster nucleation whereas greater level of high- and medium-melting TAG and SFC contributed to the more rapid crystal growth in Creamelt 701. Most importantly, the oscillation tests were able to detect the onset of nucleation that occurred before the crystallisation temperature was reached. This was found to be more difficult to observe using other static crystallisation technique like XRD and PLM. Although direct development of the β' polymorph from the melt is commonly observed for fats crystallising under slow cooling rates as seen in the case of Creamelt 701, XRD also confirmed the presence of α crystals and their transformation into β' crystals in Creamelt 601. Furthermore, observation of the crystals of the filling fats showed two different textures of spherulites with needle-like crystals on the surface and grain-like crystals for Creamelt 601 and 701, respectively.

Certain temperature-time parameters were chosen to imitate the manufacturing conditions applied to the filling fats for the rheological study. Using the chosen conditions, the crystallisation behaviour of the filling fats was studied. Under the applied processing

conditions that involved the application of shear during the crystallisation process, damage to the crystal aggregates was noticeable at a lower crystallisation temperature. Different effects of shear were seen depending on the point of application. Under shear conditions, the effect of damage to the crystal aggregates was observed at the lower crystallisation temperature. On the other hand, the effect of crystallisation under the sectional-shear conditions suggested that development of the crystal network was enhanced by the application of shear at higher temperature, which could either seed more crystal formation or allow rearrangement of the crystal network. Oscillatory rheology was also able to demonstrate the effect of processing conditions chosen to imitate manufacturing conditions for confectionery products in which the filling fat is used. The developed measurement procedure can be used to describe the rheological behaviour and deductions about the crystallisation behaviour of the filling fats during processing can be made. The crystallisation process was enhanced by the application of shear when it was applied at higher temperature. Using oscillation frequency sweep tests, it can be confirmed that the enhancement occurred due to secondary nucleation and rearrangement of the fat crystals.

Using the stop-and-return DSC technique, isothermal crystallisation behaviour of the filling fats was studied at 20 and 15 °C. Melting behaviour at the higher crystallisation temperature suggested the transformation of the α to the β' polymorph within 1 hour of the isothermal period. The rate of crystal development and transformation was faster for Creamelt 601 due to the high-melting TAGs from the crystallisation initiator and the shea stearin that promoted formation of more solid fat at higher temperature. When crystallised isothermally for a shorter period of time at the lower crystallisation temperature, it can be deduced that the bulk crystallisation developed into a mixture of lower- or medium-melting TAGs. The lower peak temperature for Creamelt 701 was due to smaller amounts of high-melting TAGs in the formulation. Furthermore, a longer isothermal time allowed clear differentiation between

TAG fractions in Creamelt 601 and further growth by crystallisation of the bulk fat in Creamelt 701. Growth of the α crystals followed by transformation into β' crystals was also seen at 15°C. In contrast to the finding at 20°C, the rate of α to β' transformation was faster in Creamelt 701 that may be due to the higher POP and lower SOS contents.

During the polymorphism study at 20°C, different sensitivity and cooling conditions (static cooling with narrow tube diameter) in XRD hindered the ability to identify any crystal formation from both filling fats. However, the presence of α polymorph and the transformation into β' as well as development of β' from the melt was observed during isothermal crystallisation at 15°C. The XRD pattern confirmed the earlier development of β' crystals and more SFC in Creamelt 701.

An oscillation frequency sweep test was used to study the viscoelastic properties of the filling fats at every step of the developed processing conditions. At the start of the processing procedure, strong liquid characteristics were exhibited by the melted fat with increasing frequency-dependent behaviour. As soon as the first crystallisation temperature was reached, slightly less frequency-dependent behaviour indicated development of a weak crystal network. The effect of shear during the first cooling step was shown by the higher elastic modulus and better separation between the two moduli, exhibiting a more ordered network. Different effects of shear during the second cooling step were detected. While the rheological response of Creamelt 701 suggested potential rearrangement of crystals by the shear applied, that of Creamelt 601 implied some damage of the crystal network by the shear applied. Under sectional-shear cooling conditions, frequency-independent behaviour and no structure loss were recognised. Overall, these findings indicate that the application of shear at higher crystallisation temperatures showed enhancement of the crystallisation process by secondary nucleation and rearrangement of the crystal network whereas shear application at lower

crystallisation temperatures may result in destruction of the network. For filled chocolate manufacturer, this shows the importance of shear application during the manufacturing process. Under static or sectional-shear cooling conditions, less-structured crystal network was developed by the filling fats. This may, then, produce softer filling that required more time to crystallise into the preferred solid fat content. Too low solid fat content can lead to fat migration that brings undesired softening of the end product and hardening of the filling (Vereecken *et al.*, 2007).

