CONTROL OF FAT CRYSTALLISATION BY ADDING ADDITIVES AND CHANGING THE PROCESS

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ABSTRACT

Saturated fat has a good potential for improving emulsion stability and products texture. However in the past ten years, food industry has intended to reduce its amount to prevent its impact on heart diseases. Therefore a better understanding of fat crystallisation has been studied to design the final properties of fat systems by changing the formulation and process of crystallisation.

Fat crystallisation occurs in several stages like nucleation, crystal growth and fat network formation. Adding emulsifiers or waxes has demonstrated the possibility to change the process of crystallisation by promoting primary or secondary nucleation as a function of additive concentration. The head group size of emulsifiers has also exhibited an influence on fat crystallisation by promoting secondary nucleation with glycerol or primary heterogeneous nucleation with sorbitol. Furthermore waxes differing by their single or multi-component nature, have induced secondary and primary nucleation respectively.

The process of crystallisation has been changed by applying different cooling rates and shear rates; increasing the cooling rate increased the number of nucleation sites and shear could enhance the interactions between fat and emulsifiers.

Finally formulation and process have displayed the design of the final texture and allowed a reduction in saturated fat of 50 % while keeping the same network strength.

To my Family

ACKNOWLEDGEMENTS

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Enfin, ce PhD est dédié à toute ma famille et surtout à mes parents, Sophie, Eric, et ma soeur préférée (!), Marion, qui m’ont soutenue depuis le début de cette thèse. Merci pour vos nombreuses visites chaque année malgré le mauvais temps tant attendu…! Mes grand-parents ont aussi été d’un très grand soutien et auront été mon pilier surtout dans certains moments difficiles.

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NOMENCLATURE

Amount of elongation when force applied	ΔL
Avrami constant	k
Avrami exponent	n
Candellila wax	*CLW*
Carnauba wax	*CW or CLW*
Chain length of triglyceride	L
Chemical potential difference between crystallised and supersaturated triglycerides	$\Delta\mu$
Concentration of soluble triglyceride at saturation	$[c_i^*]$
Concentration of triglyceride at supersaturation	$[c_i]$
Constant depending on cone geometry	C
Crystallisation temperature	T
Cryo-Transmission Electron microscopy	*Cryo-TEM*
Diameter of fat cluster	d_i
Differenctial scanning calorimetry	*DSC*
Distance of penetration	p
Dynamic supercooling time exposure	β
Elastic modulus	G'
Energy in excess resulting from triglyceride aggregation	G_{ex}
Enthalpy	H
Enthalpy of melting	ΔH_m
Entropy	S

Equivalent mean volume diameter	$D_{4,3}$
Force applied to the system	F
Gas constant	R
Gibbs free energy	G
Gibbs free energy of substrate	G_s
Glycerol monostearate	*MG*
Half-time for crystallisation	$t_{1/2}$
Hardness	H_1
Heat capacity	C_p
Hydrophilic-lipophilic balance	*HLB*
Linear viscoelastic region	*LVR*
Mass of the cone geometry	M_1
Maximum nucleation rate	J_{max}
Melting temperature	$T_{m,i}$
Molar volume	$V_m^{\ s}$
Nucleation rate	J
Number of fat clusters	n_i
Number of molecules	M
Phase angle	δ
Pulsed-nuclear magnetic resonance	*p-NMR*
Rate constant for nucleation	k_1
Rice bran wax	*RBX*
Solid fat content	*SFC*
Sorbitol palmitate	*Sorb-P*

Sorbitol stearate	*Sorb-S*
Strain	γ
Stress	σ
Sunflower oil wax	*SW or SFW*
Surface area of nucleus	A_n
Surface free energy per unit area	ρ
Temperature of nucleation	T_n
Time for nucleation	t_n
Total energy of the substrate in the absence of n-sized clusters	$\varphi_{s,0}$
Total energy of the substrate in the presence of n-sized clusters	$\varphi_{s,(n)}$
Tristearin	*SSS*
Viscous modulus	G''
Volume of nucleus	V_n
Work for nucleation	*W(n)*

1. GENERAL INTRODUCTION

1.1 CONTEXT OF THE STUDY

Developed countries have changed their diet in the past twenty years and higher demands in healthier products have arisen. This has been a good opportunity for food and beverage industries to provide either functional foods that have a direct impact on health issues (high cholesterol level, lack of calcium) or healthier products with reduced sugar or fat (Nixon *et al.*, 2015). As a result, the challenge for food industry is to develop healthier products while keeping their essential properties (taste, texture, stability).

A study has shown a worldwide repartition of deaths for men and women in 2014 (Figure 1.1) and has demonstrated the need to study more cardiovascular diseases in order to prevent them (Bhatnagar *et al.*, 2015); almost 30 % of the population is concerned by cardiovascular diseases that can be genetic or linked to smoking, drinking or diet (Figure 1.2).

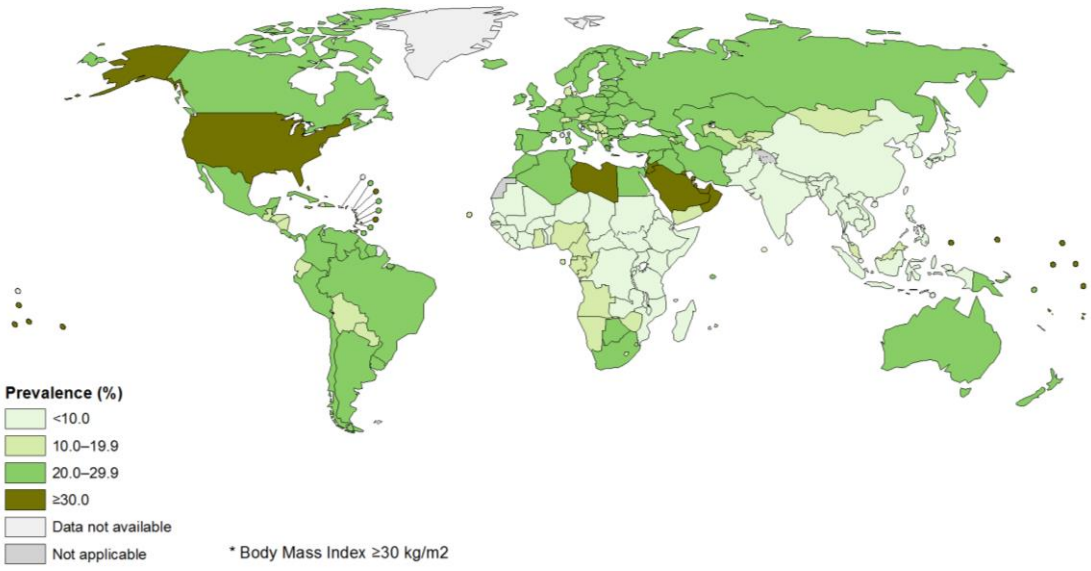

Figure 1.1. Worldwide obesity in 2014 for both sexes (WHO, 2015).

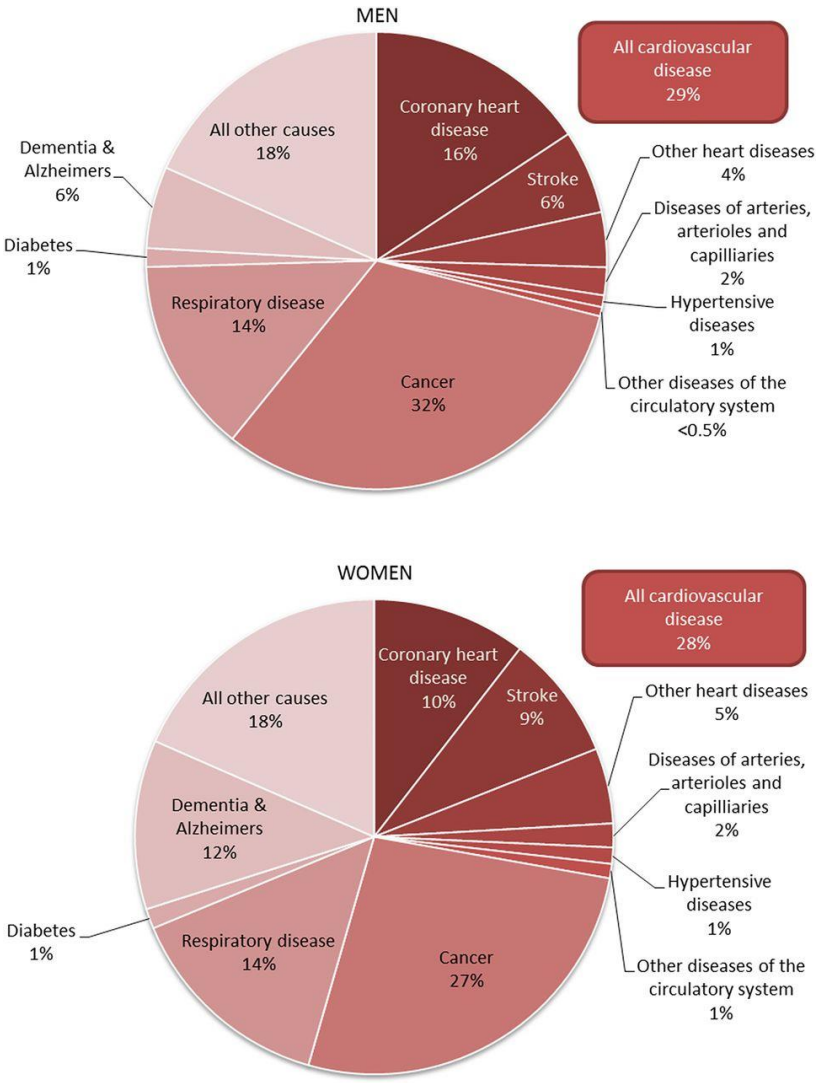

Figure 1.2. Deaths in the UK in 2014 by cause and sex (Bhatnagar *et al.*, 2015)

In addition, the worldwide increase in obesity (Body Mass Index = weight (Kg) / height $(cm)^2$ > 30) and overweight (25< BMI < 30) since 1980 has been of great interest in developed countries (WHO, 2016). Therefore, obesity and overweight have been defined such as The obesity has more than double and represents 13 % of the population, while 39 % was overweight in 2014. Moreover it has been shown that obesity is associated with cardiovascular diseases but also cancer and respiratory diseases (Nixon *et al.*, 2015). Therefore improving the diet and educating the society have been the first concern of food

companies. An example is the diet Weight Watcher which provides meetings with nutritionists and allocates points with regards to the amount of saturated fat, sugar, proteins and total calories intake over the day, also called SmartPoints® (Weightwatchers.com, 2016); the aim is to respect the SmartPoints® threshold according to your objective of weight loss. Another way of limiting the daily calorie intake has been a better packaging labelling by adding the quantity of calories, fat, carbohydrates, proteins and salt per portion (Food and Drug Administration, 2016). However a global concern is the education of the population for having the right consumption with an appropriate intake of each nutrient such as vitamins, calcium, proteins but also fat and sugar. Indeed many diets advise to eliminate either fat or carbohydrates for a specific period. This trend has also been promoted by governments like in Denmark (Vallgarda *et al.*, 2015) by launching surplus taxes for products containing high saturated fat or sugar levels (soda). On the other hand, the population needs to be educated on the benefits of each nutrient; fat brings hydrophobic vitamins and carbohydrates give energy (Fernstrom and Wurtman, 1971, Wolbach and Howe, 1925). Therefore it is important for food industry to keep the desire nutrition balance and provide healthy and delightful products.

In the past thirty years, new correlations between diet and health issues have been exhibited; the fact that saturated fat would increase cardiovascular diseases by raising the cholesterol level (Connor *et al.*, 1986, Tan *et al.*, 1980). Saturated fat has been used in many food products as it enhances their stability (emulsions, foams) and textural properties. As a consequence, food companies have tried to reduce the amount of saturated fat through its replacement by other components which can increase the product viscosity thus improving

their stability and texture; carbohydrates. However the effect of saturated fat on health has been recently controversial (Puaschitz *et al.*, 2015, Nettleton *et al.*, 2015, de Souza *et al.*,

2015, Lamarche and Couture, 2014, Micha and Mozaffarian, 2010); studies have mostly shown that replacing saturated fat by carbohydrates increases the chance to develop cardiovascular diseases compared to replacing it by polyunsaturated fat. Furthermore keeping the amount of fat constant in the diet allows the intake of some nutrients that cannot be provided by other components.

Therefore food industry has been studying fat crystallisation in order to better structure the oil in reduced saturated fat systems and tailor the final properties of the products such as ice cream, butter (replaced by margarine) or bakery products.

1.2 AIM OF THE RESEARCH

The aim of this study is to provide a better understanding of the process of crystallisation of low saturated fat systems in order to tailor the structure according to the formulation and process of crystallisation. Thus the objective is to control nucleation, crystal stability and crystal sintering to design the final textural properties.

In order to understand the effect of emulsifiers on fat crystallisation, three emulsifiers differing by the head group size (glycerol monostearate, Span 60 and Tween 60) but having the same carbon chain length as tristearin (18 carbons) are to the fat system. The results discuss their ability to crystallise with tristearin and their effect on nucleation, crystal stability and the evolution of crystallisation and viscoelastic properties aiming to propose a mechanism of tristearin / emulsifier interactions function of the moiety size.

After better understanding of the effect of emulsifiers on fat crystallisation at different stages of crystallisation, the nucleation step is controlled through the addition of two types of

wax (Sunflower oil wax and Carnauba wax) to modify the microstructure thus the final texture. The results consider the possible interactions between each wax with tristearin and how its effect on nucleation could impact the microstructure and final hardness of the system.

The process of crystallisation is modified in two ways by applying shear or by changing the cooling rate. Shear is used to increase the interactions between the emulsifier and tristearin at different stages of crystallisation; the microstructure and the evolution of crystallisation analysed by rheology allowed better comprehension of the effect of shear in the presence of emulsifiers. Changing the cooling rate in the presence of waxes results in controlling the nucleation step of wax thus tristearin crystallisation; the results show the consequence on the final textural properties.

1.3 RELEVANCE TO CARGILL

Cargill is a multinational company which manufactures food ingredients such as proteins, sweeteners or oil and fats and is the leading supplier of food industries in the world. Its main activity is to investigate how to optimise food ingredients in order to tailor efficiently the final product qualities.

This thesis has been conducted to provide a better understanding of the effect of formulation and process on fat crystallisation and maintain the texture of food products while decreasing the amount of saturated fat and emulsifiers.

1.4 THESIS LAYOUT

This thesis presents tristearin crystallisation in sunflower oil in the presence of additives and with different crystallisation processes aiming to give a better understanding of the different steps of crystallisation.

The following chapter (Chapter 2) is a literature review detailing the different steps of fat crystallisation from nucleation to the formation of a fat network and further describes the methods used for controlling fat crystallisation by changing formulation and process.

Chapter 3 shows the effect of the moiety size of emulsifiers on tristearin crystallisation. The effect of emulsifier and tristearin concentrations is analysed in order to understand the role of each emulsifier. The thermal behaviour in terms of crystallisation and crystal stability is measured by differential scanning calorimetry, the microstructure by polarized light microscopy, the evolution of crystallisation by measuring the evolution of the solid fat content by pulsed-NMR and of the viscoelastic properties by rheology upon cooling.

Chapter 4 focuses on one distinct process of crystallisation such as the effect of shear in the presence of emulsifiers. Shear rate is modified and was applied at specific stages of tristearin crystallisation. Moreover the effect of the carbon chain length compatibility with tristearin is explored in order to better understand how shear impacts crystallisation with additives. The results are discussed in terms of microstructure and evolution of the viscoelastic properties by rheology.

Chapter 5 describes the possibility to influence tristearin crystallisation by adding carnauba and sunflower oil waxes and by modifying the process of crystallisation in terms of cooling rate. The change in tristearin nucleation with waxes is discussed through the thermal

behaviour, fat cluster size, microstructure and evolution of the viscoelastic properties. A relationship between the type of nucleation and final hardness of the system is finally proposed.

1.5 PUBLICATIONS

To be submitted:

- Costard E, Douaire M. Hancocks R., Norton I.T. Tristearin crystallisation in the presence of emulsifiers: influence of the moiety size, concentration of emulsifier and tristearin.
- Costard E, Hancocks R., Spyropoulos F., Norton I.T. Shear as a precursor of triglyceride – emulsifier interactions during crystallisation.
- Costard E, Spyropoulos F., Norton I.T. Fat network properties after controlling nucleation by seeding tristearin with waxes.

Conferences attended:

- ICEF12 (Canada, 2015): The effect of wax seeding on fat crystallisation.
- Food Colloids (Germany, 2014): Controlling triglyceride crystallisation by adding emulsifiers.

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2. STATE OF THE ART

The process of fat crystallisation has been widely studied but is still under investigation due to its high complexity. Crystallisation takes place in different stages such as nucleation, crystal growth and the formation of a fat network and can be controlled by changing the formulation and the process of the system.

The first part of the state of the art reviews the process of crystallisation upon cooling from a melting phase to the transition to a solid phase. This section identifies the key stages of crystallisation that can be controlled such as the metastable zone, nucleation step, crystal growth and the formation of a fat network. Moreover the crystal structure in terms of type of polymorphs is also presented.

The second part of the state of the art reports the methods used for controlling the process of crystallisation. It shows the possibility to change the formulation by changing fat concentration or by adding additives such as emulsifiers and waxes. The process for crystallising fat is also studied with regards to the application of shear and the effect of cooling rate.

2.1 FAT CRYSTALLISATION: THEORY

Fats are present in many food products and it is very important to understand how they crystallise in order to achieve the desired texture and therefore the appropriate number of crystals, crystal size distribution, type of polymorphs and crystal sintering.

Fat is mainly composed of triglycerides and lipids such as phospholipids, mono and diglycerides, free fatty acids and other components. Each type of lipid provides different carbon chains in terms of length (from 4 to 30 carbons, Table 2.1) and number of unsaturation

bound to a skeleton such as glycerol, sorbitol *etc*. Moreover the position of the carbon chain on a glycerol can differ from a glyceride to another one and can be attributed to three positions *sn-1*, *sn-2* and *sn-3* (Karupaiah and Sundram, 2007). The considerable variety for each type of lipids gives the possibility to obtain a large number of fat systems. For instance, milk fat contains at least thirteen types of fatty acid that make this product very complex and a real challenge for understanding its behaviour (Mazzanti *et al.*, 2009, Foubert *et al.*, 2004, Martini *et al.*, 2002, Wright *et al.*, 2000a, Herrera and Hartel, 2000a, Breitschuh and Windhab, 1998).

Fatty acid	Chain length	Unsaturation
Butyric acid	4	0
Caproic acid	6	0
Caprilic acid	8	0
Capric acid	10	0
Lauric acid	12	0
Myristic acid	14	0
Palmitic acid	16	0
Stearic acid	18	0
Oleic acid	18	1
Linoleic acid	18	2
Arachidic acid	20	0
Behenic acid	22	0

Table 2.1. Nomenclature of current fatty acids.

Jensen and Mabis (1963) have discovered for the first time the structure of triglyceride by studying the tricaprin (Figure 2.1). The configuration of the molecule is in a “tuning fork” and is composed of three carbon chains attached in three positions to a glycerol. The carbon chains can be saturated or mono and poly-unsaturated in conformation *cis* or *trans*. *Trans*-

fatty acids have been banned from food products for twenty years because of their side effects and the development of heart diseases. Nowadays, it is the saturated fatty acids that are put into questions and a lower concentration is recommended (*1.1. Context of the study*).

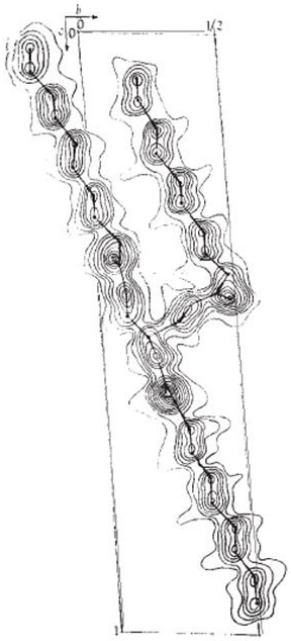

Figure 2.1. Electron density of carbon and oxygen atoms of a single triglyceride of tricaprin (Jensen and Mabis, 1963).

Thus the wide disparity in fatty acid compositions and types of triglyceride gives a good opportunity for tailoring the final texture of food products by modifying the crystal types due to different formulations and processes.

2.1.1 FAT CRYSTALS AND POLYMORPHISM

Most of fat systems such as cocoa butter and milk fat are composed of a mixture of triglycerides with different carbon chain lengths attached to a specific position on the glycerol. During crystallisation, triglycerides are bound together by Van der Waals interaction and have the particularity to be arranged in different configurations.

Firstly, the crystal lattice can have a width of two, three or four chain lengths of triglycerides (Figure 2.2). This corresponds to long spacings in crystallography that increase when increasing the carbon chain length and decrease when increasing the tilt angle (Marangoni and Wesdorp, 2013a).

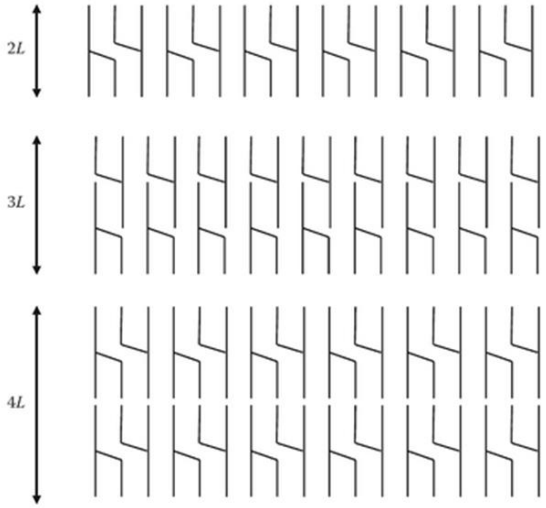

Figure 2.2. Different arrangements of triglycerides into the crystal lattice. "L" corresponds to the chain length of triglycerides (Marangoni and Wesdorp, 2013a).

Lastly triglycerides can be better arranged in the crystal lattice through better packing during crystallisation called polymorphic transitions (Lutton, 1945, Larsson, 1972). The most common polymorphs are the α-form, β'-form and β-form and their stability increases respectively. Figure 2.4 shows the sub-cells for each polymorph with an hexagonal cell for the α-form with a lattice spacing of 0.42 nm, orthorhombic cell for the β'-form with a strong lattice spacings of 0.42 nm and 0.37 nm and a triclinic cell for the β-form with a strong lattice spacing of 0.46 nm (Sato *et al.*, 1999).

The driving force needed for crystallisation differs function of the type of polymorph and is characterised by the Gibbs free energy as followed:

$$G = H - T \cdot S \tag{2.1}$$

where H is the enthalpy, T the temperature and S the entropy of the system at specific temperatures (Sato, 1999). Figure 2.5 shows the Gibbs free energy versus melting temperature of each main polymorph. The α-polymorph would be the first crystal formed during crystallisation with the highest crystallisation rate before being better arranged through polymorphic transitions solid-solid mediated or solid-melt mediated with partial melting of the first crystals created and their crystallisation in more stable forms (Koyano *et al.*, 1991, Koyano *et al.*, 1989, Kellens *et al.*, 1992).

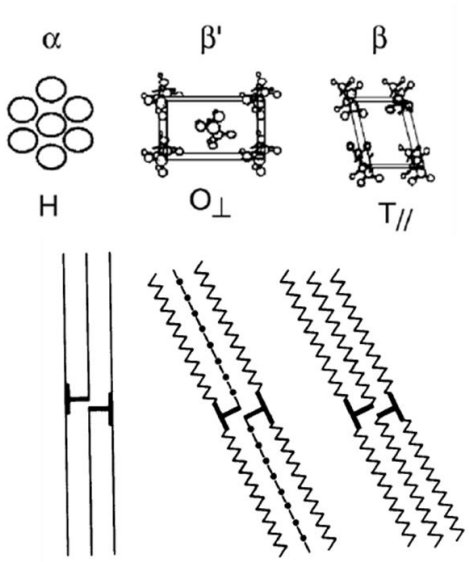

Figure 2.3. Subcell structure of the three main polymorphs of triglyceride: α – vertical oscillating chains, β' – tilted chains with adjacent chains in different planes, β – tilted chains in the same plane (Sato *et al.*, 1999, Timms, 1984).

In addition, it has been shown that the type of polymorph created impacts the crystal morphology exhibiting a spherulic growth for the α-form, feathery and lamellar growth for the β'-form and needle-like crystals for the β-form (Bunjes *et al.*, 2003, Garti and Zour, 1997, Kellens *et al.*, 1992) by comparing X-ray and polarised light microscopic analyses.

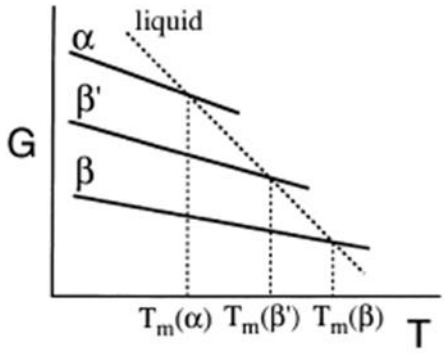

Figure 2.4. Gibbs free energy vs Temperature diagram of the three main polymorphs (Sato *et al.*, 1999).

Finally the type of polymorphs created is of great importance as it has been related to specific properties of food products such as the presence of blooming if there is no polymorph V or sandiness in margarine in the presence of β-crystals (Krog, 1977). This is why controlling the type of polymorphs allows the control of the final properties of food products and this can be achieved either by changing the formulation and adding additives or by changing the process of crystallisation as developed in the second part of the State of the Art.

Moreover mixture of triglycerides with different chain lengths or number of unsaturations can provide diverse types of crystal (Timms, 1984). Figure 2.2 represents the phase behaviour of systems containing two types of triglyceride. The simplest system corresponds to a monotectic behaviour with triglycerides very similar in melting point, molecular volume and polymorph (Figure 2.3.a). The most common system called eutectic (Figure 2.3.b) shows the segregation of crystallisation by type of triglycerides and is related to triglycerides with different molecular volume and shape but with similar melting point. When triglycerides have different melting points, the crystallisation behaviour tends to be eutectic and can shift to monotectic behaviour (Figure 2.3.c). Finally when a triglyceride is composed of at least two unsaturated fatty acids, the phase behaviour becomes peritectic (Figure 2.3.d).

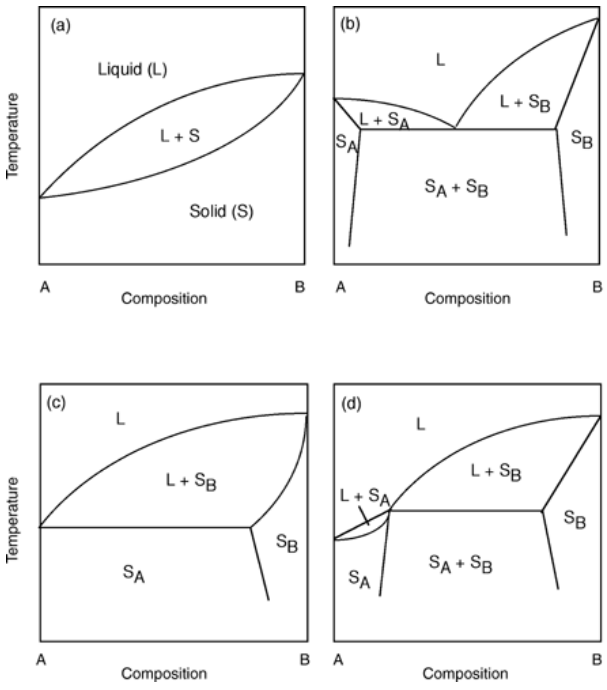

Figure 2.5. Phase behaviour in system containing two types of triglyceride (Timms, 1984); (a) monotectic, (b) eutectic, (c) eutectic and monotectic and (d) peritectic systems.

2.1.2 LIQUID LIPIDS

Larsson (1972) has been the first one reporting a specific organisation of triglycerides when the temperature is above the melting point. By using X-ray diffraction, some long-spacing lines showed the presence of a lamellar structure in the melted system. A first explanation was a crystallite size reduction or lamellar distortion (Figure 2.6). This model has been confirmed by the study of the melting state of triglycerides with different carbon chain lengths (Hernqvist, 1984); the bilayer thickness was analysed by X-ray and raised when increasing the chain length of triglycerides while it did not show any change when only increasing the temperature of the melt. Moreover, melting would modify the size of the lamellar unit and triglycerides would be linked only by their glycerol with free carbon chains.

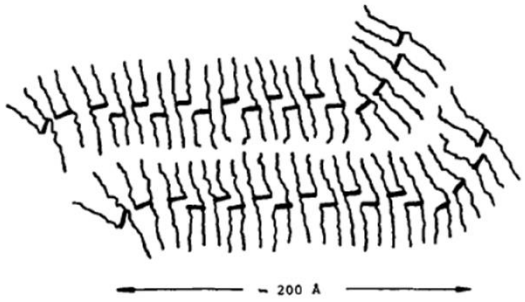

Figure 2.6. Lamellar structure of liquid crystals (Larsson, 1972).

Later a new model has been proposed and added by Corkery *et al.* (2007) with triglycerides presenting a Y-shape and forming a discotic mesophase when assembled together (Figure 2.7). This Y-shape would minimise the free energy by creating a sphere-shape and would occur at a higher temperature than the lamellar structure.

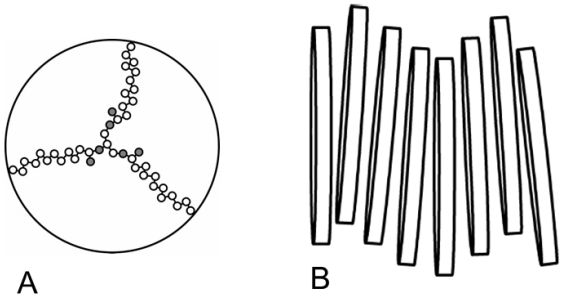

Figure 2.7. "Y-conformation" within a disc with an angle of 120 ° between each carbon chain (A). Stacking of triglycerides in a rod (B) (Corkery *et al.*, 2007).

Those experiences have demonstrated the presence of crystal memory and the importance of erasing it by melting the system long enough in order to avoid any effect of triglyceride pre-organisation during crystallisation.

2.1.3 METASTABLE ZONE

The crystallisation process starts when a fat system is cooled below its melting point. However the required driving force for crystallisation depends on many parameters such as saturation, presence of additives, cooling rate and shear rate (Chaleepa *et al.*, 2010, O'Grady *et al.*, 2007). At low driving force, the Brownian motion is predominant over triglyceride aggregation and hinders nucleation; this stage is called the metastable phase. The analysis of the overall system density by using ultrasound velocity showed more accurate results than using beam reflectance measurement as used by O'Grady *et al.* (2007) for analysing the induction time of benzoic acid in ethanol-water mixtures under shear. Chaleepa *et al.* (2010) showed that the medium density allowed the detection of micro density instead of the detection of particles that should already possess a well-defined size. Their study presented a shorter metastable zone when decreasing the cooling rate or by shearing the system upon cooling. Moreover fluorescence polarization spectroscopy revealed the possibility to study the pre-nucleation of tripalmitin in different oils by studying the anisotropy of the system (Dibildox-Alvarado *et al.*, 2010b, Dibildox-Alvarado *et al.*, 2010a). When increasing the concentration of palmitic fatty acid, the induction time for crystallisation was longer; this would be due to the development of a mixed lamellar liquid structure that would delay tripalmitin crystallisation as a result of a longer time for tripalmitin segregation and nucleation.

2.1.4 NUCLEATION

The transition from the metastable phase to a more stable phase such as solid crystal phase is a first order phase transition and starts with the nucleation of the new phase into the old phase (Kashchiev, 2000). When the driving force for nucleation is high enough (high

supersaturation or high undercooling), the first stable nuclei with a specific size are formed. The formation of a nucleus is associated with a change in surface and volume and is represented by a change in the Gibbs free energy (Marangoni and Wesdorp, 2013b):

$$\Delta G = A_n \delta - V_n \frac{\Delta \mu}{V_m^s} \tag{2.2}$$

where A_n is the surface area of a nucleus, δ is the surface free energy per unit area, V_n is the volume of a nucleus, $\Delta\mu$ is the chemical potential difference between the liquid and solid and V_m^s is the molar volume of the new solid formed. Therefore the surface area of a nucleus has a positive contribution on the Gibbs free energy while the volume changes show a negative contribution. As a result, the Gibbs free energy of nucleation reaches a maximum when a critical surface and volume of nucleus occur, e.g. at a specific nucleus size. From this point, any smaller nuclei will be breakdown while nuclei which are larger than the critical nucleus size can start growing. Subsequently nucleation ends and a new stage of crystallisation starts called crystal growth. Figure 2.8 is a schematic representation of triglyceride nucleation in the bulk with the creation of the first crystals and their growth over the creation of new nuclei.

The chemical potential difference between crystallised triglycerides and supersaturated triglycerides in the solution, $\Delta\mu_i$, is used to quantify the driving force for crystallisation and is the gain of Gibbs free energy per molecule M of triglycerides related as (Kashchiev, 2000):

$$\Delta\mu \equiv \frac{(G_{old} - G_{new})}{M} \equiv \mu_{old} - \mu_{new} \tag{2.3}$$

The chemical potential can be expressed function of the supersaturation degree:

$$\Delta\mu_i = RTln\frac{[c_i]}{[c_i^*]} \quad (2.4)$$

where R is the gas constant (R = 8.314 J/mol.K), T is the crystallisation temperature, $[c_i]$ is the concentration of triglycerides in solution at supersaturation and $[c_i^*]$ is the soluble concentration of triglycerides at saturation.

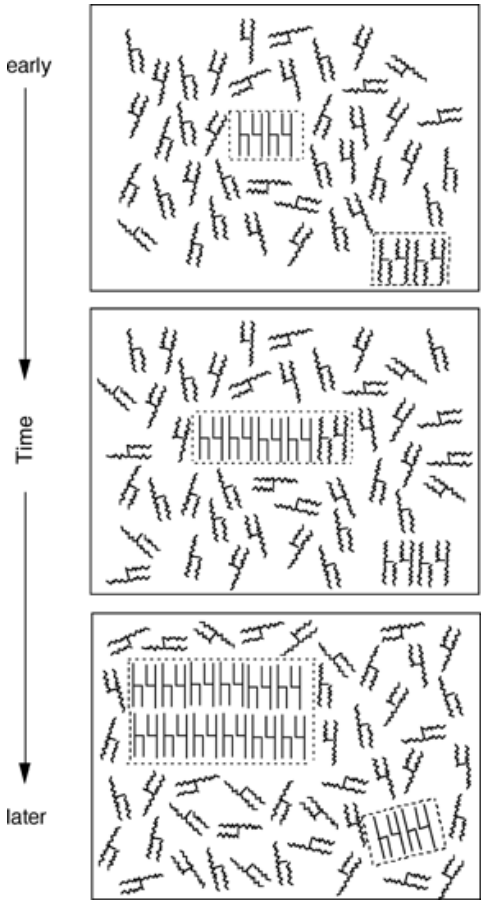

Figure 2.8. Schematic nucleation of triglycerides, straight chains – crystallised triglycerides, bent chains – melted triglycerides (Metin and Hartel, 2005).

However, the chemical potential for crystallisation is more commonly described as a function of the supercooling or degree of undercooling:

$$\Delta\mu_i = \Delta H_{m,i}\frac{(T_{m,i}-T)}{T_{m,i}} \quad (2.5)$$

where $\Delta H_{m,i}$ is the enthalpy of melting, $T_{m,i}$ is the melting temperature and T is the crystallisation temperature.

Thus combining the equations (2) and (5) shows that high degree of supercooling would increase the chemical potential and consequently would increase the contribution of the nucleus volume and would therefore decrease the Gibbs free energy. Higher degree of supercooling generates then faster nucleation step directly followed by crystal growth. Accordingly, the lower the crystallisation temperature, the faster the nucleation step is and more nuclei are formed, hence the smaller are the crystals in the system at the end of the crystallisation.

The nucleation kinetics in a non-isothermal cooling has been investigated by Marangoni *et al.* (2006) and has shown a correlation between the normalized nucleation rate (J/J_{max}) and the dynamic supercooling-time exposure (β):

$$\beta = \frac{1}{2}\Delta T_n t_n \tag{2.6}$$

where ΔT_n is the temperature of subcooling and t_n the time for the crystallisation of the first nucleus. Therefore the nucleation rate can be defined as:

$$J = J_{max} k e^{-k\sqrt{\beta}} \tag{2.7}$$

where k is the rate constant for nucleation.

Nucleation can occur in various ways such as homogeneous and heterogeneous and two distinct categories are described as primary and secondary. Primary nucleation consists in

the crystallisation of the first nuclei directly from the melt while the secondary nucleation is the crystallisation of crystals on the surface of first crystals from different origins.

Nucleation can be homogeneous, meaning that the aggregation of a specific type of triglycerides is due only to its supersaturation in the system with regards to the primary nucleation. Homogeneous secondary nucleation corresponds to the formation of new nucleation sites by breaking the first crystals created; this would increase the interface for crystallisation and the new nuclei formed have the same molecular composition as the first crystals.

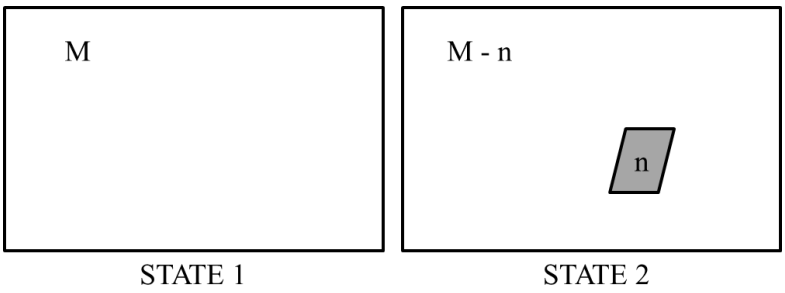

Figure 2.9. Schematic adaptation of an old phase with M molecules and a new phase with (M-n) liquid triglycerides and n crystallised triglycerides by homogeneous nucleation (Kashchiev, 2000).

Figure 2.9 represents a state 1 with a uniform density before crystallisation and the formation of clusters composed of *n* molecules of triglyceride (state 2). The free energy in both states can be defined by:

$$G_1 = M\mu_{old} \tag{2.8}$$

$$G_2(n) = (M - n)\mu_{old} + G(n) \tag{2.9}$$

$$G(n) = n\mu_{new} + G_{ex}(n) \tag{2.10}$$

where $G_{ex}(n)$ is the energy in excess resulting from the aggregation of triglycerides. As a consequence, the work $W(n)$ for homogeneous nucleation of clusters of *n*-sized is:

$$W(n) = -n\Delta\mu + G_{ex}(n) \tag{2.11}$$

When nucleation is heterogeneous, triglycerides crystallise by catalysis with a foreign surface like a stirrer, air bubbles in the case of sonication (Alig *et al.*, 1998) or other molecules that would not crystallise at this temperature. Once again if the substrate is not a crystal, this corresponds to primary nucleation while if the substrate is a crystal from the same or different origin this would be secondary nucleation (Figure 2.10).

The free energy in both states can be expressed as:

$$G_1 = M\mu_{old} + G_s + \phi_{s,0} \tag{2.12}$$

$$G_2(n) = (M-n)\mu_{old} + G(n) + G_s + \phi_s(n) \tag{2.13}$$

where $\Phi_{s,0}$ is the total surface energy of the substrate in the absence of n-sized clusters and $\phi_s(n)$ in the presence of n-sized clusters. Thus the work $W(n)$ for heterogeneous nucleation can be defined as:

$$W(n) = -n\Delta\mu + G_{ex}(n) + \phi_s(n) - \phi_{s,0} \tag{2.14}$$

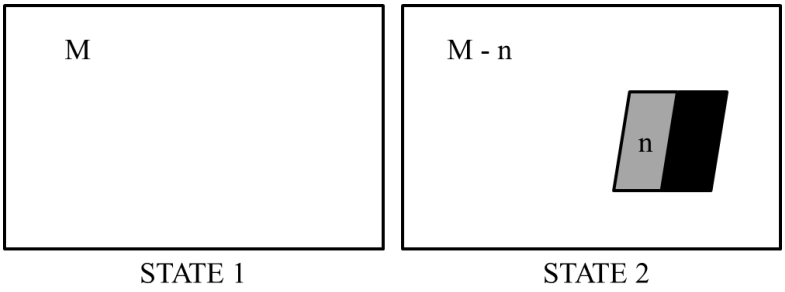

Figure 2.10. Schematic adaptation of an old phase with M molecules and a new phase with (M-n) liquid triglycerides and n crystallised triglycerides on a substrate by heterogeneous nucleation (Kashchiev, 2000).

Nucleation has been studied using different techniques with regards to the analysis of the induction time for crystallisation referred as the creation of the first nuclei. Those techniques include differential scanning calorimetry (Toro-Vazquez *et al.*, 2000, Ng, 1990, Ng, 1989, Toro-Vazquez *et al.*, 2002), pulsed NMR (Wright *et al.*, 2000b, Wang *et al.*, 2011), turbidimetry (Wright *et al.*, 2000a), viscosimetry (Chen *et al.*, 2002), diffusive light scattering (Toro-Vazquez *et al.*, 2002, Liu *et al.*, 1993), polarized light microscopy (Martini *et al.*, 2002, Wright *et al.*, 2000b, Herrera *et al.*, 1998), polarized light microscopy supplemented by a CDS photo sensor (Koyano *et al.*, 1991, Koyano *et al.*, 1989) and laser polarized light turbidimetry (Kerr *et al.*, 2011, Cerdeira *et al.*, 2005, Cerdeira *et al.*, 2004, Wright *et al.*, 2000b, Herrera *et al.*, 1998). It has been shown that some techniques such as polarized light microscopy, laser light polarized turbidimetry and light scattering are more accurate for determining the induction time for crystallisation (Cerdeira *et al.*, 2004, Toro-Vazquez *et al.*, 2002, Wright *et al.*, 2000b, Herrera *et al.*, 1998). Differential scanning calorimetry, p-NMR and viscosimetry do not offer the possibility to analyse a solid fat content below 0.1 % and are recommended for studying the beginning of the crystal growth instead.

2.1.5 CRYSTAL GROWTH

The crystal growth takes place as soon as the first nucleus is formed as the energy required for generating a nucleus from the melt is higher than the energy required for triglyceride aggregation on a nucleus. Crystal growth has been studied mostly upon isothermal cooling by using the Avrami model. This model is used for quantifying the kinetics of crystallisation and provide information on the type of crystal growth mechanism. The Avrami equation can be written as followed for fat crystallisation (Avrami, 1939, Avrami, 1941, Avrami, 1940):

$$\frac{SFC}{SFC_{max}} = 1 - e^{-kt^n} \tag{2.15}$$

where *SFC* is the solid fat content at a specific time (%), SFC_{max} is the maximum solid fat content reached at a specific temperature (%), *k* is the Avrami constant and *n* is the Avrami exponent. The constant *k* refers to the crystallisation rate and the exponent *n*, to the number of dimensions of the crystal growth; crystal growth can be in one, two or three dimensions and this is characterised by crystals with the shape of rods, disk or sphere respectively.

2.1.6 FORMATION OF FAT CRYSTAL NETWORKS

Once crystals starts growing there is the possibility of interaction between fat clusters and their sintering can lead to the formation of a fat network. Many studies have tried to correlate the solid fat content to the final viscoelastic properties of fat systems. A first approach has shown a linear contribution of the solid fat content to the elastic modulus G' (Nederveen, 1963, Vdtempel, 1961). Taking into account the presence of fat aggregates in the network has developed a power law correlation instead of a linear correlation (Vandentempel,

1979). Furthermore a scaling theory has been borrowed to the network formed by polymers and has introduced the concept of inter and intra connexions between the flocs of colloidal gels (Shih *et al.*, 1990). Finally the notion of fractals has been introduced by Vreeker *et al.* (1992) for fat systems by using light scattering with tristearin; they have shown the possibility to study the aggregates restructuration during aging by observing an increase in the fractal dimension and therefore more compact aggregates. The use of this notion has been emphasized by Marangoni and Rousseau (1996) who have displayed the possibility to use fractals to determine the structure of plastic fat; they have related a decrease in hardness to a decrease in the fractal dimension. However this model has been useful only in homogeneous system as Braga *et al.* (2015) have demonstrated that it cannot be used in systems with particles heterogeneously sized.

To summarise fat crystal networks are composed of several entities at different scales (Figure 2.11); the largest entity is fat aggregates that can be spherical or needle-like and can vary from 10 to 100 μm. Those aggregates are composed of nano-platelets that are the association of triglyceride lamella (Marangoni *et al.*, 2012).