Microstructural observation after rapid cooling to 22°C showed the same small grain-like texture for both filling fats. At the beginning of the isothermal crystallisation of Creamelt 601, larger crystals from the high-melting TAGs in the crystallisation initiator can clearly be seen. However, after 60 minutes, a more structured network was seen in Creamelt 701 compared to Creamelt 601. Despite these clear differences, a similar structure between the two filling fats was seen after 24 hours of isothermal crystallisation. For industrial purposes, small fat crystals are necessary to ensure clean-melting sensation upon consumption of confectionery products. In comparison to the microstructure images under slow cooling rate, the desirable crystal structure can clearly be achieved using rapid cooling rate.

7.2 Future Work

Further establishment of the developed method of using rheology for quality control purposes should be done using a range of different qualities of fats. This can be done by studying different batches of the filling fats. Using the same procedure, further crystallisation studies on other types of filling fat are also recommended. Since substantial amounts of sugar and milk solids are often present in confectionery products, valuable information on the effect of

incorporation of these materials on the final product would be beneficial to the application of these methods. A combination of optical microscopy or X-ray analysis with rheological testing can be useful to enable direct observation of fat structures or polymorphism under shear deformation.

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APPENDIX A: Rheological changes in the crystallisation of filling fats under static cooling conditions
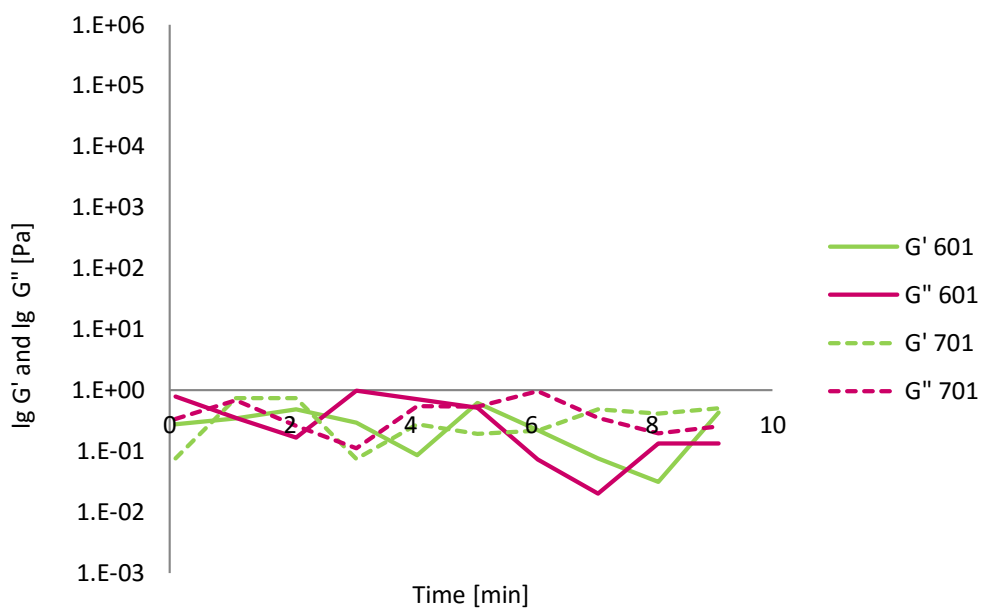
Figure A.1 Average (n=3) rheological properties of Creamelt 601 and 701 observed during isothermal at 70°C
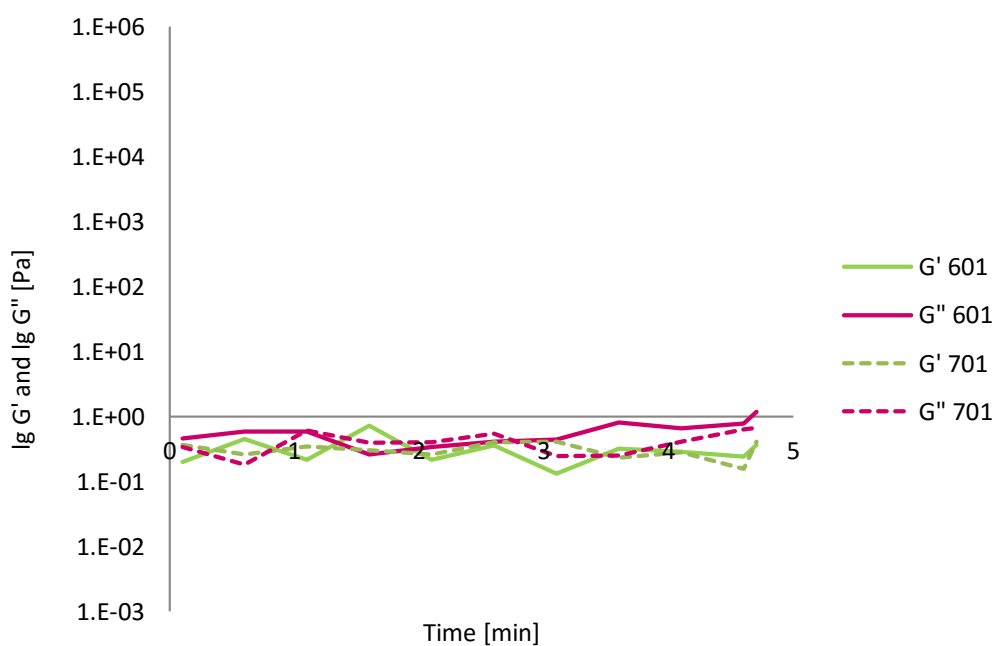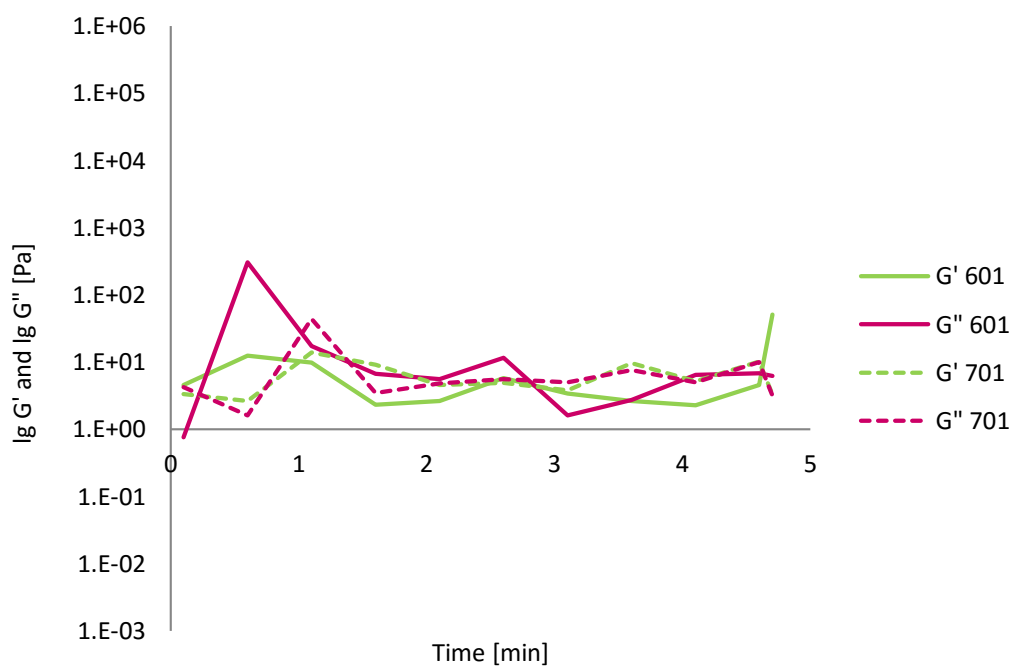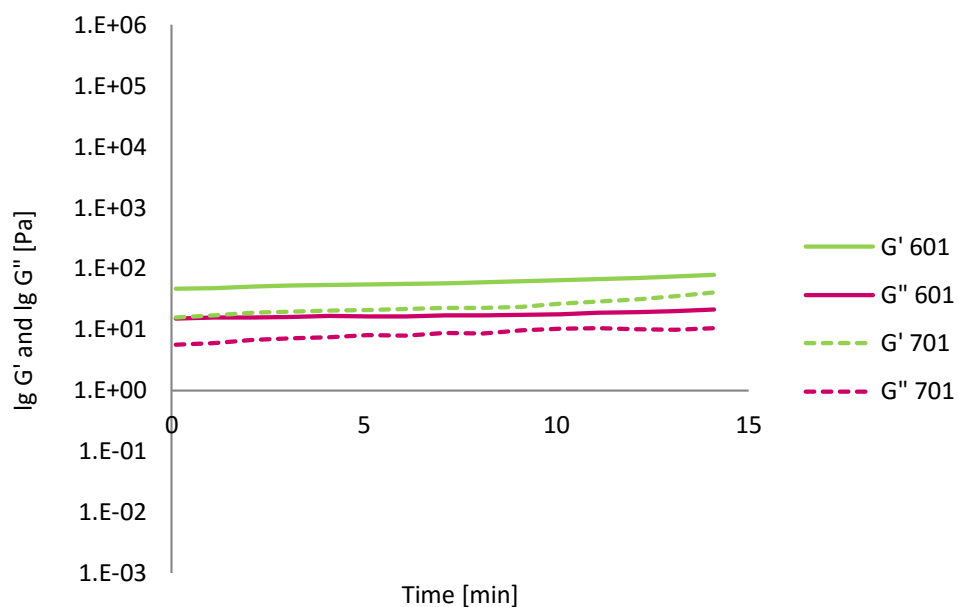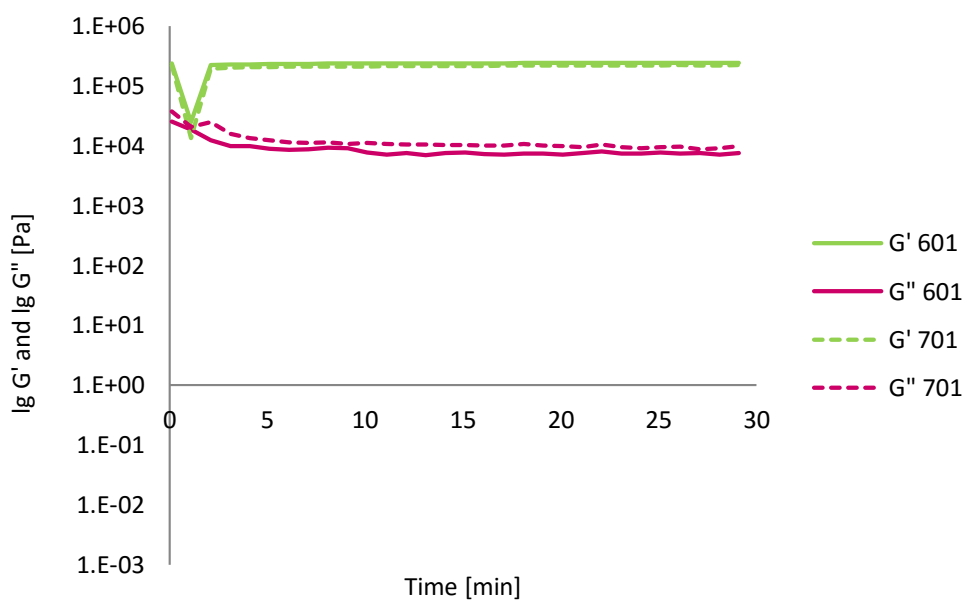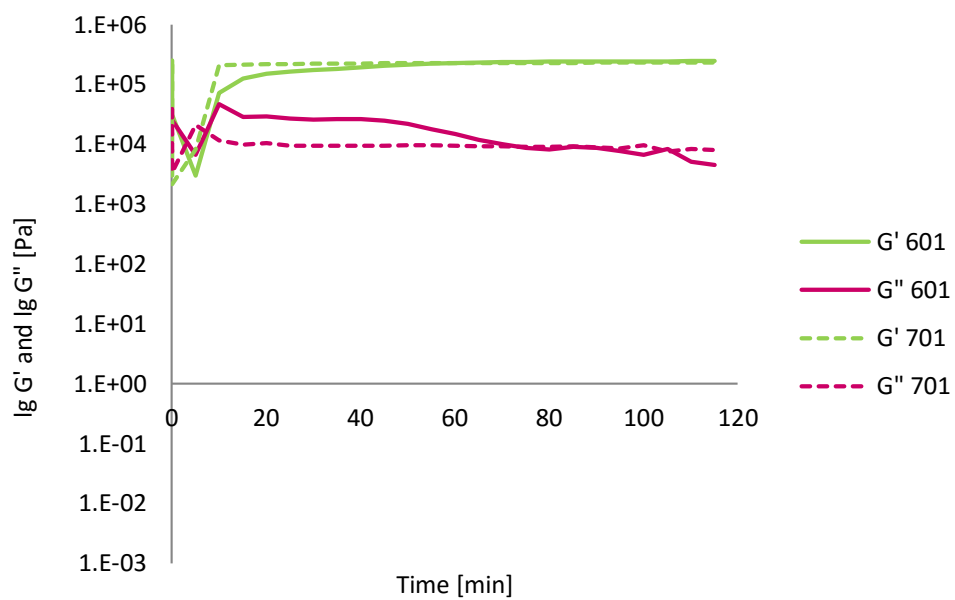