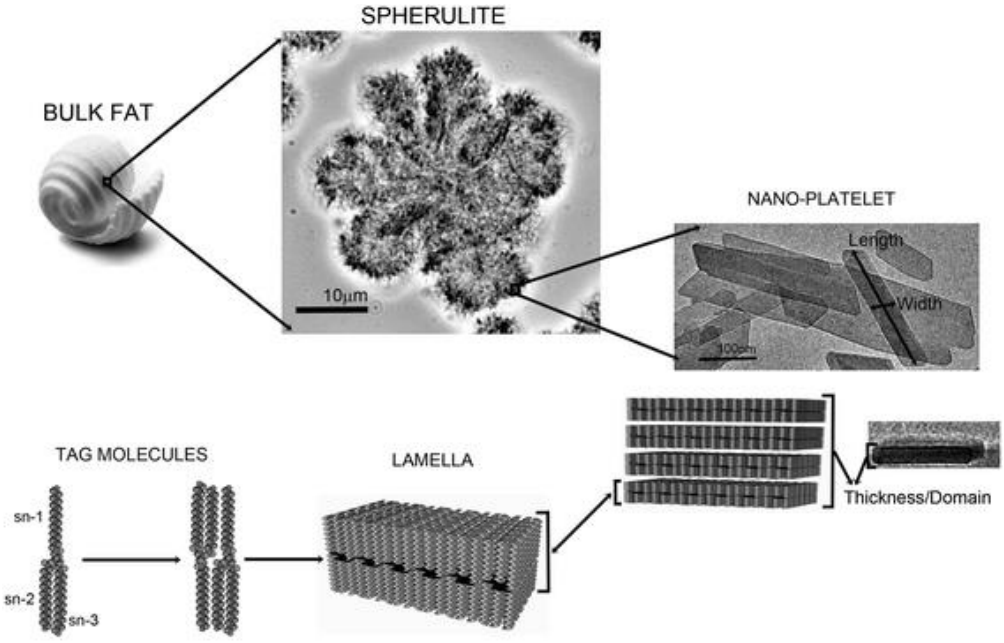

Figure 2.11. From nano to macro structure of fat crystal networks (Marangoni *et al.*, 2012)

Crystal sintering has been studied by analysing the level of sedimentation of tristearin (Johansson, 1995b, Johansson and Bergenstahl, 1995, Johansson and Bergenstahl, 1992a); the higher the level of sedimentation, the stronger the sintering between crystals and the less oil could be trapped by the crystals. They have reported that the sedimentation is higher when tristearin is only in the β'-form compared to mixture of β' and β-forms. This has revealed that the fat network is influenced from the state of the nanoscale.

Fat networks have also been studied in terms of viscoeslasticity (Maleky *et al.*, 2012, Toro-Vazquez *et al.*, 2005, Herrera and Hartel, 2000b). This refers to the Hooke's law as such:

$$F = k\Delta L \tag{2.16}$$

where F is the applied force, k is a constant and ΔL is the amount of elongation when the force applied is low enough and provide small deformations (Marangoni and Narine, 2013). In this case materials are analysed as springs. A crucial information coming out viscoelasticity is the formation of a gel, i.e. the formation of a system which displays no steady-state flow, cross-linked by crystals (Ferry, 1980a); when the elastic modulus G' becomes higher than the viscous modulus G'', the system shows a solid-like behaviour indicating the formation of a fat network (Winter and Chambon, 1986). The moduli can be expressed function of the stress applied to the system, σ, the strain resulting from it, γ, and the phase angle, δ, as (Ferry, 1980b):

$$G' = \frac{\sigma}{\gamma}\cos\delta \tag{2.17}$$

$$G'' = \frac{\sigma}{\gamma}\sin\delta \tag{2.18}$$

Lastly the hardness of the system has been investigated using cone penetrometry by analysing the yield force (Hayakawa and Deman, 1982, Dixon and Parekh, 1980, Haighton, 1959); the cone penetrates the sample for a specific time at a constant speed and the penetration distance is analysed (Hayakawa and Deman, 1982). The hardness of the system can be expressed as:

$$H_1 = C\frac{M}{p^n} \tag{2.19}$$

where H_1 is the yield value or hardness, C is a constant that depends on the cone geometry, M is the mass of the cone, p is the distance of penetration and n is a constant close to two and vary functions of the type of network. However most of the studies have used the maximum force at the end of the penetration for a specific distance as the hardness of the network.

2.2 TAILORING THE STRUCTURE AND TEXTURE OF FAT SYSTEMS

Understanding the process of fat crystallisation is necessary in order to investigate ways of controlling crystallisation. The textural and hedonic properties of food products can be influenced by the structure of the oil phase such as blooming in chocolate, emulsion destabilisation or changes in margarine consistency. Nowadays controlling the structure of fat systems has been studied by modifying the formulation like changing the type of triglycerides and their concentration or adding additives; changing the process has also shown an effect on the final structure of the product and the following paragraph will review the effect of cooling rate and shear rate especially.

2.2.1 FORMULATION OF FAT SYSTEMS

2.2.1.1 SUPERSATURATION

This paragraph presents the effect of supersaturation in terms of change in the formulation by varying the concentration of triglycerides.

It has been shown a decrease in the melting temperature by differential scanning calorimetry when diluting the system (Ng, 1989, Basso *et al.*, 2010, Maleky *et al.*, 2012). Moreover 100 % fully hydrogenated canola oil has exhibited different behaviour at lower concentrations by displaying the presence of α-crystals except when cooled very slowly at 0.1 °C/min (Maleky *et al.*, 2012); therefore the solvent plays a key role in polymorphic transition by allowing a re-arrangement of triglycerides into more stable forms at higher scan rates than in pure triglyceride systems.

The induction time for crystallisation is affected by fat concentration and is longer when diluting the tripalmitin as referred by Ng (1989) and Basso *et al.* (2010). They have also shown lower crystallisation rates by analysing the solid fat content using *p*-NMR.

As expected, diluting the system has presented an increase in the crystal size with lower nucleation rate (Herrera and Hartel, 2000a, Martini *et al.*, 2002). In addition the nanoscale dimensions analysed by cryo-TEM have also exhibited the same trend and larger nano-platelets have been observed when diluting fully hydrogenated canola oil in sunflower oil from 20 to 100 % (Acevedo and Marangoni, 2010b, Acevedo and Marangoni, 2010a).

Finally increasing fat concentration has shown an increase in material in the bulk and thus more connexions between crystals; this is in agreement with higher elastic modulus observed by rheology when increasing the concentration of high melting fraction of milk (Herrera and Hartel, 2000b).

2.2.1.2 ADDITION OF ADDITIVES

The entire process of crystallisation can be influenced by the addition of additives such as nucleation, crystal growth, fat crystal network and the type of polymorphs and their creation (Krog, 1977, Smith *et al.*, 2011, Wassell *et al.*, 2010, Pernetti *et al.*, 2007). It is therefore important to understand how physical and chemical properties of additives can impact each step of crystallisation.

2.2.1.2.1 Emulsifiers

Emulsifiers are mostly employed in food industry in order to stabilise interfaces due to their hydrophilic and lipophilic characteristics (Krog, 1977). Indeed emulsifiers are usually

composed of ester of fatty acids bound to a polar moiety such as glycerol, sorbitol or sucrose. There is then the possibility to design the required emulsifier with regards to the phase that needs to be stabilised by changing the hydrophilic-lipophilic balance (HLB); modifying the fatty acid chain length or the moiety size will change the HLB and the properties of the emulsifiers. Figure 2.12 shows that lower or higher HLB values promotes partial stabilisation of the interface and emulsifiers are mostly in the lipid phase or aqueous phase respectively; for medium HLB values, the interface can be stabilised from both phases and would lead to the best stabilisation of the interface.

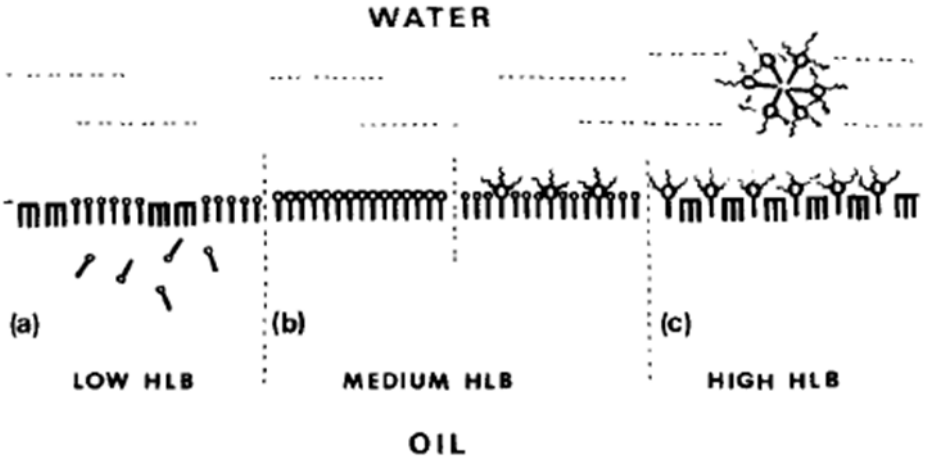

Figure 2.12. HLB scale (Low: 1-4; medium: 8-12; high: 15-20) and emulsifier interaction at the oil - water interface (Krog, 1977).

As a result, emulsifiers are required for stabilising emulsions; although they interact with the interface, they can also interact with the lipid phase and change the process of fat crystallisation.

Nucleation

Emulsifiers are also known for having an impact on the process of fat crystallisation by reducing the induction time for nucleation (Lupi *et al.*, 2012, Maruyama *et al.*, 2014) or by

lengthening it (Maruyama *et al.*, 2014, Cerdeira *et al.*, 2003, Bunjes *et al.*, 2003). Cerdeira *et al.* (2003) has proposed that longer induction time with emulsifiers can be attributed to a structural dissimilarity that would hinder triglyceride aggregation. Other studies have compared the type of emulsifier in order to understand better their impact on nucleation.

It has been demonstrated that diglycerides lengthen the induction time of milk fat crystallisation (Wright and Marangoni, 2002, Foubert *et al.*, 2004), so does low concentration of monoglycerides (Foubert *et al.*, 2004). However Foubert *et al.* (2004) has also shown that monoglycerides play a different role when added in higher concentrations; they decrease the induction time for crystallisation by crystallising at higher temperature and acting like a template for milk fat.

Moreover carbon chain length similarity between emulsifier and triglyceride is essential to decrease the induction time for crystallisation. Basso *et al.* (2010) has presented that although behenic-monoglyceride crystallises at higher temperature than palmitic-monoglyceride, the latter is more efficient and sharply shortened the induction time of tripalmitin. Likewise the use of saturated and unsaturated monoglycerides has displayed the ability to shorten the induction time of saturated triglycerides of coconut oil (Sonwai *et al.*, 2016) and palm oil (Fredrick *et al.*, 2008). They have shown that unsaturated monoglycerides would increase the induction time with higher concentrations. Thus the compatibility between emulsifier and triglyceride would allow a seeding effect, while an incompatibility such as unsaturation would hinder fat crystallisation.

Lastly the effect of the emulsifier moiety size has been studied and has revealed a shorter induction time with smaller moieties or more similarity between the moiety and triglyceride glycerol as shown by Cerdeira *et al.* (2005) by comparing the effect of sucrose

and polyglycerol fatty acids on milk fat crystallisation (Figure 15). However this study was contradicted by a more pronounced decrease of induction time when increasing the moiety from glycerol (monoglyceride) to sorbitol (Span 60) to polyoxyethylene sorbitol (Tween 60) for crystallising hydrogenated palm kernel stearin (Wang *et al.*, 2011). The use of different techniques for analysing the induction time would be the reason for such variation with higher accuracy when using polarized laser light turbidimetry (Cerdeira *et al.*, 2005) than nuclear magnetic resonance (Wang *et al.*, 2011) as described *Chapter 2.7.4 Nucleation*.

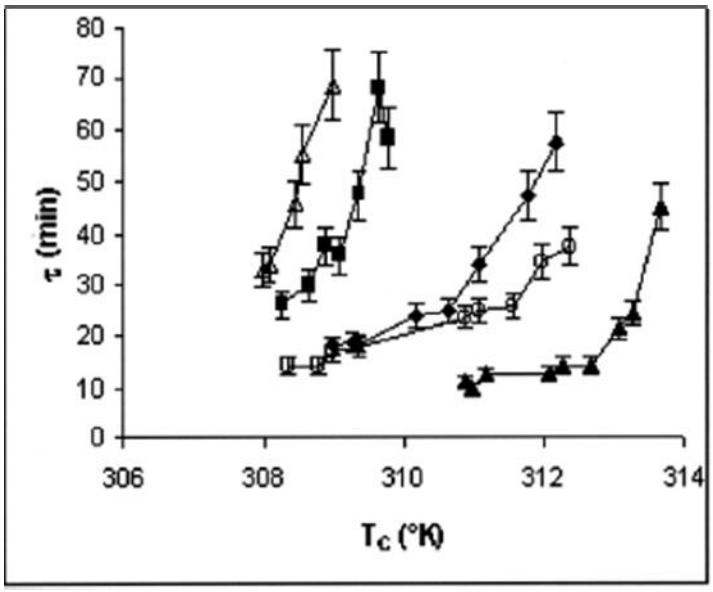

Figure 2.13. Induction time of crystallisation versus crystallisation temperature: 60 % high milk fat in sunflower oil (◆) and in the presence of 0.1 % sucrose palmitate (■), sucrose stearate (△), polyglycerol behenate (▲) and polyglycerol stearate (○) (Cerdeira *et al.*, 2005)

Polymorphic transitions

The emulsifier molecular structure has shown an effect on the ability of triglycerides to re-arrange in more or less stable polymorphs. They can create impurities in the crystal lattice of triglycerides and hinder polymorphic transitions by stabilising specific polymorphs (Cerdeira *et al.*, 2005, Bunjes *et al.*, 2003). Sorbitol monostearate and Span 60 have shown

their ability to slow down polymorphic transitions of stearic acid (Garti *et al.*, 1982) and of 1,3-distearoyl-2-oleo glycerol (Guth *et al.*, 1989). It has been demonstrated, by using the Langmuir adsorption theory, that emulsifiers can adsorb differently at the crystal lattice with regards to their concentration in the bulk (Garti and Zour, 1997); low emulsifier concentration provides adsorption of single molecules onto the crystal lattice and hinders polymorphic transitions, while higher concentration can aggregate in hemi-micelles and would be less compatible with the crystal lattice showing less effect on polymorphic transitions. Moreover it has been shown that emulsifier can interfere in the exchange of molecule between the melted and crystallised system thus delaying polymorphic transitions (Smith *et al.*, 2007). However some emulsifiers like monopalmitin have displayed a different behaviour and the crystallisation of palm oil triglycerides in the most stable polymorph directly from the melt (Verstringe *et al.*, 2014); this is due to a seeding effect of the emulsifier that crystallised at a higher temperature and created a template for triglyceride crystallisation.

The effect of the number of carbon chains per emulsifier has been considered. Increasing the number of carbon chains on a single polar head changes the ability of the emulsifier to be incorporated into the crystal lattice. It has been shown that di-glycerides and sorbitol dipalmitate or distearate and tristearate sharply delay the polymorphic transitions of triglycerides (Loisel *et al.*, 1998a, Elisabettini *et al.*, 1996, Mohamed and Larsson, 1992, Lee and De Man, 1984). Mohamed and Larsson (1992) has investigated the molecular interaction between sorbitol monopalmitate, dipalmitate and tripalmitate and tripalmitin (PPP) by using X-ray diffraction and has presented the formation of a molecular compound between sorbitan monopalmitate and PPP able to promote polymorphic transitions while the eutectic behaviour of sorbitol dipalmitate and tripalmitate created impurities and hindered polymorphic transitions.

The carbon chain length is also a parameter that can impact the polymorphic transitions. Lee and De Man (1984) has demonstrated a slight difference between sorbitan monostearate and monopalmitate on canola oil crystallisation over storage; slower polymorphic transition to the β-form occurred in the presence of sorbitol monostearate linked to its greater compatibility with the triglycerides in canola oil and thus a better adsorption to their crystal lattice with the creation of more impurities. However this difference in behaviour is very limited as the emulsifier carbon chain differs by only two carbons.

In addition, the compatibility between emulsifiers and the chain length of triglycerides has been investigated. Garti *et al.* (1985) has shown that sorbitan monostearate delays polymorphic transitions of tristearin but has no effect on trilaurin and Aronhime (1988) has demonstrated that sorbitan monostearate did not affect tripalmitin polymorphic transitions. The effect of unsaturation of monoglyceride on saturated triglycerides of palm oil has also been studied and has displayed no significant differences in the type of polymorphs created (Fredrick *et al.*, 2008); saturated and unsaturated both acted as a template for triglyceride crystallisation and generated the formation of the most stable form directly from the melt.

A last critical emulsifier characteristic to take into account in polymorphic transition is the size of emulsifier moiety. A common result has been the potential for monoglycerides to create stable polymorphs directly from the melt due to their potential to crystallise at higher temperature than triglyceride, to create seeds and thus a template for crystallisation (Shelef and Garti, 1988, Aronhime *et al.*, 1987, Garti *et al.*, 1986). However Elisabettini *et al.* (1996) has shown a delay of polymorphic transitions with glycerol monostearate; a different effect would be due to a lower concentration of emulsifier used in this study. As a result, lower concentrations of monoglyceride would not allow their crystallisation at higher temperature

than triglycerides and would promote the adsorption of single molecules in the crystal lattice creating impurities and delaying a molecular re-arrangement as discussed previously in this chapter. Besides those authors have also studied the effect of increasing the moiety size by using a sorbitol (Span 60) or polyoxyethylene sorbitol (Tween 60) and various behaviours occurred. They have demonstrated that sorbitol has the capacity to hinder polymorphic transitions of triglycerides by the adsorption of single molecules in the crystal lattice (Figure 14). Various results have come out of the study of Tween that acts like Span but less efficiently due to its lower ability to fit in the crystal lattice with lower concentration (Elisabettini *et al.*, 1996, Garti *et al.*, 1986). Higher concentration such as 10 % Tween 60 has delivered different results with more polymorphic transitions. Aronhime *et al.* (1987) has justified that imperfections in the crystal lattice can promote better re-arrangement of triglycerides and then polymorphic transitions to more stable forms.

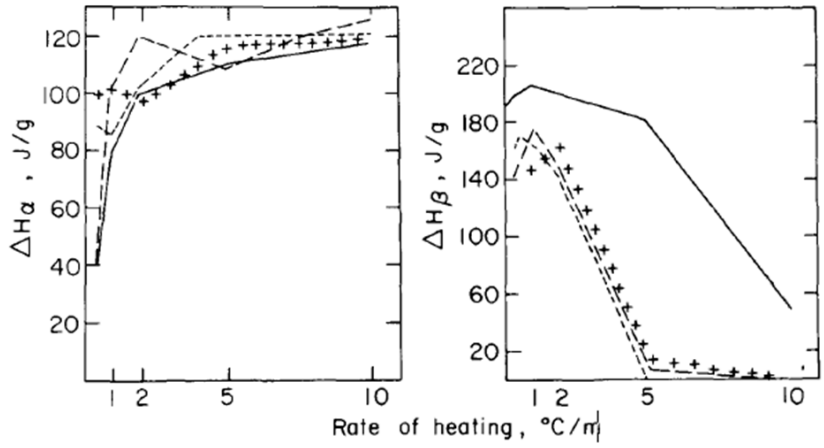

Figure 2.14. Enthalpy of melting of α-crystals ΔHα and β-crystals ΔHβ of tristearin (—) in the presence of Span 60 (---) versus heating rate (Aronhime *et al.*, 1987).

Crystallisation rate

The crystallisation rate of cocoa butter in the presence of polar lipids has been analysed by using the Avrami equation (Toro-Vazquez *et al.*, 2005); polar lipids would be responsible for slower crystallisation rate due to the stabilisation of the α-form that requires more time for crystallisation. The concentration of emulsifier can also impact the crystallisation rate as presented in the crystallisation of milk fat with glycerol monostearate (Foubert *et al.*, 2004); lower concentration of glycerol monostearate has resulted in slowing down the crystallisation rate while higher concentration (1 %) increased the crystallisation rate. Therefore increasing the supersaturation of monoglyceride allows the creation of seeds in the system and larger area for crystallisation thus higher crystallisation rate. Beyond Foubert *et al.* (2004) has studied the effect of mono-di-tri ester of fatty acids and has shown lower crystallisation rate with diglycerides than monoglycerides due to larger impurities that would lead to generation of steric hindrance for further triglyceride incorporation. This study has confirmed previous results showing lower crystallisation rate of dark chocolate with distearin than with stearic acid (Loisel *et al.*, 1998b).

Another particularity to investigate has been the saturation of emulsifiers compared to triglycerides. It has been exhibited slower crystallisation rate of saturated triglycerides of coconut oil with unsaturated sorbitol (Sonwai *et al.*, 2016) and of palm oil with unsaturated monoglycerides (Vereecken *et al.*, 2009). As mentioned previously this is due to steric hindrance in the crystal lattice of triglyceride that prevents triglycerides from being incorporated onto the crystal lattice.

Microstructure – Crystal growth

The microstructure of fat system is strongly influenced by the addition of emulsifiers whatever the type of emulsifiers added with regards to the emulsifier carbon chain length

(Basso *et al.*, 2010, Garbolino *et al.*, 2005, Cerdeira *et al.*, 2003, Lee and De Man, 1984), the number of carbon chains per polar head (Lee and De Man, 1984) and the moiety size (Garbolino *et al.*, 2005); all these studies have presented the creation of smaller crystals in the presence of emulsifiers. This is due to steric hindrance in the fat crystal lattice that would prevent the incorporation of melted triglycerides and thus the crystal growth. However the concentration has sound to have an impact on microstructure as although 0.1 % diglyceride delays the onset of crystallisation, Wright and Marangoni (2002) has shown that they did not affect the crystal size. Therefore lower concentrations of emulsifier would lessen their effect on crystal size. Furthermore more homogeneous structure has been observed when adding emulsifiers with longer carbon chain such as behenic-monoglycerides (C22) with tripalmitin (Basso *et al.*, 2010).

Formation of a fat network

The effect of emulsifiers on the final properties of a fat network have been mostly studied by analysing the hardness of the system (Garbolino *et al.*, 2005, Litwinenko *et al.*, 2004), but other techniques such as rheology (Toro-Vazquez *et al.*, 2005) or sedimentation (Johansson and Bergenstahl, 1992) have also been adopted. The study of the elastic modulus G' by low deformation rheology has presented no signification differences when cocoa butter crystallised with or without polar lipids (Toro-Vazquez *et al.*, 2005), while this technique could differentiate the crystallisation rate. Using sedimentation has allowed the analysis of the sintering strength of crystals when adding monoglycerides and Span 60 to β-crystals of tristearin in oil (Johansson and Bergenstahl, 1992a); it has demonstrated the ability of higher concentrations of monoglyceride and of all concentrations studied of Span 60 to increase the sediment volume exhibiting stronger interactions between crystals. The analysis of hardness

has presented differences when increasing the emulsifier moiety size by adding sorbitol or sucrose ester to palm oil but no differences when changing the emulsifier carbon chain length from palmitate to stearate; higher hardness has been achieved with smaller moiety such as sorbitol. In addition, Litwinenko *et al.* (2004) has attended to understand the difference in hardness between samples containing Tween 60 and glycerol; hardness has been attributed to a reduction in the crystal size in the presence of Tween 60, while glycerol increased the crystal-melt interfacial tension as analysed through the fractal theory.

As a result, various properties of emulsifiers can be used in order to tailor the process of fat crystallisation and the final properties of the system.

2.2.1.3 WAXES

Edible waxes are obtained from vegetable fats (sunflower oil waxes, carnauba wax) or animal fat (beeswax) and have been used in food industry to structure the oil phase in low-saturated fat systems and generate the formation of organogels (Martini *et al.*, 2015, Hwang *et al.*, 2014, Blake *et al.*, 2014, Hwang *et al.*, 2013, Alvarez-Mitre *et al.*, 2012, Morales-Rueda *et al.*, 2009b, Morales-Rueda *et al.*, 2009a, Dassanayake *et al.*, 2009, Toro-Vazquez *et al.*, 2007). Their composition varies function of the type of waxes (Table 2.2) but are mainly constituted of esters with two fatty acids (Blake *et al.*, 2014). The variation in their composition allows a wide window for tailoring fat systems as it is presented further.

Material	RBX	SFX	CLX	CRX
Ester content (%)	92-97	97-100	27-35	84-85
Free fatty acid (%)	0-2	0-1	7-10	3-3.5
Free fatty alcohol (%)	-	-	10-15	2-3
Hydrocarbons (%)	-	-	50-65	1.5-3
Resins/others (%)	3-8	0-3	-	6.5-10
Melting point (°C)	78-82	74-77	60-73	80-85

Table 2.2. Composition of rice bran wax (RBX), sunflower oil wax (SFX), candellila wax (CLX) and carnauba wax (CRX) (Blake et al., 2014).

Waxes in oil

Blake *et al.* (2014), Dassanayake *et al.* (2009), Martini and Anon (2003) and Martini *et al.* (2015) have studied the thermal behaviour by differential scanning calorimetry of rice bran wax, sunflower oil wax, candellila wax and carnauba wax (Figure 2.15) and beeswax and paraffin wax (Figure 2.16). By comparing the results with X-ray diffraction (Blake *et al.*, 2014, Bouzidi *et al.*, 2012, Dassanayake *et al.*, 2009), only β' polymorph has been observed revealing the multi-component characteristic of candellila wax, carnauba wax, beeswax and paraffin wax in contrast to a single-component in rice bran wax and sunflower oil wax showing a narrow melting peak.

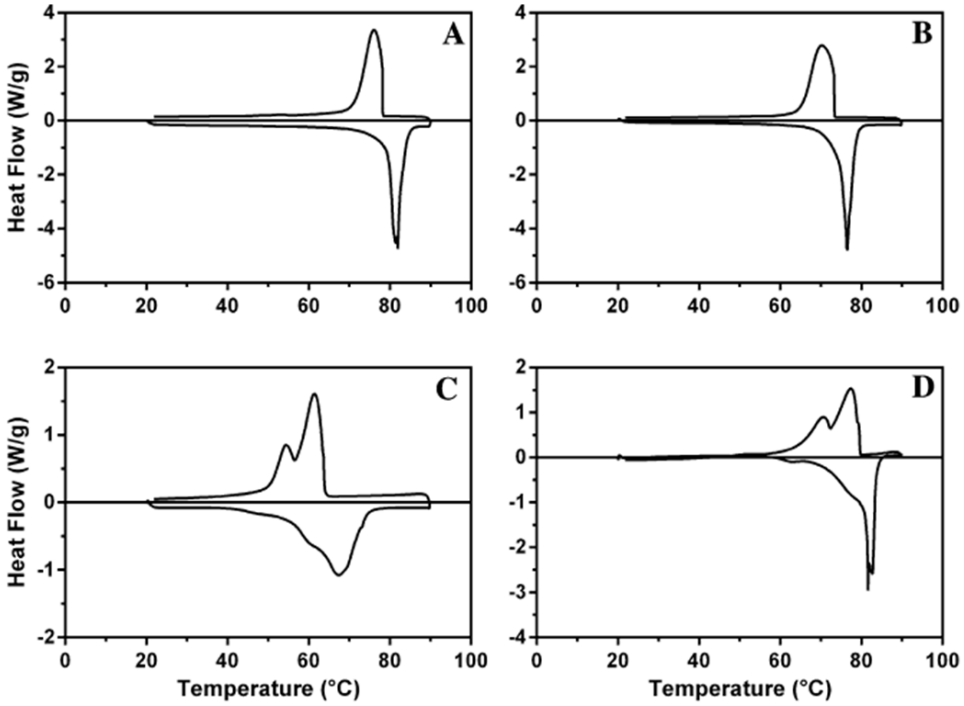

Figure 2.15. Melting and crystallisation curves analysed by DSC with a scan rate of 5 °C/min of neat RBX (A), SFX (B), CLX (C) and CRX (D) (Blake *et al.*, 2014).

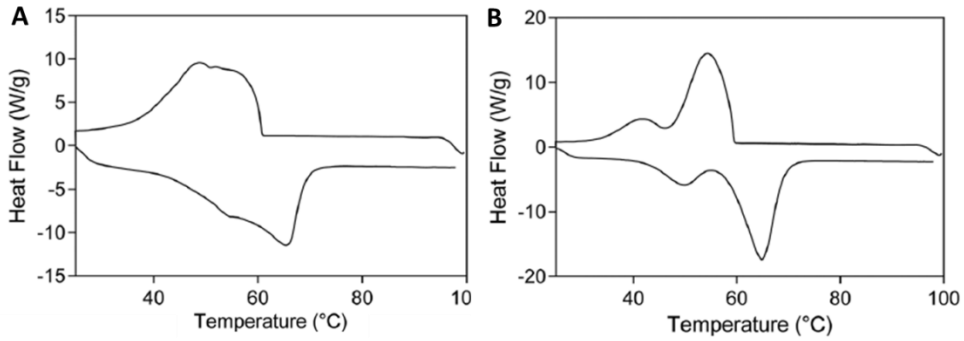

Figure 2.16. Melting and crystallisation curves analysed by DSC with a scan rate of 5 °C/min of neat beeswax (A) and paraffin wax (B) (Martini *et al.*, 2015).

Furthermore, the ability of waxes for their oil binding capacity has been investigated by first analysing the microstructure in oil (Figure 2.17) (Martini *et al.*, 2015, Blake *et al.*, 2014, Morales-Rueda *et al.*, 2009a, Dassanayake *et al.*, 2009). Rice bran waxes, sunflower oil wax have shown a fibrous morphology compared to clusters for candellila wax, carnauba wax, beeswax and paraffin wax in canola oil. This particularity has exhibited a change in the

gelling capacity of each wax by reaching the gelling point analysed by texture analysis with 1 % in oil for the fibrous waxes and 2 % for candellila wax and 4 % for carnauba wax (Blake *et al.*, 2014, Dassanayake *et al.*, 2009).

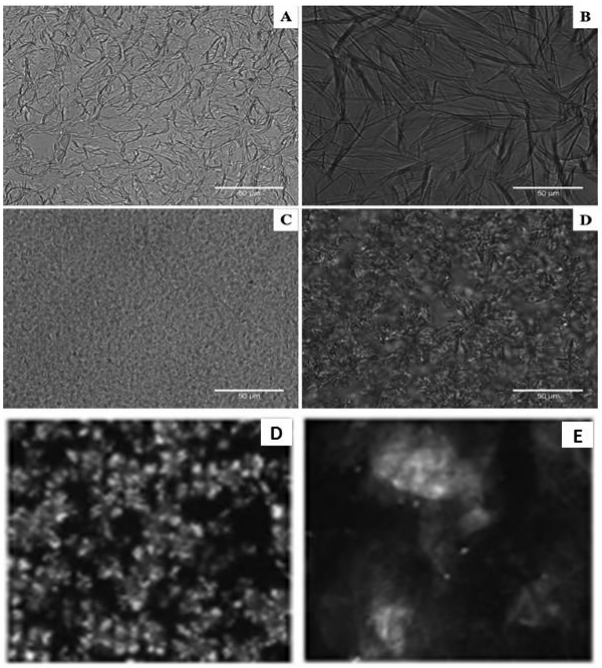

Figure 2.17. Micrograph of 10 % wax in canola oil by polarized light microscopy; RBX(A), SFX (B), CLX (C), CRX (D) (Blake *et al.*, 2014) and beeswax (D) and paraffin wax (E) (Martini *et al.*, 2015).

The effect of waxes in emulsion stabilisation and texture has been investigated in order to reduce the amount of saturated fat. It has been demonstrated that 2 to 6 % sunflower oil wax instead of 18 to 30 % hydrogenated soybean oil in margarine increases the firmness of the system as analysed by compression test with higher melting point (Hwang *et al.*, 2013). In addition, better understanding of emulsion stabilisation by using carnauba wax in oil-in-water emulsion has displayed higher level of fat droplet aggregation in the presence of wax (Matsumura *et al.*, 1995); this effect has been attributed to higher carnauba wax crystal orientation into the droplet that favoured fat droplet destabilisation as required in ice cream.

As waxes have exposed their great ability to increase the gelling properties with much lower concentrations than saturated triglycerides, some studies have attended to control wax and triglyceride crystallisation by combining them in the same system. It has been shown that tripalmitin and candellila wax can co-crystallise at low concentration (< 3 %) and this impacts the melting properties of wax by reducing its increase in melting temperature when increasing wax concentration (Toro-Vazquez *et al.*, 2009). Moreover adding phospholipids and goat fat to beeswax has prevented polymorphic transition in wax crystals and therefore has improved its drug incorporation efficiency (Attama and Mueller-Goymann, 2008).

As a result, little interest has been given to the effect of wax on fat crystallisation. Martini *et al.* (2008) has shown that sunflower oil wax influences the crystallisation process of milk fat by decreasing milk fat melting temperature and by reducing the induction time for crystallisation and thus creating smaller crystals attributed to the formation of more nucleation sites.

Finally, waxes have proven their ability to tailor the texture of fat system and to affect fat crystallisation. Therefore this is a new field of investigation that would allow better control of the texture of healthier fat system.

2.2.2 PROCESS OF CRYSTALLISATION

The process of crystallisation is highly dependent of supercooling and forces applied like shear rate. These two parameters have shown an influence at each stage of crystallisation such as nucleation, microstructure fat crystal network and also the polymorphs created. It is therefore crucial to understand the effect of cooling rate or shear rate on crystallisation in order to tailor the microstructure and final texture of fat system.

2.2.2.1 COOLING RATE – SUPERCOOLING

Changing the cooling rate or the crystallisation temperature results in modifying the driving force for crystallisation. This can have consequences on the type of polymorphs created, the number of nucleation sites and then the crystal size and final hardness.

Polymorphic transitions

Triglycerides with different carbon chain lengths have been crystallised under different cooling rates and have been analysed by DSC and X-ray diffraction (Norton *et al.*, 1985, Hernqvist, 1984). It has been displayed the formation of α-crystals with faster cooling rates while the formation of more stable polymorphs, such as β'-form, with slower cooling rates. Those results have then been confirmed by different studies that analysed the presence of polymorphic transitions by inducing fast cooling followed by slower heating rate providing information on the presence of polymorphic transitions (Aronhime, 1988, Garti *et al.*, 1985). However other studies have exhibited no difference in polymorphs created when varying the cooling rate of fully hydrogenated canola oil (Maleky *et al.*, 2012) and hydrogenated sunflower oil (Herrera *et al.*, 1992); the difference with the latter studies is the type of fat used that is composed of several types of triglycerides that would be more influenced by fractionation event when changing the cooling rate.

The impact of supercooling has also been investigated by modifying the crystallisation temperature and applying an isothermal cooling. van Malssen *et al.* (1999) and Martini *et al.* (2013) have studied the crystallisation of cocoa butter and high stearic fraction of sunflower oil and showed the formation of more stable polymorphs with higher crystallisation temperatures. In addition, Breitschuh and Windhab (1998) have exposed broader melting peak

and at higher temperature by scanning differential calorimetry when lowering the crystallisation temperature of milk fat and then the presence of less stable crystals when increasing the supercooling.

Nucleation

The induction time for crystallisation has appeared to be drastically decreased when increasing the cooling rate as presented by Wang *et al.* (2011) with the crystallisation of hydrogenated palm kernel stearin at different crystallisation temperatures; from a specific temperature, cooling rate predominates over the effect of emulsifier such as monoglycerides or Span 60 on nucleation.

Microstructure

Increasing supercooling by either increasing the cooling rate or lowering the temperature of crystallisation of milk fat has exhibited the formation of smaller crystals as observed by polarized light microscopy (Martini *et al.*, 2002, Campos *et al.*, 2002, Herrera and Hartel, 2000a). Maleky *et al.* (2012) and Acevedo and Marangoni (2010b) have studied the crystal size of fully hydrogenated canola oil at the nanoscale by using cryo-TEM; they have discovered that the nanoscale is also affected by the cooling rate and higher scan rates generate smaller nano and meso-crystals (Figure 2.18). Besides the size of crystals, the shape is altered with regards to supercooling as considered by Bunjes *et al.* (2003) with the crystallisation of tripalmitin; faster cooling rate has developed round shape crystals while slower cooling rate created needle-like crystals. This can be attributed to the relationship between the polymorphic form and microstructure as discussed *Chapter 2.7.5. Crystal growth*.

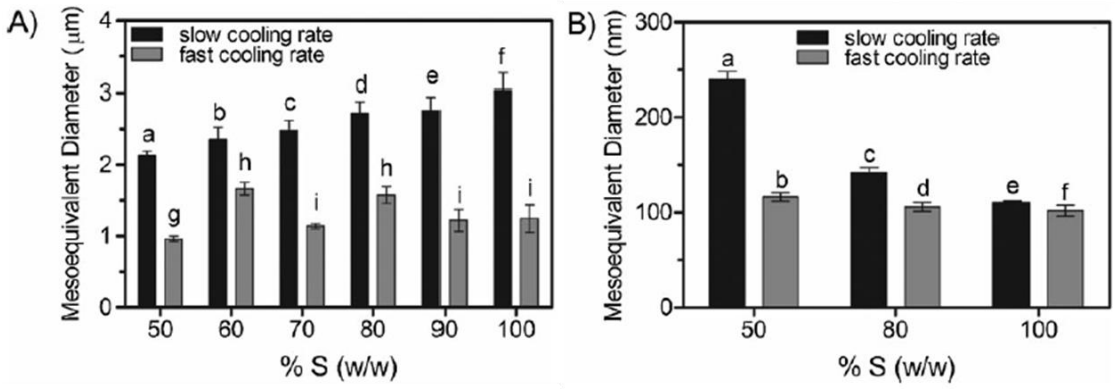

Figure 2.18. Equivalent diameter of the meso (A) and nano (B) crystals versus fully hydrogenated canola oil concentration (%) upon slow (0.7 °C/min) and fast (10 °C/min) cooling rates (Maleky *et al.*, 2012).

As a result, those latter studies are in good agreement with the hypothesis proposed in *Chapter 2.7.4 Nucleation* with the concept that higher degree of supercooling would promote faster nucleation, more nucleation sites and therefore the formation of smaller crystals.

Fat network

The fat crystal network has been widely investigated with regards to the final viscoelasticity and final hardness versus cooling rate or crystallisation temperature. Herrera and Hartel (2000b) and Maleky *et al.* (2012) have evidenced higher final G' by low amplitude deformation when decreasing the cooling rate of 100 % high melting fraction of milk fat and fully hydrogenated canola oil respectively. Higher storage temperature has also proven to increase the final G'. However the final hardness analysed by cone penetrometry decreased when decreasing the cooling rate of cocoa butter (Humphrey and Narine, 2007) and anhydrous milk fat (Campos *et al.*, 2002).

To conclude supercooling has shown decisive impact on fat crystallisation; lower supercooling has exposed the ability to crystallise in more stable polymorphs directly from

the melt, to increase the induction time for crystallisation, to create larger crystals and to impact the texture by increasing the viscoelasticity and decreasing the final hardness of the system.

2.2.2.2 SHEAR RATE

Shear rate is commonly applied in emulsions to decrease the size of droplets in the main phase and increase the surface coverage by emulsifiers in order to stabilise the emulsion (Lupi *et al.*, 2012, Kalnin *et al.*, 2004). Hence understanding the effect of shear rate on the different phases of emulsion such as the fat system is of great importance.

Polymorphic transitions

The effect of shear on the polymorphs created has been studied by Rheo-X-ray diffraction or by re-melting the system in a differential scanning calorimeter in order to analyse the final crystal stability. Cocoa butter is one of the main system investigated with regards to the effect of shear as specific polymorphs are required such as the form V to prevent blooming (Shi and Maleky, 2015, Mazzanti *et al.*, 2007, Sonwai and Mackley, 2006, MacMillan *et al.*, 2002); it has been demonstrated that shearing during cooling from 3 s^{-1} promotes earlier crystallisation of the form V. The most probable hypothesis for faster polymorphic transitions is the ability of shear to melt the crystals created by increasing the temperature locally and generating the crystallisation of more stable polymorphs directly from the melt (Sonwai and Mackley, 2006, Ojijo *et al.*, 2004). Other studies have confirmed the effect of shear versus polymorphic transitions with other fat systems such as milk fat and palm oil or sunflower oil (Martini *et al.*, 2013, Mazzanti *et al.*, 2009, Mazzanti *et al.*, 2003) with the development of more stable polymorphs with shear rate. Although shear has

manifested higher influence on polymorphic transitions, they have also referred a delay in the polymorphic transitions with the addition of polar lipids compared to a free-polar lipids system with shear.

Nucleation

The kinetics of crystallisation is also affected by the presence of shear. Increasing the shear rate has resulted in decreasing the induction time for crystallisation of the least stable forms such as form II for cocoa butter (Sonwai and Mackley, 2006) the α-form for milk fat (Mazzanti *et al.*, 2003). In addition, Martini *et al.* (2013) has displayed an increase in solid fat content from higher temperature when increasing shear rate of soft and high stearic fraction of sunflower oil

Microstructure

As presented previously, shear forces induce an increase in the temperature of the system and crystallisation of stable polymorphs directly from the melt. Moreover shearing has exhibited changes in the microstructure with the presence of spheroidal fat clusters as observed with milk fat in Figure 2.19 (Herrera and Hartel, 2000a) and fully hydrogenated canola oil (Tran *et al.*, 2014).

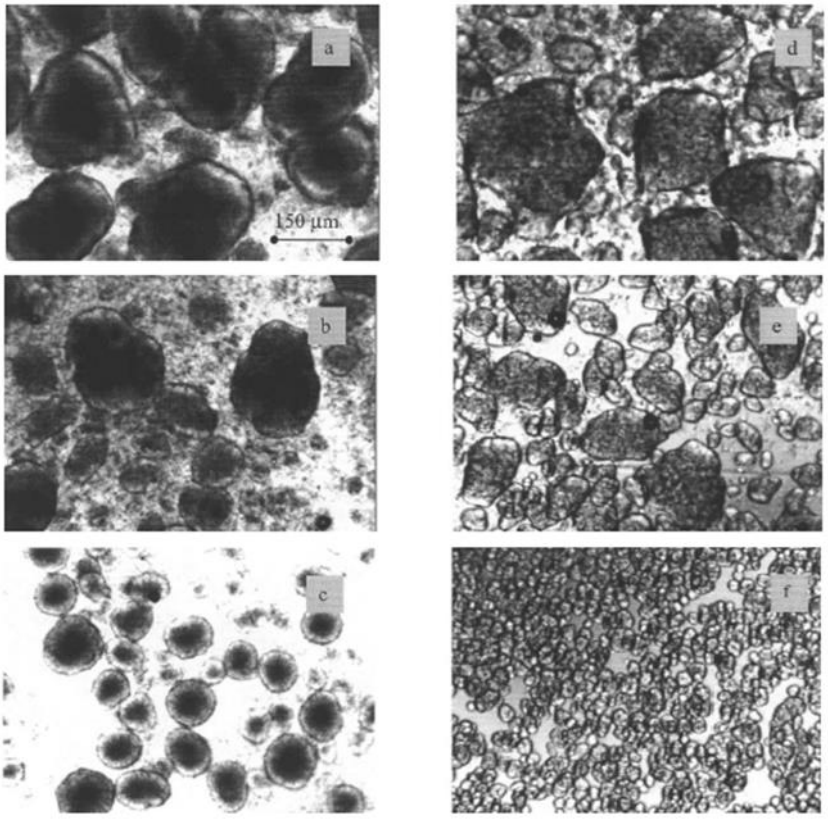

Figure 2.19. 50 % high melting milk fraction crystallised at 0.2 °C/min at (a) 50 rpm, (b) 100rpm, (c) 200 rpm, and at 5.5 °C/min at (c) 50 rpm, (b) 100 rpm and (e) 200 rpm (Herrera and Hartel, 2000a).

Besides the crystal shape, the size of fat clusters is modified by applying shear and smaller crystals are created in milk fat systems (Herrera and Hartel, 2000a) and cocoa butter systems (Sonwai and Mackley, 2006). More in depth studies of crystal size of fully hydrogenated soybean oil have been done at the nanoscale by using cryo-TEM (Acevedo *et al.*, 2012a, Acevedo *et al.*, 2012b); they have shown the presence of a critical shear rate of 30 s^{-1} until which the nano-platelets increase in size.

Fat network

The fat network can be analysed by studying the hardness or the viscoelastic properties of a system. Most of the studies described further have studied the elastic modulus

and the viscosity of the systems. Increasing the shear rate upon cooling has exhibited a reduction in the elastic modulus G'(Alvarez-Mitre *et al.*, 2012, Acevedo *et al.*, 2012a, Acevedo *et al.*, 2012b, Da Pieve *et al.*, 2010, Herrera and Hartel, 2000b) and lower final viscosity (Tarabukina *et al.*, 2009, Lupi *et al.*, 2012). However shearing has also shown an increase in the elastic modulus when applied until the crystallisation temperature of candellila wax and tripalmitin (Chopin-Doroteo *et al.*, 2011) and when applied as a short step during cooling (Sonwai and Mackley, 2006). Thus shearing until the end of fat crystallisation would create spheroidal crystals that are not able to sinter together while shearing before the creation of a network would allow better crystal distribution in the bulk with smaller crystals and therefore a better crystal sintering.