Figure A.2 Average (n=3) rheological properties of Creamelt 601 and 701 observed during cooling to 22°C (static cooling)
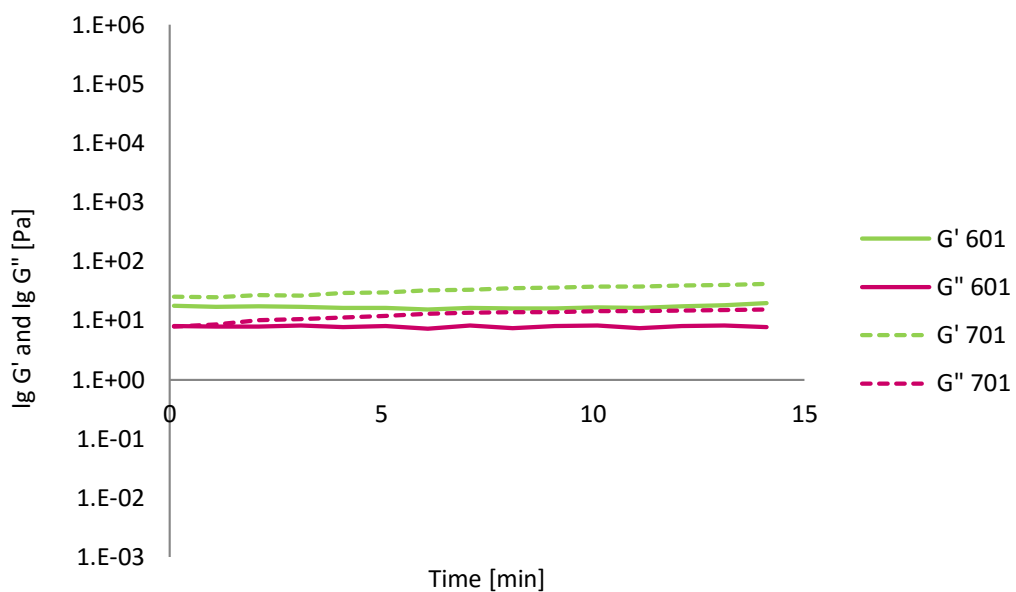
Figure A.3 Average (n=3) rheological properties of Creamelt 601 and 701 observed during isothermal crystallisation at 22°C
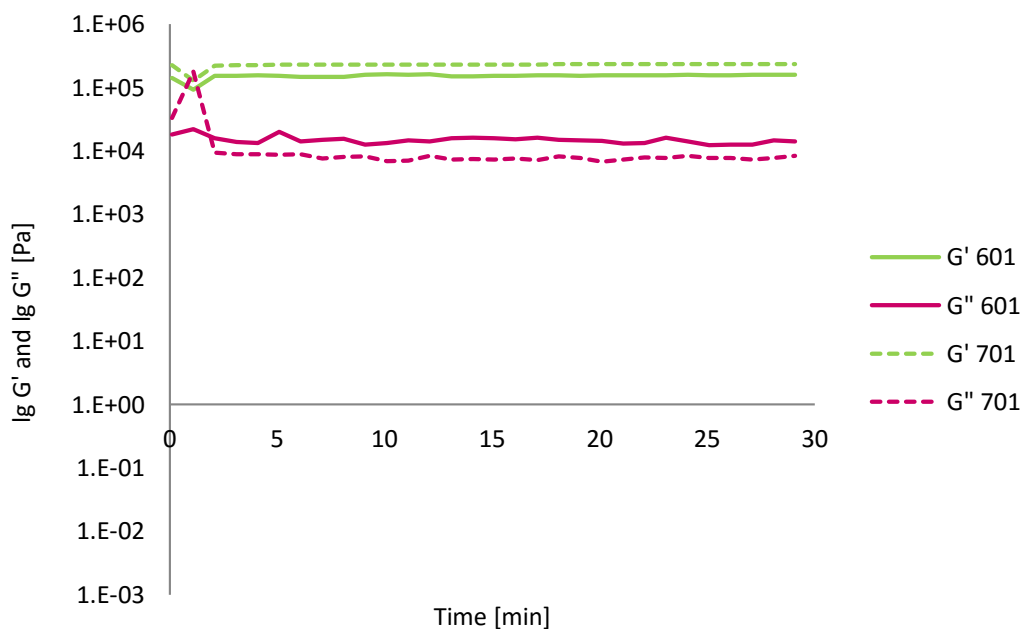
Figure A.4 Average (n=3) rheological properties of Creamelt 601 and 701 observed during isothermal crystallisation at 10°C
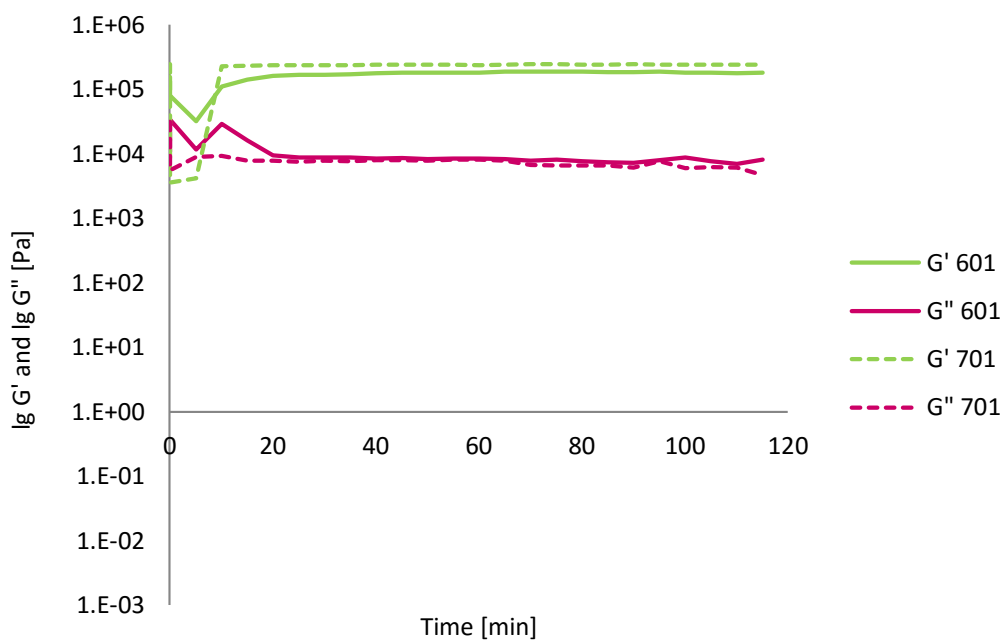
Figure A.5 Average (n=3) rheological properties of Creamelt 601 and 701 observed during isothermal crystallisation at 20°C