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3. TRISTEARIN CRYSTALLISATION IN THE PRESENCE OF EMULSIFIERS: INFLUENCE OF THE HEAD GROUP SIZE, CONCENTRATION OF EMULSIFIER AND TRISTEARIN

3.1 ABSTRACT

The effect of the size of the emulsifier polar head on tristearin (SSS) crystallisation has been investigated with respect to thermal behaviour (DSC), crystal morphology (Polarized light microscopy), rheological properties (small deformations) and solid fat content (*p*-NMR) of SSS crystals in oil at a given temperature. It was highlighted that at higher emulsifier concentration, the monoglycerides provided a good template for triglyceride crystallisation by crystallising at higher temperatures than SSS. This induced the presence of a strong seeding effect as SSS crystallised at higher temperature during cooling and showed the same crystal stability without involving polymorphic transitions. The small polar head emulsifier also allowed a more stable network after cooling at 1 and 10 °C/min as a result of an addition of crystallised material and of better crystal sintering through glycerol-glycerol interactions. The emulsifier with medium polar head size (Span 60) delayed polymorphic transitions and created smaller crystals by adsorbing at the crystal lattice with SSS. This improved the structuring of crystallised SSS by promoting better sintering between platelets. Finally, the larger polar head emulsifier (Tween 60) did not influence the thermal behaviour, the evolution of the solid fat content or the crystal morphology. However it slightly increased the strength of the fat network. Therefore, it was found that larger polar head would not co-crystallise with SSS and became a part of a second phase in the sample.

3.2 INTRODUCTION

Fats are found in various products such as food or cosmetics, where they play a key role in terms of structure and texture. The addition of high melting point lipids, such as saturated fatty acids or *trans*-fatty acids, is a common way of texturing fat products

(margarine, fillings...), imparting hardness and improving plasticity. However, saturated fat is linked to health risks, such as heart disease (Food and Drug Administration, 2003) which is recognised to be of major concern in many countries. This is why much of recent research has focused on fat crystallisation process in order to reduce the saturated and trans-fat content while maintaining textural properties of the product.

Fat is mainly composed of tri-esters of glycerol, commonly named triglycerides. Crystallisation occurs with the association of triglycerides by Van der Waals forces to form crystals. The process of crystallisation first involves the formation of nuclei by the association of melted triglyceride molecules, called primary nucleation. This step is driven by the level of supersaturation or supercooling of the system (Kloek *et al.*, 2000). Once the temperature of the system is lower than the melting temperature, the molecules have the possibility to aggregate. However some forces such as the Brownian motion hinder the crystallisation until the degree of supercooling becomes high enough. This unstable condition is called metastable zone (Chopin-Doroteo *et al.*, 2011), after which the first nuclei are formed. These nuclei act like seeds and grow into platelets that can sinter together and form fat networks. A secondary nucleation occurs when some crystalline particles are separated from the first crystals and form new nuclei. Following the formation of crystals, the raised surface tension arises between the melted and crystallised parts (Kanishchev and Barannik, 2009) induces triglycerides association until the formation of a continuous fat network (Vdtempel, 1961). Triglycerides aggregated in crystal form are able to packing in several conformations with different stabilities, called polymorphic forms. Tristearin, a commonly used triglyceride for structuring food products, has been widely studied by DSC and X-Ray and showed its crystallisation in three different polymorphs; α, β' and β-forms, depending on processing conditions (Elisabettini *et al.*, 1996, Kellens *et al.*, 1991, Cebula and Smith, 1990). The

influence of the homogeneity of polymorphs in the system has been studied by analysing the sedimentation of tristearin when only β'-crystals are in the bulk or when β' and β-forms are mixed (Johansson and Bergenstahl, 1995). A system composed of only one type of polymorph has presented a higher sedimentation volume illustrating a better compatibility for crystal sintering when the platelets are composed of the same polymorph.

Ways of controlling fat crystallisation include changing the system formulation such as the amount of crystallised material or by adding other molecules such as emulsifiers (Maleky *et al.*, 2012, Kloek *et al.*, 2000) as well as modifying the process of crystallisation by applying different cooling rates (Vuillequez *et al.*, 2010, Humphrey and Narine, 2007, Perez-Martinez *et al.*, 2005) or shear rates (Shi and Maleky, 2015, Acevedo *et al.*, 2012a, Tarabukina *et al.*, 2009). Emulsifiers adsorb differently at the crystal lattice depending on their hydrophobic chain length, their polar head size and their concentration. It has been shown that low emulsifier concentrations can co-crystallise with triglycerides while higher concentrations adsorb at the crystal lattice after being organised in hemi-micelles or crystals as observed by Garti and Zour (1997) and Johansson and Bergenstahl (1992a). These two kinds of phenomena have an impact on nucleation with a heterogeneous primary nucleation effect in the case of hemi-micelles and co-crystallisation or secondary nucleation with the creation of segregated crystals of emulsifier and therefore earlier crystallisation (Basso *et al.*, 2010, Kalnin *et al.*, 2004). Furthermore, emulsifier incorporation hinders the polymorphic transitions by creating irregularities in the crystal lattice as revealed by studies of tripalmitin or tristearin crystallisation or by adsorbing preferentially at the α-crystal surface with glutamic acid (Kalnin *et al.*, 2004, Garti and Zour, 1997, Elisabettini *et al.*, 1996, Shelef and Garti, 1988). However, it has been shown that emulsifiers do not influence the final solid fat content as observed when monopalmitate is added to tripalmitate (Basso *et al.*, 2010) or when Tween

60 is added to a high melting fraction of milk fat (Litwinenko *et al.*, 2004) while they affect the structuring and therefore the sintering between crystals by enhancing the formation of bridges between particles as observed with Span 60 through a sedimentation study (Johansson and Bergenstahl, 1992b).

Furthermore the process of crystallisation is of great importance and the effect of the cooling rate has been investigated on viscoelastic properties and microstructure principally. First of all, the elastic modulus increases when decreasing the cooling rate as observed when cooling milkfat at 0.2 °C/min instead of 5.5 °C/min (Herrera and Hartel, 2000b) or when decreasing the cooling rate of fully hydrogenated canola oil from 10 to 0.7 °C/min (Maleky *et al.*, 2012). At the micro-scale, decreasing the cooling rate has shown to create larger crystals with heterogeneous sizes with diluted milk fat (Herrera and Hartel, 2000a, Martini *et al.*, 2002) and with diluted fully hydrogenated canola oil (Maleky *et al.*, 2012). Lastly, the nano-scale has been recently studied by cryo-TEM and has shown lower nano-crystal dimensions with higher cooling rate of diluted fully hydrogenated canola oil (Acevedo and Marangoni, 2010a, Acevedo and Marangoni, 2010b). Decreasing the cooling rate would favour the growth of the first nuclei formed in the bulk rather than the creation of new nucleation sites and therefore larger crystals (Kashchiev, 2000).

The purpose of this study was to investigate tristearin crystallisation in the presence of emulsifiers, and the influence of the polar head size of the emulsifier molecules in terms of crystal stability, polymorphic transitions, solid fat content evolution and formation of a fat crystal network.

3.3 MATERIALS AND METHODS

3.3.1 MATERIALS

Glyceryl tristearate (SSS) commonly named tristearin, glyceryl monostearate (MG, > 99 %), Span® 60 and Tween® 60 were purchased from Sigma-Aldrich (Sigma–Aldrich Company Ltd., Dorset, UK). Sunflower oil was commercially purchased.

The polar head of MG, Span 60 and Tween 60 are composed of a glycerol, sorbitol and a polyoxyethylene sorbitol, with a HLB of 3.8, 4.7 and 14.9 respectively (Figure 3.1).

10 % w/w SSS in sunflower oil was used as a reference, and different concentrations of MG, Span 60 and Tween 60 were then added (0.5, 1 and 2 %). 5 % and 20 % SSS were used and 0.5 % Span 60 or 2 % Span 60 were added respectively in order to analyse the influence of the amount of crystallised material with a constant ratio [SSS : Span 60] of 10:1 w/w. Samples were heated at 80 °C for 30 min to ensure a total dissolution.

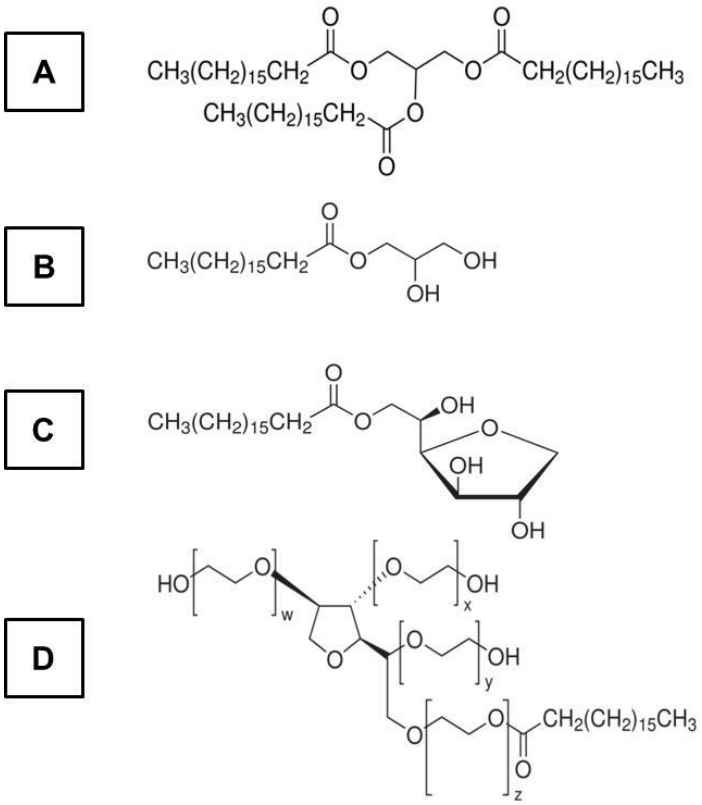

Figure 3.1. SSS (A), MG (B), Span 60 (C) and Tween 60 (D).

3.3.2 METHODS

3.3.2.1 THERMAL BEHAVIOUR

The aim was to investigate the thermal behaviour at two different scan rates, therefore a micro-DSC3 evo was used at 1 °C/min and a DSC 8000 at 10 °C/min because of a better accuracy at these specific scan rates.

Micro-DSC3 evo (Setaram Instrumentation, United Kingdom) was used to analyse the thermal behaviour at 1 °C/min. Prior to the analysis, a calibration with indium at a scan rate of 1 °C/min was performed. 35-55 mg of sample was subjected to the following thermal program: isothermal at 80 °C for 5 min to remove the crystal memory, cooling at 1 °C/min to

5 °C, isothermal at 5 °C for 2 min, heating from 5 °C to 80 °C at 1 °C/min. An empty capsule was used as a reference.

The analyses were performed using a differential scanning calorimeter (DSC) (Perkin-Elmer Delta Series Model DSC 8000) which was previously calibrated with indium at scan rate of 10 °C/min. 4-7 mg of each sample were weighted in an aluminium pan (40 µl) and covers were sealed into place. An empty aluminium pan was used as a reference. Samples were maintained at 80 °C for 5 min to remove the crystal memory, cooled to -20 °C at 10 °C/min, held for 2 min at -20 °C, and heated up to 80 °C at 10 °C/min.

The heat capacity curves obtained were normalised per gram of crystallising material. Each analysis was repeated twice per sample, and duplicates were made.

3.3.2.2 KINETICS OF CRYSTALLISATION

The analyses were performed using a pulsed nuclear magnetic resonance (*p*-NMR) spectrometer (Minispeq mq 20, Bruker) which was calibrated with sunflower oil as a function of (i) sample quantity, (ii) sample temperature and (iii) temperature gradient into the sample during cooling. The solid fat content (SFC) was obtained by an indirect method, which consisted in analysing the signal intensity given by the liquid component, the remainder of the mass therefore being the solid content.

Glass tubes of diameter 18 mm were filled with 4 g of sample and were placed in a water bath. Samples were melted and kept at 80 °C for 10 min and then cooled down at 1 °C/min to 20 °C. Two tubes per sample were subjected to the same thermal program. One tube was used for monitoring the temperature with a HH309A Omega data logger

thermometer, and the other one for analysing the SFC. Each analysis was performed in triplicate.

3.3.2.3 RHEOLOGY

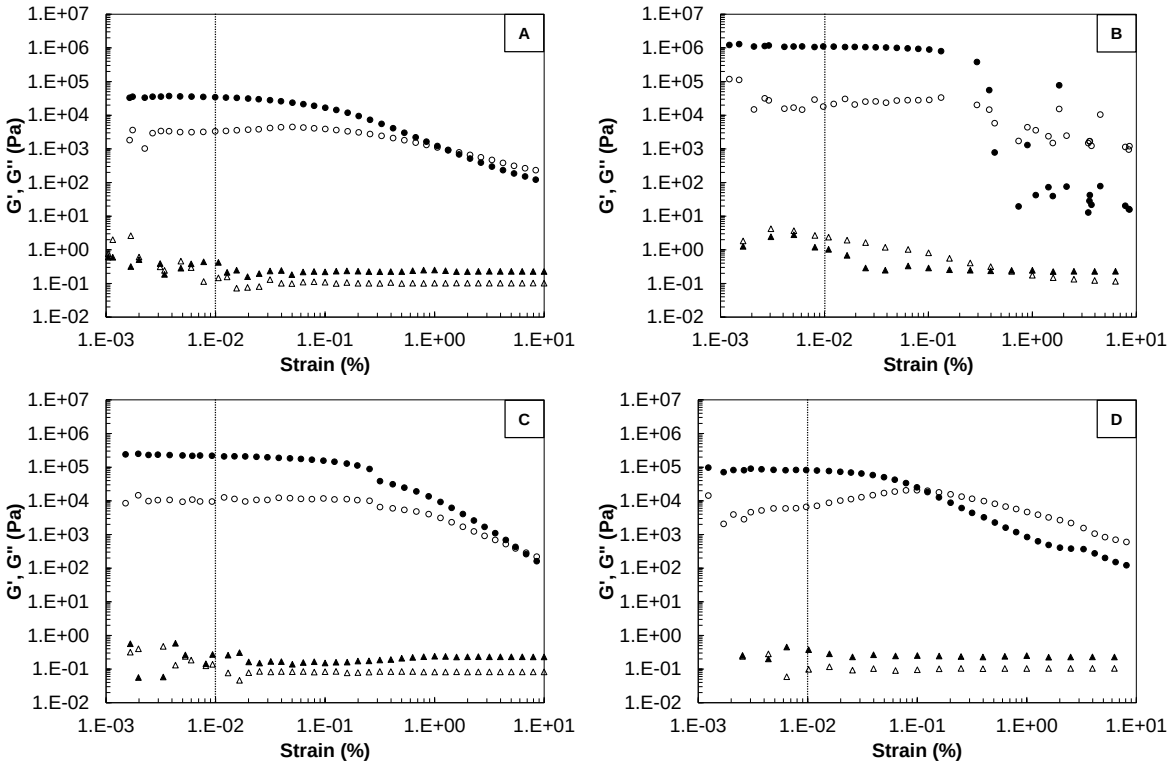

Figure 3.2. Amplitude sweep of 10 % SSS (A), in the presence of 2 % MG (B), 2 % Span 60 (C) and 2 % Tween 60 at 60 °C (∆) and at 20 °C (○). Closed symbols refer to the elastic modulus G' and open symbols to the viscous modulus G''.

The analyses were performed using a Kinexus rheometer (Malvern, United Kingdom) and a cone-plate geometry with 40 mm diameter, a conic angle of 4 ° and a gap of 0.15 mm. Samples were loaded at room temperature. The temperature was controlled by a Peltier system with a sensitivity of ± 0.2 °C. Oscillatory experiments were run within the linear viscoelastic region (LVR) determined at two stages of fat crystallisation (Figure 3.2); after maintaining the samples at 80 °C for 5 min to erase the crystal memory, cooling at 1 °C/min

until 60 °C (to analyse the LVR of the melted system) and until 20 °C where the samples were kept for 15 minutes (to analyse the LVR at the end of crystallisation).

Samples were subjected to the following program at 1Hz and shear strain of 0.01 %: 80 °C for 5 min, cooling at 10 °C/min or 1 °C/min from 80 °C to 5 °C, and thereafter heating up to 80 °C at 10 °C/min. Evolution of elastic and viscous moduli (G' and G'' respectively) was analysed during cooling and heating. Two repetitions for each sample and one duplicate were performed.

3.3.2.4 MICROSTRUCTURE

The samples were analysed by polarized light microscopy (Brunel microscopes Ltd, United Kingdom). The samples were cooled at 1 °C/min after 5 min at 80 °C until 20 °C using a Kinexus rheometer (Malvern, United Kingdom) and a cone-plate geometry with 40 mm diameter and with a conic angle of 4 ° and a gap of 0.15 mm. After being cooled, the samples were stabilised at 20 °C for 24 h before analysis. A small amount of sample was placed on a glass slide and then covered with a glass cover slip. Each analysis was duplicated, and 20 images per sample were taken.

Micrographs were analysed with ImageJ 1.49 (National Institutes of Health, Maryland, USA), processed in a 8-bit grayscale and contrast and brightness were adjusted.

3.4 RESULTS AND DISCUSSION

3.4.1 THERMAL BEHAVIOUR

Initially, the thermal behaviour of 10 % SSS in the presence of MG, Span 60 and Tween 60 was studied using a scan rate of 1 °C/min in order to investigate the effect of emulsifiers on the onset of crystallisation and crystal stability of SSS (Figure 3.3).

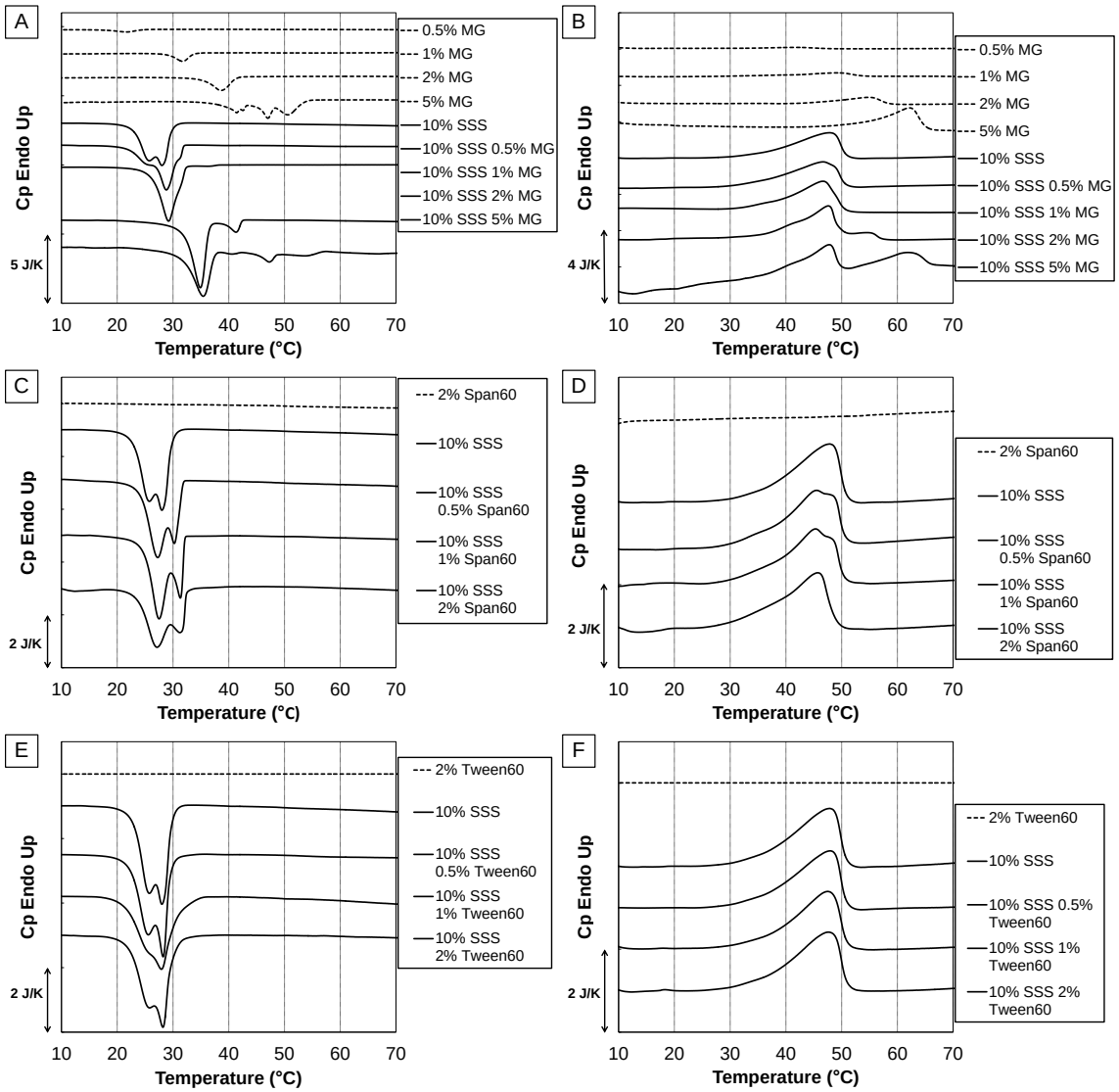

Figure 3.3. Heat capacity during a scan rate of 1 °C/min upon cooling (A, C, E) and re-heating (B. D, F).

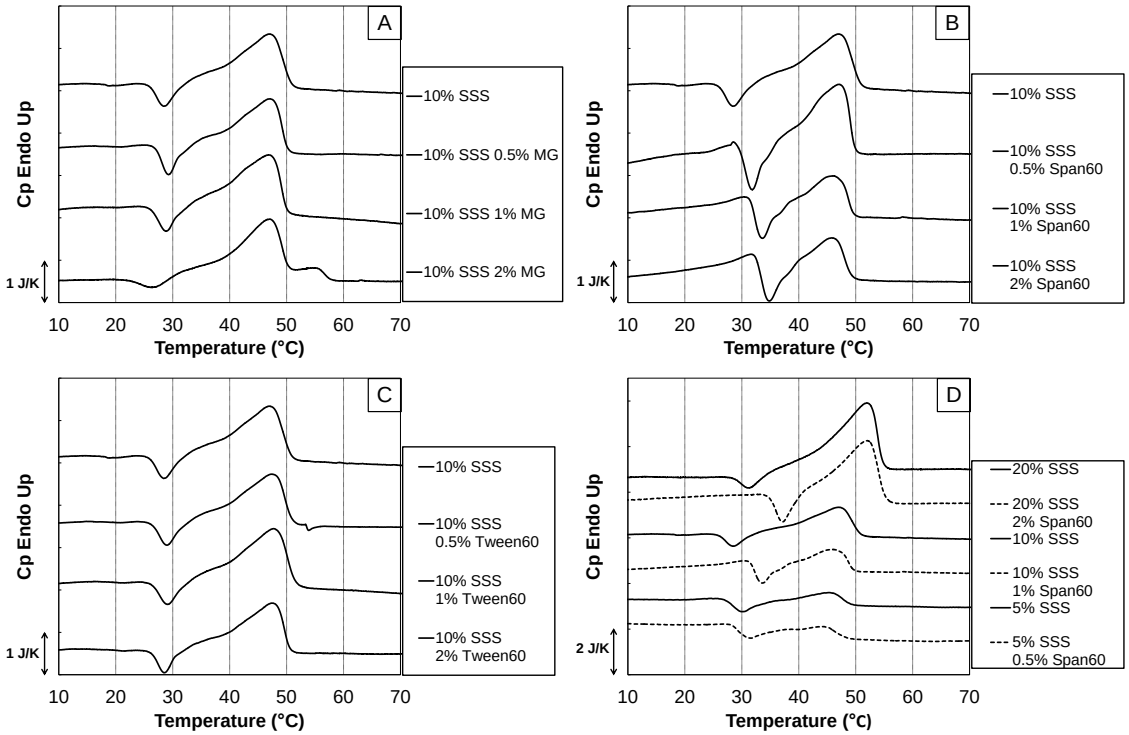

Figure 3.4. Heat capacity upon heating rate of 10 °C/min after a cooling at 10 °C/min from 80 to -20 °C.

	Cooling 1 °C/min		Re-heating 1 °C/min		Cooling 10 °C/min		Re-heating 10 °C/min	
	T_{onset} (°C)	ΔH (J/g.crystallising material)	T_{peak} (°C)	ΔH (J/g.crystallising material)	T_{onset} (°C)	T_{peak1} (°C)	T_{peak2} (°C)	T_{peak3} (°C)
5% SSS	24.5 ± 0.2	-147.7 ± 3.0	45.5 ± 0.2	137.9 ± 5.9	21.9 ± 0.2	26.9 ± 0.2	46.0 ± 0.2	-
5% SSS 0.5% Span60	27.5 ± 0.2	-147.0 ± 10.4	45.0 ± 0.2	130.6 ± 11.4	26.1 ± 0.2	28.0 ± 0.2	44.7 ± 0.2	-
20% SSS	35.4 ± 0.3	-151.7 ± 2.9	51.4 ± 0.2	149.4 ± 0.6	34.0 ± 0.2	28.4 ± 0.2	52.9 ± 0.2	-
20% SSS 2% Span60	37.3 ± 0.2	-137.8 ± 3.5	51.0 ± 0.2	143.3 ± 0.6	36.8 ± 0.2	34.8 ± 0.2	52.5 ± 0.2	-
10% SSS	30.0 ± 0.1	-152.1 ± 3.3	48.7 ± 0.1	147.2 ± 0.8	25.9 ± 0.2	25.6 ± 0.2	47.4 ± 0.2	-
10% SSS 0.5% Span60	31.8 ± 0.1	-145.3 ± 5.4	45.3 ± 0.3 48.8 ± 0.3	143.1 ± 0.5	29.0 ± 0.3	31.3 ± 0.2	47.1 ± 0.1	-
10% SSS 1% Span60	32.1 ± 0.1	-145.7 ± 4.9	45.1 ± 0.3 48.7 ± 0.3	139.1 ± 1.3	30.1 ± 0.1	29.3 ± 0.2	46.1 ± 0.2	-
10% SSS 2% Span60	32.4 ± 0.1	-132.1 ± 5.5	46.3 ± 0.1	138.2 ± 0.8	30.5 ± 0.2	32.2 ± 0.2	45.9 ± 0.5	-
10% SSS 0.5% MG	32.1 ± 0.1	-143.5 ± 4.0	46.7 ± 0.1	141.1 ± 2.3	26.9 ± 0.2	26.8 ± 0.2	47.1 ± 0.1	-
10% SSS 1% MG	32.2 ± 0.1	-137.0 ± 2.0	46.5 ± 0.1	138.8 ± 1.2	27.5 ± 0.4	26.0 ± 0.2	47.0 ± 0.1	-
10% SSS 2% MG	42.4 ± 0.1 37.8 ± 0.1	-213.7 ± 3.4 [a] -168.7 ± 5.4 [b]	48.0 ± 0.1 55.6 ± 0.2	136.0 ± 3.4	39.2 ± 0.2 31.6 ± 0.2	22.0 ± 0.2	47.1 ± 0.1	55.9 ± 0.2
10% SSS 5% MG	48.9 ± 0.1 37.6 ± 0.1	-212.0 ± 13.2 [c] -104.7 ± 10.6 [d]	47.2 ± 0.1 62.0 ± 0.2	87.5 ± 3.5 [c] 203.3 ± 33 [d]	39.2 ± 0.2 31.6 ± 0.2	22.0 ± 0.2	47.1 ± 0.1	55.9 ± 0.2
10% SSS 0.5% Tween60	29.6 ± 0.1	-144.1 ± 3.8	48.0 ± 0.1	147.1 ± 0.5	26.4 ± 0.7	26.0 ± 0.2	47.3 ± 0.1	-
10% SSS 1% Tween60	31.0 ± 0.1	-135.3 ± 6.9	48.0 ± 0.1	139.8 ± 0.7	27.6 ± 0.1	26.0 ± 0.2	47.7 ± 0.4	-
10% SSS 2% Tween60	30.0 ± 0.1	-138.5 ± 3.3	48.0 ± 0.1	148.4 ± 0.5	26.9 ± 0.2	26.0 ± 0.2	47.5 ± 0.1	-

[a] Enthalpy of crystallisation between 42.4 °C and 38 °C calculated per 1 % MG

[b] Enthalpy of crystallisation from 38 °C calculated per (10 % SSS + 1 % MG)

[c] Enthalpy of crystallisation between 48.9 °C and 38 °C calculated per 2.5 % MG

[d] Enthalpy of crystallisation from 38 °C calculated per (10 % SSS + 2.5 % MG)

Table 3.1. Crystallisation, melting temperatures and enthalpies of SSS in sunflower oil and its blends with emulsifiers during cooling and heating at 1 °C/min and 10 °C/min.

	Cooling 1 °C/min		Re-heating 1 °C/min	
	T_{onset} (°C)	ΔH (J/g.crystallising material)	T_{peak} (°C)	ΔH (J/g.crystallising material)
0.5% MG	24.4 ± 0.6	-150.0 ± 14.1	45.9 ± 5.3	120.0 ± 1.0
1% MG	33.7 ± 0.5	-190.0 ± 1.0	49.2 ± 0.1	190.0 ± 14.1
2% MG	41.5 ± 0.1	-214.3 ± 2.4	55.5 ± 0.1	161.2 ± 2.3
5% MG	45.1 ± 0.2	-209.0 ± 1.4	62.2 ± 0.1	196.0 ± 2.8
100% MG	45.5 ± 0.2	-125.3 ± 2.1	42.8 ± 0.1 58.5 ± 0.1	103.3 ± 0.2

Table 3.2. Crystallisation, melting temperatures and enthalpies of MG at different concentration in sunflower oil.

10 % SSS crystallised from 30 °C in a two-step process; Figure 3.4 is a re-heating thermogram at 10 °C/min of 10 % SSS after cooling at 10 °C/min. This showed the lower melting temperature at 25.6 °C referred as the crystallisation of the least stable polymorph α. Therefore, the crystallisation of 10 % SSS at 1 °C/min shows the formation of crystals above the melting temperature of the least stable polymorph; 10 % SSS first crystallise in the β' form at 1 °C/min and then to the β-form (Table 3.1). The effect of MG on SSS crystallisation is shown Figure 3.3.A. 0.5 % and 1 % MG in sunflower oil crystallised within the same temperature range as 10 % SSS. When MG and SSS were mixed together, the crystallisation temperature increased slightly (up to 32.2 °C) and the process of crystallisation occurred in one predominant step. However, 2 % MG in sunflower oil crystallised at a higher temperature than 10 % SSS (41.5 °C). As a result the process of crystallisation of 10 % SSS with 2 % MG differed and two distinct crystallisation steps occurred with first MG and then SSS crystallisation in one step. Extrapolating from the energy released per gram of crystallised MG (Table 3.2), the first crystallisation peak accounted for half of the MG present, and the second peak (from 37.8 °C) was due to the other half of MG and to SSS crystallisation

together. Moreover, the co-crystallisation of 10 % SSS with the rest of MG released more energy than 10 % SSS showing an enthalpy of crystallisation of -168.7 J/(g.crystallising material) and -152.1 J/(g.crystallising material) respectively. When adding a higher concentration of MG (5 % MG to 10 % SSS), MG crystallised prior to SSS and an extrapolation from the energy released per gram of crystallised MG revealed first the crystallisation of 2.5 % MG followed by the co-crystallisation of 2.5 % MG with SSS. Accordingly, with a low MG concentration such as 1 %, MG did not display a seeding effect for SSS crystallisation and co-crystallised with SSS while higher MG concentrations such as 2 % or 5 % lead to SSS crystallisation at a higher temperature. However only half of MG acted like a seed for SSS crystallisation. The crystal stability upon re-heating is shown Figure 3.3.B. The co-crystallisation of SSS with low MG concentrations resulted in a lower melting temperature (46.5 °C instead of 48.7 °C for 10 % SSS). However, the seeding effect of 2 % and 5 % MG revealed two distinct types of crystal created during cooling with the presence of two melting peaks. Furthermore the melting temperature of SSS with higher MG concentrations was comparable to 10 % SSS in sunflower oil (48 °C instead of 48.7 °C). Consequently, low MG concentration lessened the crystal stability while higher concentrations resulted in creating the same crystal stability as in absence of emulsifier. Accordingly, low MG concentration would co-crystallise with SSS and these impurities in the crystal lattice would hinder polymorphic transitions. When higher concentrations of MG acted like seeds for SSS crystallisation, SSS co-crystallised in one step with half of the MG present in the bulk and thus was directly arranged in the most stable polymorph. These results clarified the discrepancies observed in the literature; either saturated monoglycerides delay the polymorphic transitions of SSS to β-form (Elisabettini *et al.*, 1996) or accelerate them (Basso *et al.*, 2010, Fredrick *et al.*, 2008).

SSS crystallisation with Span 60 is shown Figure 3.3.C. 2 % Span 60 in sunflower oil did not show any crystallisation step in the temperature range used in this study. However, increasing the concentration of Span 60 with 10 % SSS exhibited a more predominant d=second peak of crystallisation compared to the reference. Therefore, even though Span 60 did not crystallise, the thermal behaviour indicated a change in the process of SSS crystallisation. Figure 3.3.D shows the re-melting of 10 % SSS in the presence of Span 60. Adding Span 60 shifted the melting peak to lower temperature (i.e. 46.3 °C) with 2 % Span 60 and the melting peak revealed two shoulders, so two melting events when adding 0.5 and 1 % Span 60 to 10 % SSS. As a result Span 60 affected the crystal stability of 10 % SSS and delayed the polymorphic transitions. This was demonstrated several times with SSS, SOS or canola oil by coupling DSC and X-Ray analysis (Guth *et al.*, 1989, Shelef and Garti, 1988, Lee and De Man, 1984). This was ascribed to the incorporation of Span 60 molecules into the fat crystal lattice allowing steric hindrance thus delaying better molecular re-arrangement. Keeping a constant [SSS : Span 60] ratio whilst decreasing SSS concentration to 5 % did not show any differences to 10 % SSS in sunflower oil alone. This might be due to the error of determination of the melting temperature and melting enthalpy of a broad endothermic peak. Nonetheless increasing SSS concentration to 20 % and adding 2 % Span 60 lead to the same melting temperature but a lower energy was absorbed by the system to break all the bonds. Hence, Span 60 had less of an effect on the crystal stability of SSS for higher concentration of crystallised material.

The thermal behaviour of 10 % SSS in the presence of Tween 60 revealed a slight difference in the process of crystallisation with a more predominant first crystallisation peak, although 2 % Tween 60 did not crystallise in sunflower oil (Figure 3.3E and F). However

adding Tween 60 to 10 % SSS revealed the same final crystal stability as in the absence of Tween 60 with regards to the melting temperature and the enthalpy for melting.

The ability of each system to undergo polymorphic transitions was studied by crystallising them at 10 °C/min and by re-heating the samples at 10 °C/min thereafter (Figure 3.4). Cooling at 10 °C/min induced the formation of α polymorph (Aronhime, 1988) and allowed the determination of liquid or solid-mediated polymorphic transitions during re-heating. Each sample underwent two endothermic transitions, corresponding to the melting of different types of crystal, and one exothermic transition in between those corresponding to either polymorphic transitions during the melting of the least stable crystals or to a process of crystallisation of more stable polymorphs directly from the melt. In agreement with Elisabettini et Al. (1996), the melting of α- crystals of SSS first occurred, then polymorphic transitions or liquid-mediated crystallisation to β' and β-crystals and followed by the melting of β'-crystals around 35 °C and then of the β-crystals at around 47 °C.

Adding Tween 60 or 0.5 and 1 % MG did not alter either the temperature for polymorphic transitions of SSS upon re-heating or the melting temperature of the most stable crystals. Increasing MG concentration to 2 % resulted in the crystallisation of, first half of MG present in the bulk, and then SSS with the other half of MG. Moreover a lower amount of α-form crystals were created during cooling and almost no polymorphic transitions or crystallisation occurred during re-heating. In addition the melting temperature was the same as in absence of MG. Thus, higher concentration of MG favoured the crystallisation of the most stable polymorph of SSS directly from the melt after nucleation by the initial crystallisation of MG during cooling.

Span 60 created more stable α polymorphs that melted at higher temperatures than in its absence. The polymorphic transitions also occurred at higher temperatures but lead to the same crystal stability as the most stable polymorph created at 10 % SSS in sunflower oil alone. Increasing the amount of SSS while keeping the ratio [SSS : Span 60] constant, showed a higher crystal stability for the α-form created during cooling in presence of Span 60 and an increase of polymorphic transitions showed by a sharpest exothermic peak upon re-heating. The same thermal behaviour was observed with 5 % SSS regardless of the presence of Span 60. This could be due to a higher error of the analysis with the DSC due to the low amount of crystallised material.

The purity of tristearin used in this study was lower than 80 % and this could be shown by lower melting temperature, 47 °C as mentioned *Chapter 2.2.1 Formulation of fat systems* (Ng, 1989, Basso *et al.*, 2010, Maleky *et al.*, 2012), instead of 72 °C as shown by Norton *et al.* (1985). Therefore, the effect of tristearin impurities on the impact of Span 60 on tristearin crystallisation was analysed by DSC by cooling the system at 10 °C/min and re-heating it at 10 °C/min. The ability of pure tristearin to undergo polymorphic transitions in the presence of 2 % Span 60 was then compared with the tristearin used previously in this study (Figure 3.4).

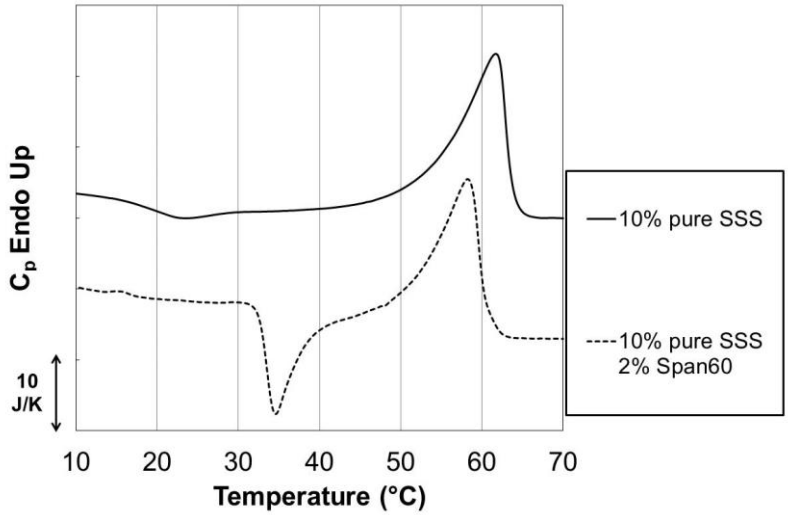

Figure 3.5. Melting thermograms at 10 °C/min of 10 % pure tristearin in the absence and in the presence of 2 % Span 60 in sunflower oil after cooling at 10 C/min from 80 °C to -20 °C.

Figure 3.5 shows higher melting temperature for 10 % pure tristearin (62.0 °C). Moreover, tristearin exhibited polymorphic transitions from 20 °C to 25 °C, but smaller than with the tristearin used in this study. However, the addition of 2 % Span 60 showed lower melting temperature of the most stable crystals (68.0 °C) and the presence of polymorphic transitions between 32 °C and 40 °C. This result presented the same conclusion than previously with the creation of less stable crystals when 2 Span 60 was added to the system. As a conclusion, the impurities present in the tristearin used in this study did not hinder the effect of Span 60, thus of emulsifiers, on tristearin crystallisation. This result proved the possibility to analyse the crystallisation of the tristearin provided by Sigma and the effect of emulsifiers on its crystallisation.

3.4.2 KINETICS OF CRYSTALLISATION

The kinetics of crystallisation and induction time were studied by analysing the evolution of the SFC upon cooling by *p*-NMR (Figure 3.6).

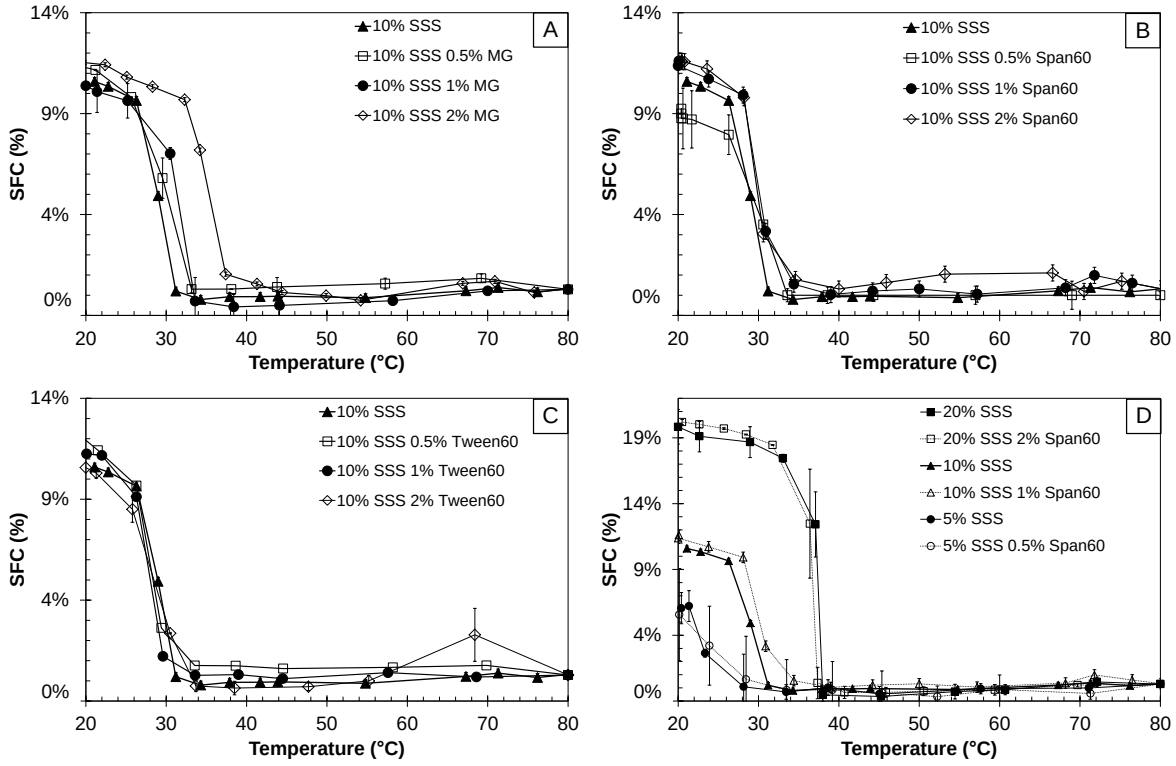

Figure 3.6. SFC versus temperature during crystallisation at cooling 1 °C/min of SSS in sunflower oil in presence of 0.5, 1 and 2 % emulsifier.

Adding lower concentrations of MG such as 0.5 and 1 % did not change the kinetics of SSS crystallisation while 2 % MG sharply impacted the onset of crystallisation (Figure 3.6.A). However the addition of Span 60 or Tween 60 did not expose any significant differences in the kinetics of SSS crystallisation which is in agreement with the results obtained by DSC (Figure 3.2). Furthermore, no effect of Span 60 on SFC evolution of SSS occurred when keeping a constant [SSS : Span 60] ratio and increasing SSS concentration. Finally, the onset of SFC increase, related to a decrease in molecule mobility, corresponded to the beginning of crystallisation observed by DSC at a cooling rate of 1 °C/min for all the samples (Figure 3.2).

3.4.3 MICROSTRUCTURE

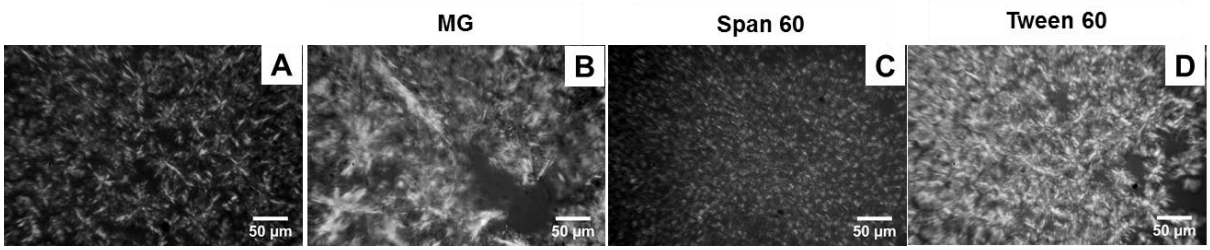

Figure 3.7. Micrographs after a cooling at 1 °C/min from 80 to 20 °C and isothermal at 20 °C for 24 h of 10 % SSS in sunflower oil (A), 10 % SSS with 2 % MG (B), 10 % SSS with 2 % Span 60 (C), 10 % SSS with 2 % Tween 60 (D).