APPENDIX B: Rheological changes in the crystallisation of filling fats under shear cooling conditions
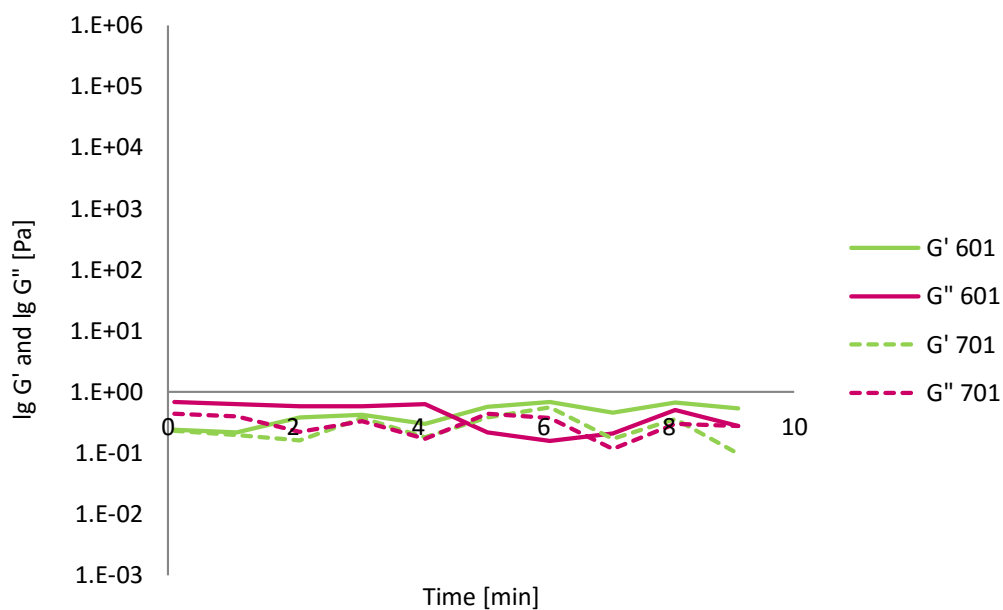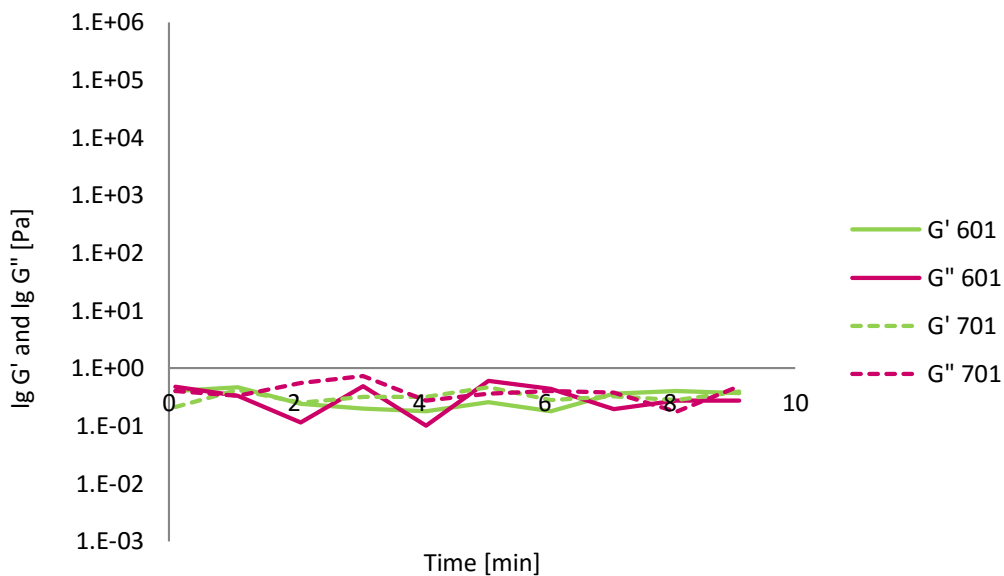