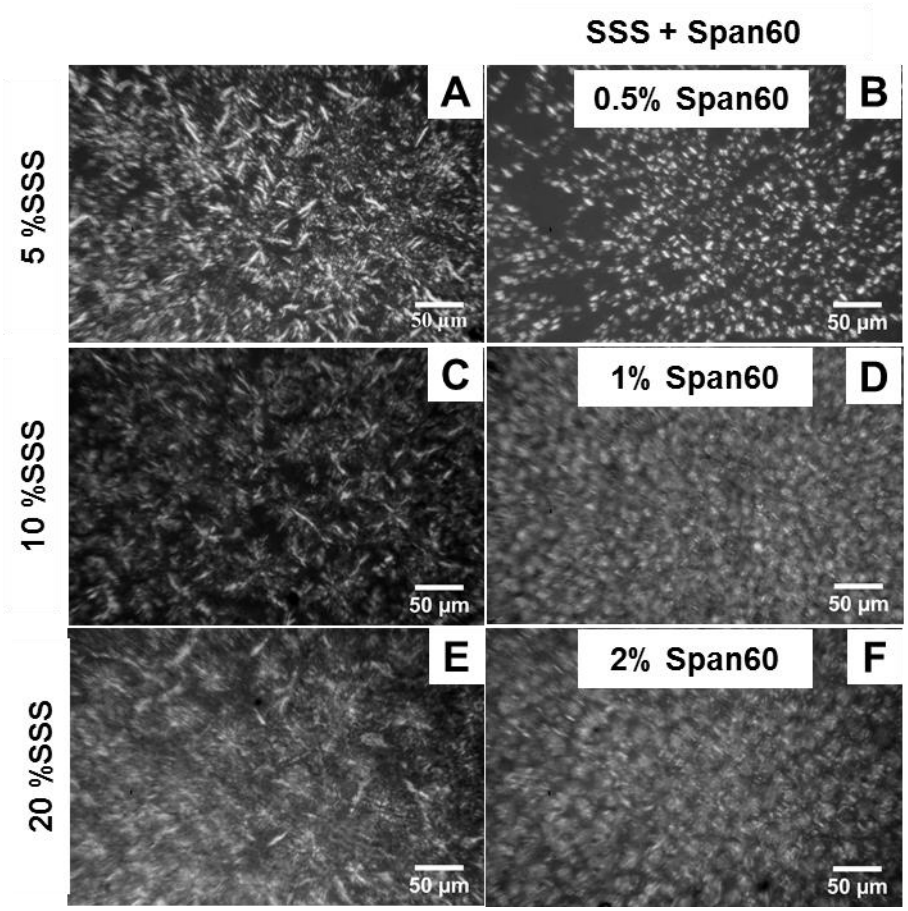

Figure 3.8. Micrographs after a cooling at 1 °C/min from 80 to 20 °C and isothermal at 20 °C for 24 h of 5 % SSS, 10 % SSS and 20 % SSS in sunflower oil (A, C, D respectively), and 5 % SSS with 0.5 % Span 60 (B), 10 % SSS with 1 % Span 60 (D) and 20 % SSS with 2 % Span 60 (F).

The microstructure of the systems was analysed by using polarized light microscopy after a quiescent cooling at 1 °C/min from 80 to 20 °C and an isothermal step at 20 °C for 24 h. Figures 3.7 shows micrographs of 10 % SSS in sunflower oil and in presence of 2 % MG, 2 % Span 60 and 2 % Tween60. 10 % SSS formed crystals in the range of 10 to 20 μm and adding 2 % emulsifier promoted several different crystal structures; needle-like crystals with MG (50 μm), small crystals of less than 5 μm arranged in spherical aggregates with Span 60 and similar crystal morphology with or without Tween 60. The creation of smaller crystals observed with MG compared to the literature (Wang *et al.*, 2011, Basso *et al.*, 2010, Lee and De Man, 1984) are due to lower proportion emulsifier to fat used in their studies. Increasing the amount of crystallised material whilst maintaining the ratio [SSS : Span 60] constant (Figures 3.8) showed the same trend with smaller crystals arranged in aggregates in the presence of Span 60. Therefore, 2% MG generated crystal growth whereas Span 60 hindered it. Furthermore, 2 % Tween 60 did not influence the crystal morphology of 10 % SSS.

3.4.4 RHEOLOGICAL BEHAVIOUR

The rheological behaviour under low deformations of SSS with and without emulsifiers was investigated upon cooling and re-heating in order to analyse the evolution from a liquid-like behaviour, when the system was totally melted, to a solid-like behaviour, after crystallisation.

The effect of type of crystal formed and the presence of polymorphic transitions on the viscoelasticity of the network is illustrated upon cooling (Figure 3.9) and upon re-heating (Figure 3.10).

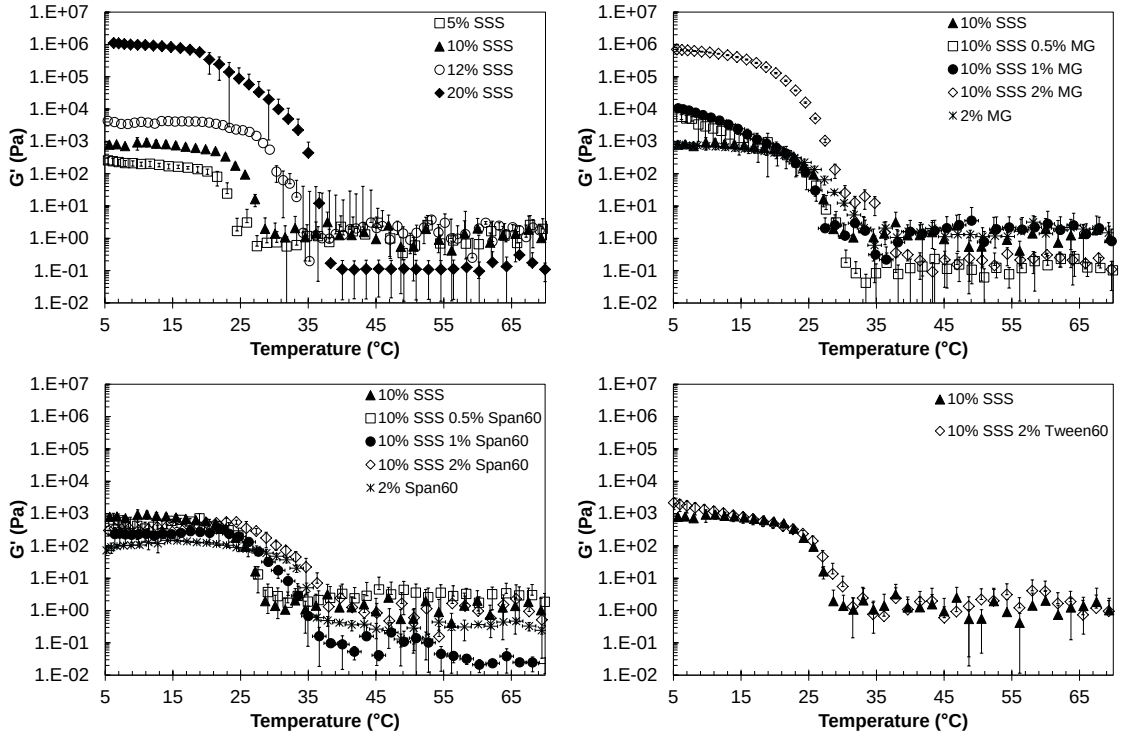

Figure 3.9. Evolution of the elastic modulus G' of 5 %, 10 %, 12 %and 20 % SSS in sunflower oil (A), 10 % SSS with 0.5 %, 1 % and 2 % MG (B) and Span 60 (C), and with 2 % Tween 60 (D) under cooling rate of 10 °C/min.

Figure 3.9.A shows the evolution of the elastic modulus G' of various SSS concentrations in absence of emulsifiers. G' increased during the cooling of all the samples (Table 3.3). Higher G' was observed around the crystallisation temperature as shown previously by DSC (Table 3.1). Therefore SSS crystallisation was illustrated by higher G' with the formation of a fat network showing a solid-like behaviour (G' higher than G'' at the end of the cooling (Winter and Chambon, 1986)).

10 % SSS in the presence of Tween 60, Span 60 and MG all presented a solid-like behaviour at higher temperatures during cooling at 10 °C/min than 10 % SSS in sunflower oil alone. Adding 2 % emulsifier to 10 % SSS in sunflower oil decreased the volume of liquid oil significantly enough to improve the elastic modulus of the systems at higher temperature.

However, their effect on the final G' differed by increasing sharply G' with MG, while no change occurred with Span 60 and Tween 60. The sharp increase of G' in presence of 2 % MG could be due to the addition of crystallised material to the system. In order to check this hypothesis, lower concentrations of MG were added to 10 % SSS and Figure 3.9.B shows a much lower effect of 0.5% or 1% MG on the final G' of 10 % SSS than when 2 % MG was added. Moreover 10 % SSS with 2 % MG and 20 % SSS in sunflower oil reached a very similar final G'. Therefore 2 % MG induced the same increase in G' compared to higher SSS concentrations from 10 % in sunflower oil, suggesting that it was not due to the crystallised material alone. MG improved G' by increasing the amount of crystallised material on one hand, and by contributing to the sintering of the crystals by glycerol-glycerol interactions in the other hand.

The lower G' obtained with larger polar head emulsifiers such as Span 60 during fast crystallisation might be attributed to steric hindrance reducing the time for organisation between crystals thus limiting crystal sintering. A similar trend was observed when increasing the amount of crystallised material which showed lower final G' in the presence of Span 60. Moreover it was noticeable that adding Span 60 to 5 % SSS or to 20 % SSS did not affect the onset temperature of the solid-like behaviour, whereas it increased it at 10 % SSS in sunflower oil; this could be explained by insufficient amount of crystallised material with 5 % SSS. Conversely 20 % SSS crystallised at a higher temperature than the sorbitol-sorbitol interaction of Span 60 during cooling at 10 °C/min. This implied that the onset of solid-like behaviour observed with higher amount of SSS was dominated by SSS crystallisation rather than Span 60 organisation.

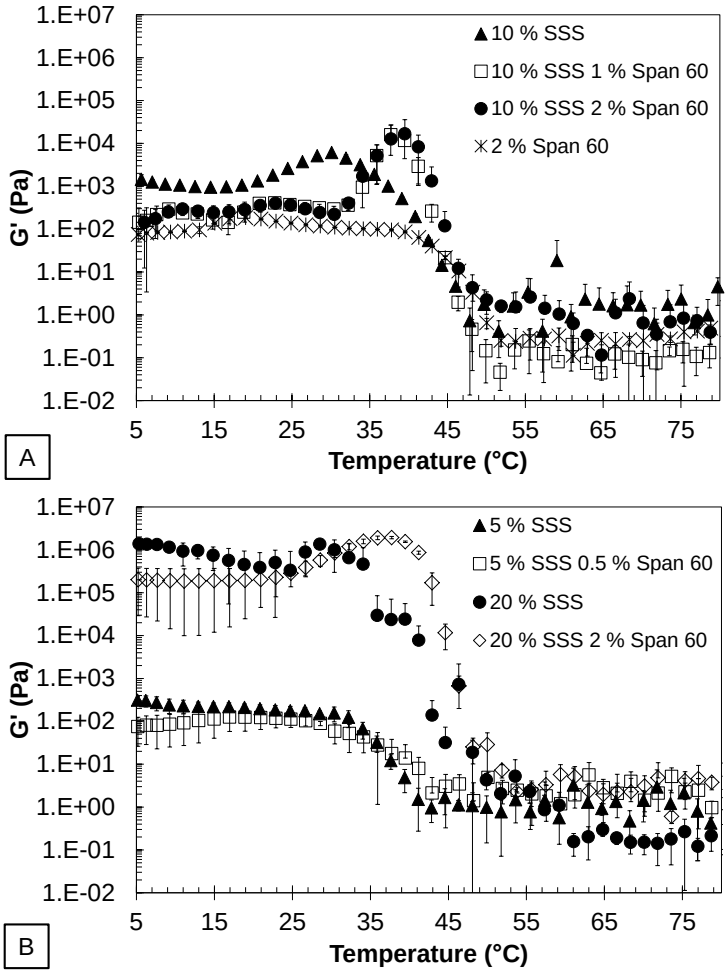

Figure 3.10. Evolution of the elastic modulus G' during re-heating at 10 °C/min after a cooling at 10 °C/min. effect of Span 60 on 10 % SSS in sunflower oil (A) and when increasing the amount of crystallised material keeping a constant ratio [SSS : Span 60].

The stability of SSS fat network in the presence of emulsifier is shown figure 3.10.A with the evolution of G' during re-heating at 10 °C/min after a fast cooling step at 10 °C/min. 2 % Span 60 in sunflower oil remained stable during re-heating until the end of melting. G' of 10 % SSS in sunflower oil was initially stable during melting and then increased in the temperature range corresponding to polymorphic transitions as shown by DSC. Therefore, the type of polymorph created played a role in G' as already shown by coupling rheology and DSC (Toro-Vazquez *et al.*, 2004). Indeed the more homogeneous the bulk of fat crystals, the better was the sintering between the crystals with β' and β form of SSS and the higher the

sedimentation level (Johansson and Bergenstahl, 1995). In the same way, 10 % SSS with 2 % Span 60 showed an increase in G' during re-heating in the same temperature range where polymorphic transitions occurred. However G' increased of two orders of magnitude instead of only one order as for 10 % SSS in sunflower oil. According to the study of the effect of emulsifiers on fat crystal sedimentation Span 60 could adsorb at the crystal lattice of SSS and then form polar bridges between crystals, thus helping to create a fat network (Johansson, 1995a). Therefore although 2 % Span 60 hindered polymorphic transitions of 10 % SSS, it allowed a packing involving better bridging between crystals thanks to sorbitol-sorbitol interactions (Johansson and Bergenstahl, 1992b).

Decreasing the amount of crystallised material resulted in diminishing the possibility of improving the network strength with the presence of Span 60 (Figure 3.10.B) while increasing SSS concentration from 5 % to 10 % and 20 % in the presence of Span 60 lead to an increase in G' in the same temperature range where polymorphic transitions occurred (Figure 3.2).

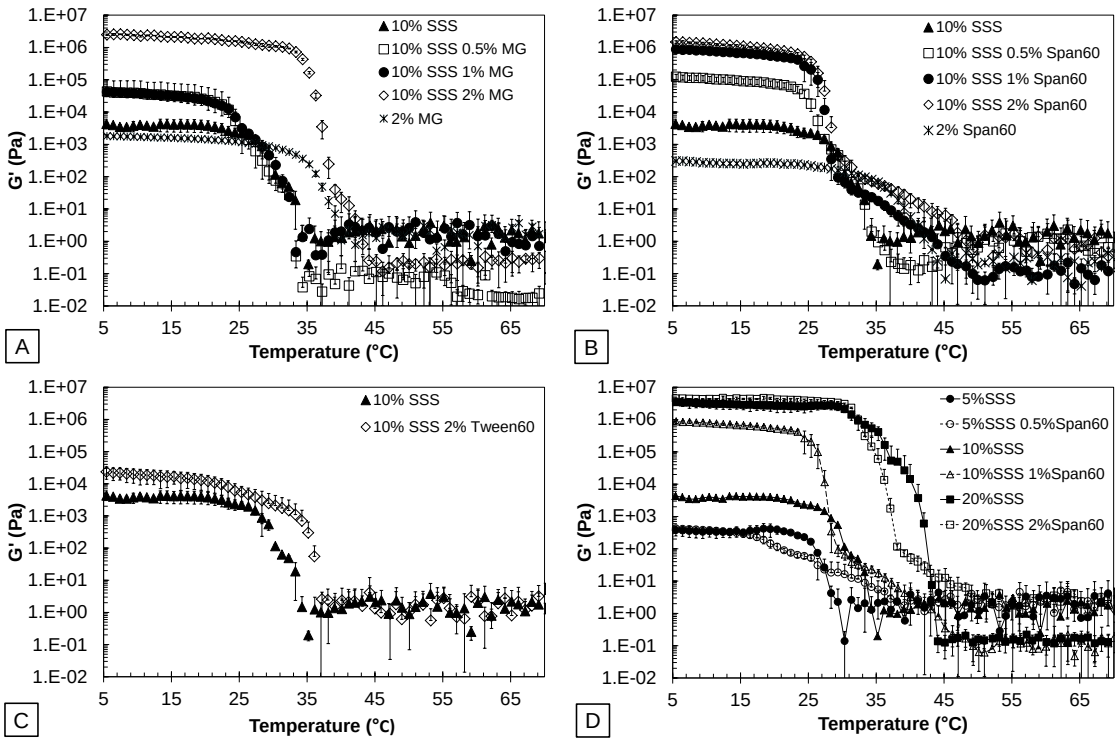

Figure 3.11. Evolution of the elastic modulus G' of 10 % SSS in sunflower oil with 0.5, 1% and 2 % MG (A) and Span 60 (B), with 2 % Tween 60 (C) and when increasing the amount of crystallised material and keeping a constant [SSS : Span 60] ratio of 10 : 1 w/w (D) under cooling rate of 1 °C/min.

To discriminate the effect of each emulsifier on the fat network formation, the evolution of the elastic modulus was studied during a slow cooling step at 1 °C/min (Figure 3.11). Adding low concentrations of MG such as 0.5 % and 1 % to 10 % SSS revealed the same onset temperature for an increase in G' as 10 % SSS in sunflower oil but a slightly higher final G' (Figure 3.11.A). Higher MG concentration such as 2 % with 10 % SSS sharply increased the final G' and generated a solid-like behaviour at higher temperature as shown on Table 3.2 with the temperature of the cross-over of G' and G''; the evolution of G' of 10 % SSS with 2 % MG first followed the same trend as 2 % MG in sunflower oil before rising at the temperature of SSS crystallisation as analysed by DSC. Thus, those results suggested that

the sintering between crystals would be linked to the type of crystals as adding 2 % MG to 10 % SSS created the most stable polymorph directly from the melt upon cooling.

An emulsifier with a larger polar head such as a sorbitol of Span 60 did not show any crystallisation in sunflower oil by DSC. However cooling at 1 °C/min displayed an increase in G' and a solid-like behaviour from 38 °C related to the interactions between Span 60 molecules (Figure 3.11.B). Lower Span 60 concentrations (i.e. 0.5 % with 10 % SSS) showed the same evolution of G' at high temperature but a higher final G' than 10 % SSS in sunflower oil. However, increasing the Span 60 concentration to 1 and 2 % with 10 % SSS presented an earlier increase in G' corresponding to the organisation of Span 60 into the bulk. Furthermore, the larger polar head of Tween 60 hindered a sharp increase of G' even with 2 % Tween 60 with 10 % SSS. As a result it was concluded that Tween 60 would not interact with tristearin so G' remained the same.

The final G' reached after cooling 10 % SSS in the presence of 2 % Span 60 or 2 % MG at 1 °C/min was comparable and almost three orders of magnitude higher than with 10 % SSS in sunflower oil. As previously described with a cooling at 10 °C/min, MG enhanced SSS network strenght in sunflower oil by first increasing the amount of crystallised material and then by creating bridging between fat crystals with glycerol-glycerol interactions. However 2 % Span 60 did not add crystallised material to 10 % SSS in sunflower oil; either sorbitol-sorbitol interactions confirmed a stronger bridging between fat crystals or Span 60 molecules were better distributed into the crystal lattice showing a better repartition of polar bonds, instead of co-crystallising with SSS within more concentrated areas into the crystal lattice as noticed with 2 % MG.

Increasing the amount of crystallised material but keeping the same [SSS : Span 60] ratio of 10 : 1 w/w resulted in the same G' evolution with low and high SSS concentrations and the same final G' when adding Span 60 (Figure 3.11.D). Moreover the onset temperature of solid-like behaviour was higher with Span 60 regarding the three SSS concentrations used in this study. Nevertheless both 5 % SSS and 20 % SSS with Span 60 exhibited another similarity consisting of a delay of the increase in G' in the range of SSS crystallisation .This evolution was distinctly different to the one observed with 10 % SSS, which showed a sharp increase in G' ascribed to SSS crystallisation.

Finally MG enhanced structuring, producing a more elastic structure by increasing the concentration of crystallised material and by creating bridging between fat crystals. Although Span 60 hindered polymorphic transitions of SSS, the low amount of polymorphic transitions was enough to favour crystal sintering during re-heating at 10 °C/min by creating bridges between crystals. Larger polar head emulsifiers such as Tween 60 promoted a slightly stronger and earlier stabilisation of the structure upon cooling (Figure 3.11.C).

	Temperature when G' > G'' (°C)		G' at 5°C (Pa)		Apparent viscosity (Pa.s)	
	-1°C/min	-10°C/min	-1°C/min	-10°C/min	-1°C/min	-10°C/min
2%Span60 in sunflower oil	38.0 ± 0.5	30.0 ± 0.5	$3.0 \pm 0.1 \cdot 10^{2}$	$8.7 \pm 1.5 \cdot 10^{1}$	$4.7 \pm 0.1 \cdot 10^{1}$	$1.4 \pm 0.1 \cdot 10^{1}$
2%MG in sunflower oil	39.0 ± 0.5	29.0 ± 0.5	$1.8 \pm 0.1 \cdot 10^{3}$	$8.2 \pm 1.5 \cdot 10^{2}$	$2.9 \pm 0.1 \cdot 10^{2}$	$1.3 \pm 0.2 \cdot 10^{2}$
5%SSS	29.0 ± 0.5	24.0 ± 0.5	$3.7 \pm 1.3 \cdot 10^{2}$	$2.4 \pm 0.6 \cdot 10^{2}$	$6.0 \pm 2.1 \cdot 10^{1}$	$4.0 \pm 1.0 \cdot 10^{1}$
5%SSS 0.5%Span60	37.0 ± 0.5	23.0 ± 0.5	$4.3 \pm 0.2 \cdot 10^{2}$	$9.5 \pm 6.0 \cdot 10^{1}$	$6.9 \pm 0.3 \cdot 10^{1}$	$1.6 \pm 1.0 \cdot 10^{1}$
20%SSS	44.0 ± 0.5	37.0 ± 0.5	$3.5 \pm 0.8 \cdot 10^{6}$	$1.1 \pm 0.1 \cdot 10^{6}$	$5.8 \pm 1.2 \cdot 10^{5}$	$1.8 \pm 0.2 \cdot 10^{5}$
20%SSS 2%Span60	48.0 ± 0.5	37.0 ± 0.5	$4.5 \pm 0.2 \cdot 10^{6}$	$1.7 \pm 1.5 \cdot 10^{5}$	$1.2 \pm 0.5 \cdot 10^{6}$	$1.5 \pm 1.1 \cdot 10^{5}$
10%SSS	33.0 ± 0.5	28.0 ± 0.5	$4.3 \pm 0.5 \cdot 10^{3}$	$7.8 \pm 1.7 \cdot 10^{2}$	$6.9 \pm 0.7 \cdot 10^{2}$	$1.3 \pm 0.3 \cdot 10^{2}$
10%SSS 0.5%Span60	33.0 ± 0.5	28.0 ± 0.5	$1.2 \pm 0.2 \cdot 10^{5}$	$5.7 \pm 3.4 \cdot 10^{2}$	$2.2 \pm 0.4 \cdot 10^{4}$	$9.9 \pm 5.7 \cdot 10^{1}$
10%SSS 1%Span60	43.0 ± 0.5	34.0 ± 0.5	$8.7 \pm 1.0 \cdot 10^{5}$	$2.3 \pm 1.3 \cdot 10^{2}$	$1.4 \pm 0.2 \cdot 10^{5}$	$3.8 \pm 2.1 \cdot 10^{1}$
10%SSS 2%Span60	46.0 ± 0.5	36.0 ± 0.5	$1.4 \pm 0.1 \cdot 10^{6}$	$3.1 \pm 0.1 \cdot 10^{2}$	$2.4 \pm 0.2 \cdot 10^{5}$	$5.0 \pm 0.8 \cdot 10^{1}$
10%SSS 0.5%MG	32.0 ± 0.5	26.0 ± 0.5	$4.2 \pm 5.5 \cdot 10^{4}$	$5.3 \pm 1.0 \cdot 10^{3}$	$1.1 \pm 1.0 \cdot 10^{4}$	$8.7 \pm 1.8 \cdot 10^{2}$
10%SSS 1%MG	33.0 ± 0.5	26.0 ± 0.5	$4.2 \pm 1.2 \cdot 10^{4}$	$9.8 \pm 0.9 \cdot 10^{3}$	$6.7 \pm 1.9 \cdot 10^{3}$	$1.6 \pm 0.2 \cdot 10^{3}$
10%SSS 2%MG	42.0 ± 0.5	35.0 ± 0.5	$2.6 \pm 0.1 \cdot 10^{6}$	$6.8 \pm 0.2 \cdot 10^{5}$	$4.1 \pm 0.2 \cdot 10^{5}$	$1.1 \pm 0.1 \cdot 10^{5}$
10%SSS 2%Tween60	40.0 ± 0.5	35.0 ± 0.5	$2.4 \pm 1.0 \cdot 10^{4}$	$2.2 \pm 0.8 \cdot 10^{3}$	$3.8 \pm 1.5 \cdot 10^{3}$	$3.5 \pm 1.5 \cdot 10^{3}$

Table 3.3. Temperature at the cross-over of G' – G'' indicating a change in the viscoelastic properties of the system and final G' after cooling at 10 °C/min and 1 °C/min.