Figure B.1 Average (n=3) rheological properties of Creamelt 601 and 701 observed during isothermal at 70°C

Figure B.2 Average (n=3) rheological properties of Creamelt 601 and 701 observed during cooling to 22°C (shear cooling)

Figure B.3 Average (n=3) rheological properties of Creamelt 601 and 701 observed during isothermal crystallisation at 22°C

Figure B.4 Average (n=3) rheological properties of Creamelt 601 and 701 observed during isothermal crystallisation at 10°C

Figure B.5 Average (n=3) rheological properties of Creamelt 601 and 701 observed during isothermal crystallisation at 20°C

APPENDIX C: Rheological changes in the crystallisation of filling fats under sectional-shear cooling conditions

Figure C.1 Average (n=3) rheological properties of Creamelt 601 and 701 observed during isothermal at 70°C
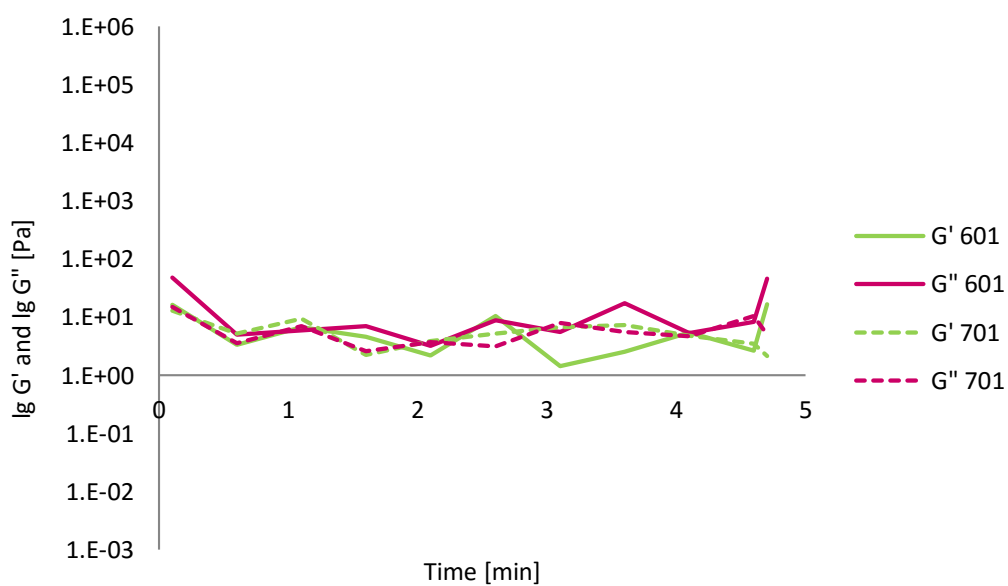

Figure C.2 Average (n=3) rheological properties of Creamelt 601 and 701 observed during cooling to 22°C (shear cooling)
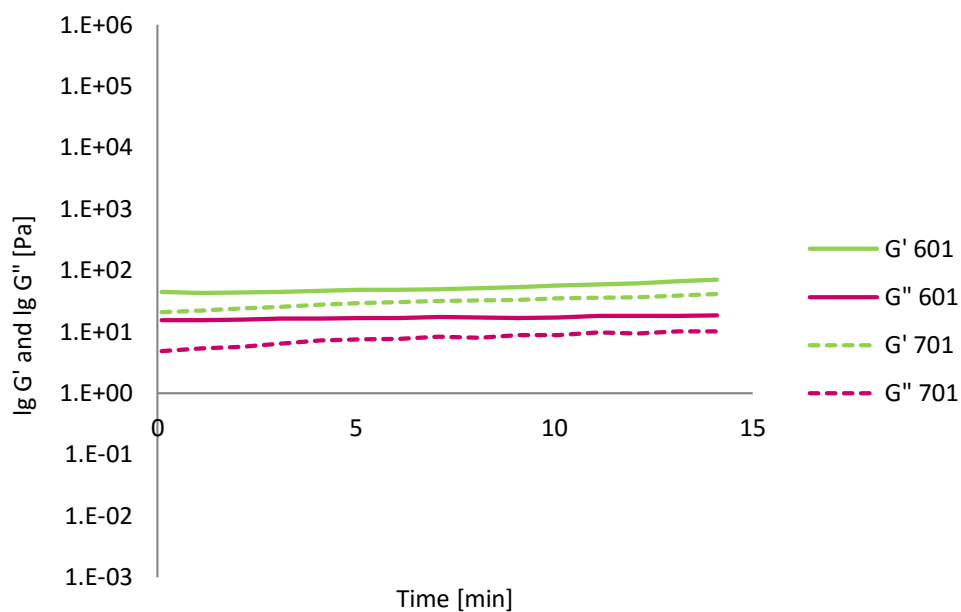
Figure C.3 Average (n=3) rheological properties of Creamelt 601 and 701 observed during isothermal crystallisation at 22°C
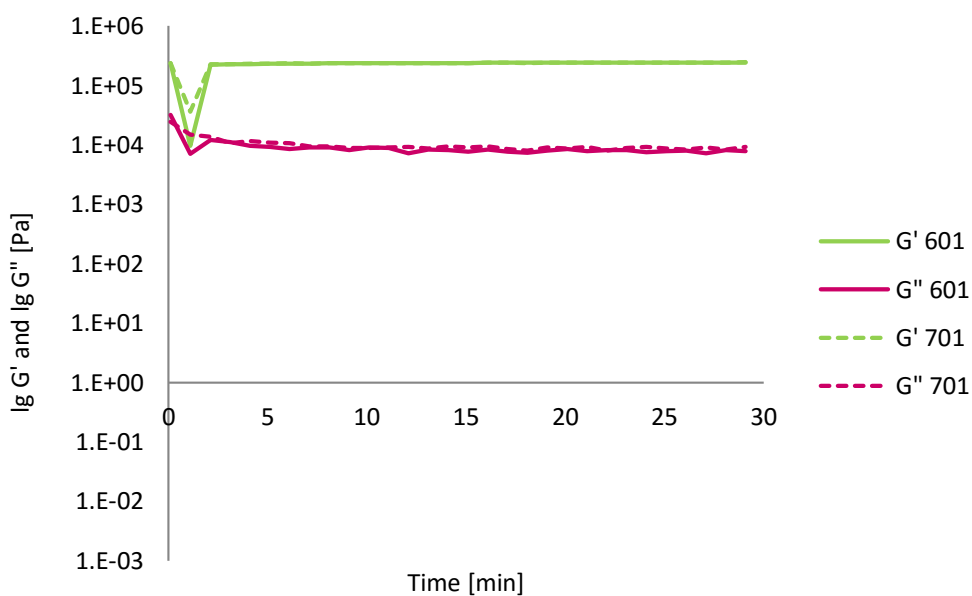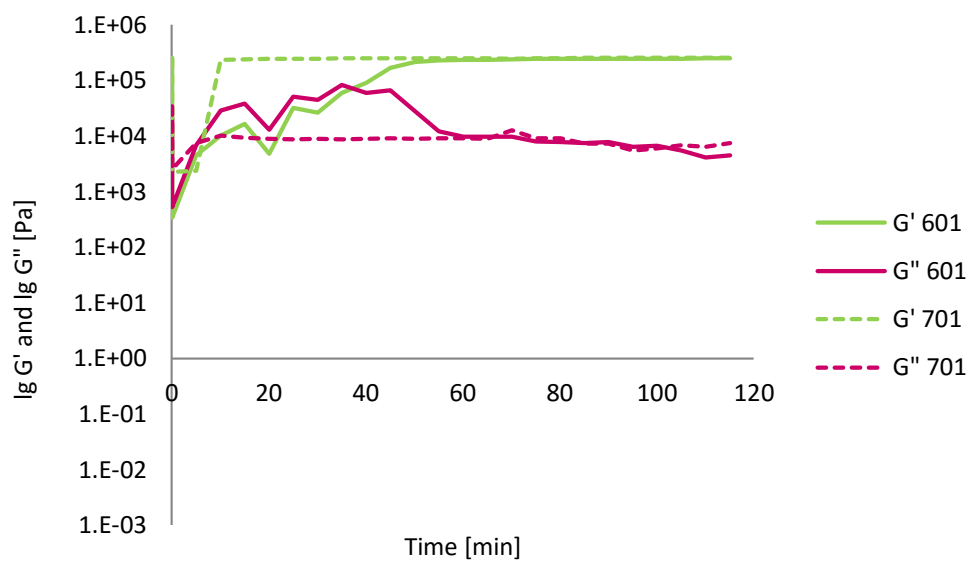

Figure C.4 Average (n=3) rheological properties of Creamelt 601 and 701 observed during isothermal crystallisation at 10°C

Figure C.5 Average (n=3) rheological properties of Creamelt 601 and 701 observed during isothermal crystallisation at 20°C