3.5 CONCLUSION

In this study the influence of the polar head size of emulsifier was shown on the thermal behaviour, microstructure, SFC evolution and fat network formation of SSS in sunflower oil. Different crystallisation processes exhibited seeding effect, co-crystallisation with SSS and no effect on SSS crystallisation. When small polar head emulsifier acted like a seed for SSS crystallisation it promoted the formation of stable polymorphs directly from the melt, while the medium polar head emulsifier co-crystallised with SSS, promoted more stable crystals and delayed the polymorphic transitions. Moreover only seeding effect displayed a change in the SFC evolution, while co-crystallisation and seeding effect affected the microstructure and the fat network formation. Crystal growth was promoted by the small polar head emulsifier while it is hindered by the medium polar head emulsifier. However the dissimilarity of behaviour of the small and medium polar head emulsifiers on SSS crystallisation induced the same G' for the fat network formed improving it of three orders of magnitude.

In conclusion, our results showed the possibility to decrease the quantity of saturated fat of 50 % but keeping the same fat network strength by adding small or medium polar head emulsifier and changing the microstructure at the nano and meso-scale of the network.

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4. SHEAR AS A PRECURSOR OF TRIGLYCERIDE – EMULSIFIER INTERACTIONS DURING CRYSTALLISATION

4.1 ABSTRACT

This chapter examined the effect of shear at three different stages of triglyceride crystallisation in the presence of emulsifiers. The thermal behaviour was studied upon quiescent cooling (Differential Scanning Calorimetry), while the evolution of the viscoelastic properties (low deformation rheology) and the microstructure (Polarized Light Microscopy) were both analysed upon quiescent and dynamic cooling. The objective of the study was to enhance the triglyceride-emulsifier interactions by modifying the heterogeneous primary nucleation and by promoting secondary nucleation. This could be achieved by understanding the effect of shear on emulsifier aggregation by shearing until the end of their aggregation, by analysing the effect of shear until the end of triglyceride crystallisation and by optimizing the triglyceride-emulsifier interaction by shearing until the beginning of the formation of triglyceride crystal network. The effect of the carbon chain length compatibility between the emulsifier and triglyceride was also studied and a predominant effect was related to the size of the carbon chain; the longer the emulsifier carbon chain, the higher the shear rate was to break down the emulsifier aggregates and to better disperse the emulsifier molecules in the bulk.

4.2 INTRODUCTION

Manufacturing water-in-oil emulsions such as margarine (O'Brien, 2009a) or incorporating colloidal structures into fat such as chocolate (Glicerina *et al.*, 2016) requires the stabilisation of water droplets under shear within a fat network mostly composed of triglycerides and additives. Besides the emulsion stabilisation, the fat network also determines the texture and mouthfeel of the final product which is in turn driven by the crystal morphology and crystal sintering. These two parameters are influenced by formulation like the type of triglycerides, their level of saturation, the presence of additives and the process of crystallisation.

Thus, fat crystallisation is a significant field of study and the process involves the following stages upon cooling (Himawan *et al.*, 2006, Metin and Hartel, 2005). When triglycerides of a fat system are held for a specific time above their melting point, they are all dissociated in the bulk. Subsequently the potential for crystallisation starts when the temperature reaches the melting temperature. When cooling the system below the melting point, the driving force for crystallisation rises and saturation increases. If the driving force is too low, there is no crystallisation and the system is in a metastable zone where triglycerides tend to create bonds that are quickly broken by Brownian motion. The onset of crystallisation takes place when the system becomes supersaturated, corresponding to the formation of the first nuclei. These nuclei generate an interface for crystallisation and grow into platelets due to the incorporation of triglycerides onto the crystal lattice. Two main types of nucleation are described in the literature such as homogeneous and heterogeneous nucleation. Homogeneous nucleation corresponds to the association of molecules in the bulk while heterogeneous nucleation coincides with the catalysis of molecule association by the presence of foreign nucleation sites that would generate an interface for crystallisation. Fat crystals are known for

being arranged in different conformations called polymorphs. The most common polymorphic forms are α, β' and β, increasing their stability. If the driving force for crystallisation is high, triglycerides crystallise in the α-form and can be better-packed through polymorphic transitions that are either solid-mediated or by melting the α-form and crystallising in a more stable polymorph directly from the melt. Furthermore, platelets sinter together and form fat clusters that are eventually linked together and form a fat network.

This process of crystallisation is influenced by the type of triglyceride in terms of carbon chain length, unsaturation level and symmetry of the carbon chain. In this work, tristearin was studied as it is commonly used in food industry and is composed of a glycerol attached to three carbon chains with eighteen carbons. Some studies have shown the crystallisation of pure tristearin to be α polymorph upon fast cooling, followed by a transition to the β'-form before final polymorphic transitions to the β-form during re-melting (Elisabettini *et al.*, 1996, Cebula and Smith, 1990).

The addition of emulsifiers to fat systems leads to changes in the process of crystallisation at different stages; nucleation, crystal growth and crystal sintering. Earlier nucleation can be promoted with heterogeneous nucleation by seeding tripalmitin with monoglycerides that would crystallise at higher temperature than tripalmitin (Basso *et al.*, 2010). Moreover this effect is more predominant when the chain length of additives and triglycerides are compatible. Indeed emulsifiers with larger polar head such as Tween 60 and Span 60 have also shown the ability to decrease the induction time for crystallisation of hydrogenated palm kernel stearin (Wang *et al.*, 2011). In addition it has been demonstrated an effect on polymorphic transitions (Aronhime, 1988, Aronhime *et al.*, 1987, Garti *et al.*, 1986, Lee and De Man, 1984, Mohamed and Larsson, 1992, Elisabettini *et al.*, 1996); sorbitol fatty acids, polyoxyethylene sorbitol fatty acids and sugar fatty acids have exhibited a delay

in the polymorphic transitions by creating impurities in the crystal lattice and hindering triglyceride rearrangement in the crystal lattice. Studies on crystal growth have displayed the effect of emulsifiers with a sucrose or sorbitol as polar head on palm oil crystallisation (Garbolino *et al.*, 2005), a sorbitol polar head on canola oil crystallisation (Lee and De Man, 1984) and monoglycerides on tripalmitin crystallisation (Basso *et al.*, 2010); the creation of irregularities in the crystal lattice would hinder triglyceride aggregation, prevent crystal growth and produce smaller fat clusters. Lastly these changes in the crystal habits allow the formation of diverse fat networks; higher sedimentation levels have been reported for tristearin in the presence of Span 60 (Johansson and Bergenstahl, 1992a) and harder fat networks for palm oil systems with sorbitol monoesters (Garbolino *et al.*, 2005).

The effect of shear on fat crystal formation is of great interest as this is used for the preparation of emulsions and for decreasing the water droplet size. Therefore its impact on fat crystallisation has been studied in terms of polymorph creation, microstructure and hardness of crystal network. Increasing shear rate has been reported to enhance polymorphic transitions in various types of fat like cocoa butter (Shi and Maleky, 2015, Sonwai and Mackley, 2006, Toro-Vazquez *et al.*, 2004, MacMillan *et al.*, 2002), milk fat (Mazzanti *et al.*, 2009, Mazzanti *et al.*, 2003) and soft and high stearic fraction of sunflower oil (Martini *et al.*, 2013). The majority of these studies have concluded that shearing reduces the chance of local rise of temperature during crystal growth and would maintain the local supercooling (Sonwai and Mackley, 2006). Moreover shearing has been found to affect the crystal size at different scales; larger nanocrystals are formed at low shear rates and smaller nanocrystals are generated above a specific shear rate (Acevedo *et al.*, 2012a, Acevedo and Marangoni, 2010b). At the mesoscale, it has been shown a decrease in the fat cluster size decreased when increasing shear rate and exhibited a spherical shape (Sonwai and Mackley, 2006, Herrera

and Hartel, 2000a). At last, shearing upon cooling influences the final fat network properties. Shearing until the end of crystallisation of milk fat, *trans*-fatty acids and monoglyceride organogels has resulted in lowering the final viscoelastic properties (Da Pieve *et al.*, 2010, Bell *et al.*, 2007, Herrera and Hartel, 2000b). Besides, shearing until different stages of candelilla wax crystallisation has presented a lower elastic modulus when shearing until the end of crystallisation while no changes have occurred when shearing until the metastable zone (Alvarez-Mitre *et al.*, 2012).

However, little attention was paid to the influence of shear on fat crystallisation in the presence of additives. Some studies have exposed that crystals are less orientated in the presence of milk polar lipids (Mazzanti *et al.*, 2009, Mazzanti *et al.*, 2003), the onset of crystallisation is mainly affected by additives when seeding effect (Lupi *et al.*, 2012) and shear predominates over the addition of additives on the network strength (Acevedo *et al.*, 2012b).

Within this framework, the objective of this research was to investigate the interaction between tristearin and emulsifiers. For this purpose, emulsifiers with different chain lengths were added to 10 % tristearin and shear rate was applied upon cooling until specific stages of crystallisation in order to tailor the microstructure and the final crystal network. This could be achieved by applying shear until three stages of crystallisation; the end of the emulsifier aggregation, the beginning of the fat network formation and the end of crystallisation.

4.3 MATERIAL AND METHODS

4.3.1 MATERIAL

Glyceryl tristearate (SSS, C18) commonly named tristearin, and emulsifiers present in Span 60 such as sorbitol monostearate (Sorb-S, C18) and sorbitol monopalmitate (Sorb-P, C16) were purchased from Sigma-Aldrich (Sigma–Aldrich Company Ltd., Dorset, UK). Sunflower oil was commercially purchased.

10 % w/w SSS in sunflower oil was used as a reference and 2 % w/w of sorb-S or sorb-P were then added. Samples were heated at 80 °C for 30 minutes to ensure a total dissolution.

4.3.2 METHODS

4.3.2.1 THERMAL BEHAVIOUR

The thermal behaviour was studied upon quiescent cooling rate of 1 °C/min by DSC (Micro-DSC3 evo, Setaram Instrumentation, United Kingdom). Prior to the analysis, a calibration with indium at a scan rate of 1 °C/min was performed. 35-55 mg of sample was subjected to the following thermal program: isothermal at 80 °C for 5 minutes to remove the crystal memory, cooling at 1 °C/min to 5 °C, isothermal at 5 °C for 2 minutes, heating from 5 °C to 80 °C at 1 °C/min. An empty capsule was used as a reference.

Thermograms were then analysed in terms of heat capacity evolution versus temperature, enthalpies of crystallisation and melting, temperature at the onset of crystallisation and temperature at the peak of the melting curve. Each analysis was repeated twice per sample, and duplicates were made.

4.3.2.2 RHEOLOGY

The analyses were performed using a Kinexus rheometer (Malvern, United Kingdom) and a cone-plate geometry with 40 mm diameter, a cone angle of 4 ° and a gap of 0.15 mm. Samples were loaded at room temperature. The temperature was controlled by a Peltier system with a sensitivity of ± 0.2 °C. Samples were cooled at 1 °C/min from 80 °C to 5 °C after 5 minutes at 80 °C in order to remove the crystal memory.

Shear rates of 5, 50 and 500 s^{-1} were first applied from 80 °C to three different temperatures (39 °C, 31 °C and 20 °C) followed by low deformation rheology (Figure 4.1). Oscillatory experiments were run within the LVR (*3.3.2.4 Rheology*) at 1 Hz and shear strain of 0.01 %. A reference sample was quiescently cooled and only subjected to low deformation from 80 °C to 5 °C.

Evolution of elastic and viscous moduli (G’ and G’’ respectively) was analysed during cooling after the shearing step. Two repetitions and one duplicate were performed.

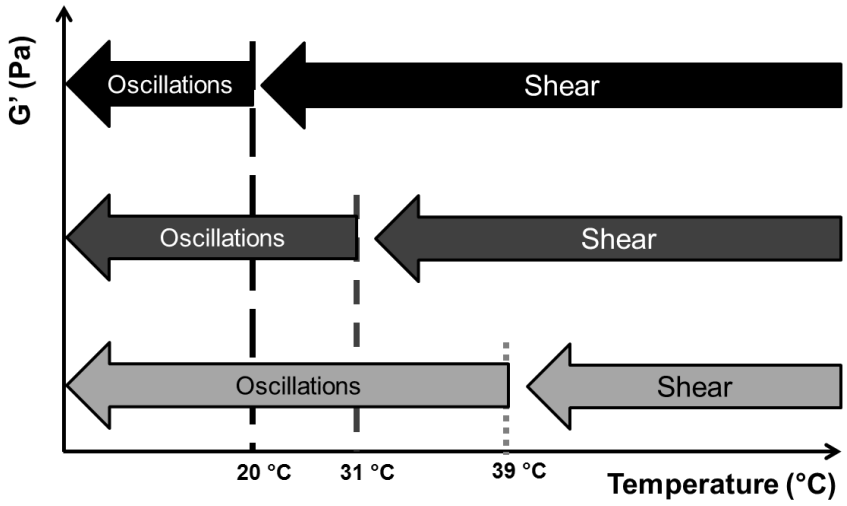

Figure 4.1. Process of crystallisation, cooling at 1 °C/min from 80 °C to 5 °C with a first step of shear until 39 °C, 31 °C or 20 °C, followed by oscillations. The elastic modulus G' was analysed.

4.3.2.3 MICROSTRUCTURE

The samples were analysed by polarized light microscopy (Brunel microscopes Ltd, United Kingdom) equipped with a camera (Canon EOS 1000D, Taiwan), after being cooled in the Rheometer and stabilised at 20 °C for 24 h (*3.3.2.4 Rheology*). A small amount of sample was placed on a glass slide and then covered with a glass cover slip. Each analysis was duplicated and 20 images per sample were taken.

Micrographs were analysed with ImageJ 1.49 (National Institutes of Health, Maryland, USA), processed in a 8-bit grayscale and the contrast and brightness were adjusted.

4.4 RESULTS AND DISCUSSION

4.4.1 EFFECT OF EMULSIFIER CHAIN LENGTH COMPATIBILITY ON SSS UPON QUIESCENT COOLING

The effect of chain length of emulsifiers on the crystallisation and crystal stability of 10 % SSS was studied by DSC. Data for the crystallisation and melting behaviour of 10 % SSS in the presence of 2 % sorb-S or sorb-P upon a scan rate of 1 °C/min are shown Figure 4.2. These emulsifiers were composed of the same head group (sorbitol) but differed in their chain length; sorb-S had the same number of carbons as SSS is the hydrophobic chain, while sorb-P had two carbons less than SSS.

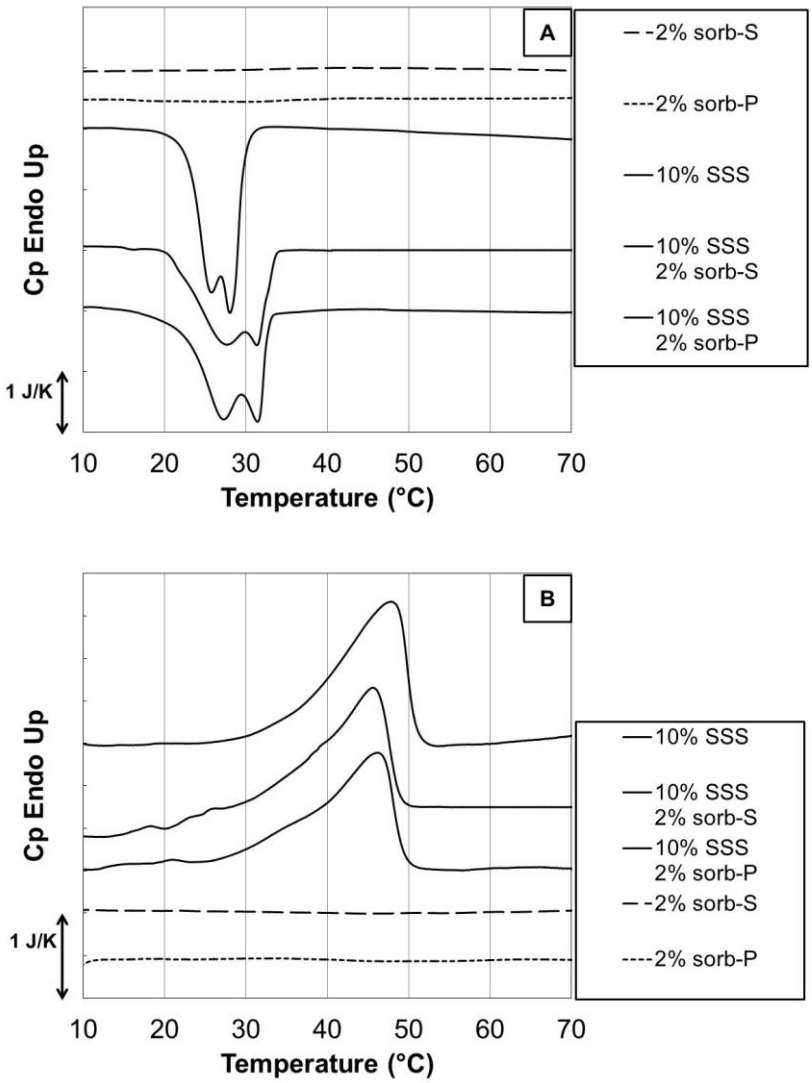

Figure 4.2. Heat capacity of 10 % SSS in the presence of 2 % sorb- S and sorb-P upon cooling at 1 °C/min (A) and melting at 1 °C/min (B).

Figure 4.2.A shows the thermal behaviour upon cooling. No crystallisation peak occurred with 2 % emulsifier in sunflower oil. Moreover 10 % SSS crystallised in two steps in the absence and presence of emulsifiers in the same temperature range. Adding 2 % sorb-S or sorb-P lead to broader crystallisation peaks occurring at higher temperatures. A detailed study of crystallisation and melting temperatures and the enthalpy of phase transitions is presented Table 4.1. 10 % SSS crystallised at 32.7 and 32.8 °C with 2 % sorb-S and sorb-P respectively instead of 30.0 °C in the absence of emulsifiers. The energy released by

crystallisation was lower when sorb-S or sorb-P were added to the system. Moreover, the melting temperature was lower with emulsifiers and even more with sorb-S (45.5 °C instead of 48.7 °C with 10 % SSS) although their melting profiles remained very similar (Figure 4.2.B). Therefore crystallising SSS with those emulsifiers hindered the crystallisation process and would generate less stable crystals. Accordingly, those results are in good agreement with the ones obtained with Span 60, composed of 1:1 [sorb-S: sorb-P] (*Chapter 3*). Lower induction times for crystallisation would then be associated to heterogeneous nucleation; emulsifier molecules would locally increase the triglyceride saturation in the bulk and promote earlier crystallisation. In addition, this type of heterogeneous nucleation would create less stable crystals by incorporating impurities in the crystal lattice.

	Cooling		**Heating**	
	T_{onset} (°C)	Enthalpy (J/g)	T_{peak} (°C)	Enthalpy (J/g)
10% SSS	30.0 ± 0.1	-15.2 ± 0.3	48.7 ± 0.1	14.7 ± 0.1
10% SSS 2% sorb-S	32.7 ± 1.1	-13.1 ± 0.6	45.5 ± 0.1	13.8 ± 2.1
10% SSS 2% sorb-P	32.8 ± 0.4	-13.3 ± 0.2	46.2 ± 0.2	14.3 ± 0.8

Table 4.1. Temperature at the onset of crystallisation and at the melting peak; enthalpies of crystallisation and melting upon a scan rate of 1 °C/min.

Using low deformation by rheology, the evolution of the viscoelastic properties of the blends were determined upon cooling at 1 °C/min. Figure 4.3 shows the evolution of the viscous and elastic moduli of 10 % SSS in sunflower oil which have been described in *3.4.4 Rheological behaviour*; 2 % emulsifier in sunflower oil also induced an increase in the viscoelastic properties, from 46 °C for sorb-S and from 42 °C for sorb-P. As the absence of crystallisation was demonstrated by DSC, an increase in G' of three orders of magnitude would correspond to the emulsifier aggregation through the formation of bonds between the polar heads in the bulk. However shorter induction times for emulsifier aggregation upon

cooling were attributed to longer carbon chains for sorb-S. Therefore 2 % sorb-S or sorb-P did not crystallise but would be aggregated in hemi-micelles due to polar bonds sorbitol - sorbitol and Van der Waals bonds between the carbon chains. As a result, the addition of 2 % sorb-S or sorb-P to 10 % SSS lead to a two-step increase in the viscoelasticity from higher temperatures, 48 °C and 43 °C respectively instead of 32 °C for 10 % SSS. According to previous statements, the first step of increase in the viscoelasticity corresponded to emulsifier aggregation in the bulk and the second step to the contribution of SSS crystallisation to the fat network formation. The final elastic modulus at 5 °C was two orders of magnitude higher in the presence of emulsifier than 10 % SSS in sunflower oil. This was due to higher crystal sintering with sorbitol – sorbitol interactions, as noted in *3.4.4 Rheological Behaviour*. Furthermore, similar carbon chain length compatibility between SSS and sorb-S enhanced their surface contact and would better disperse the emulsifiers onto the triglyceride crystal lattice. This would increase the crystal sintering by creating sorbitol-sorbitol bonds and therefore would build stronger networks.

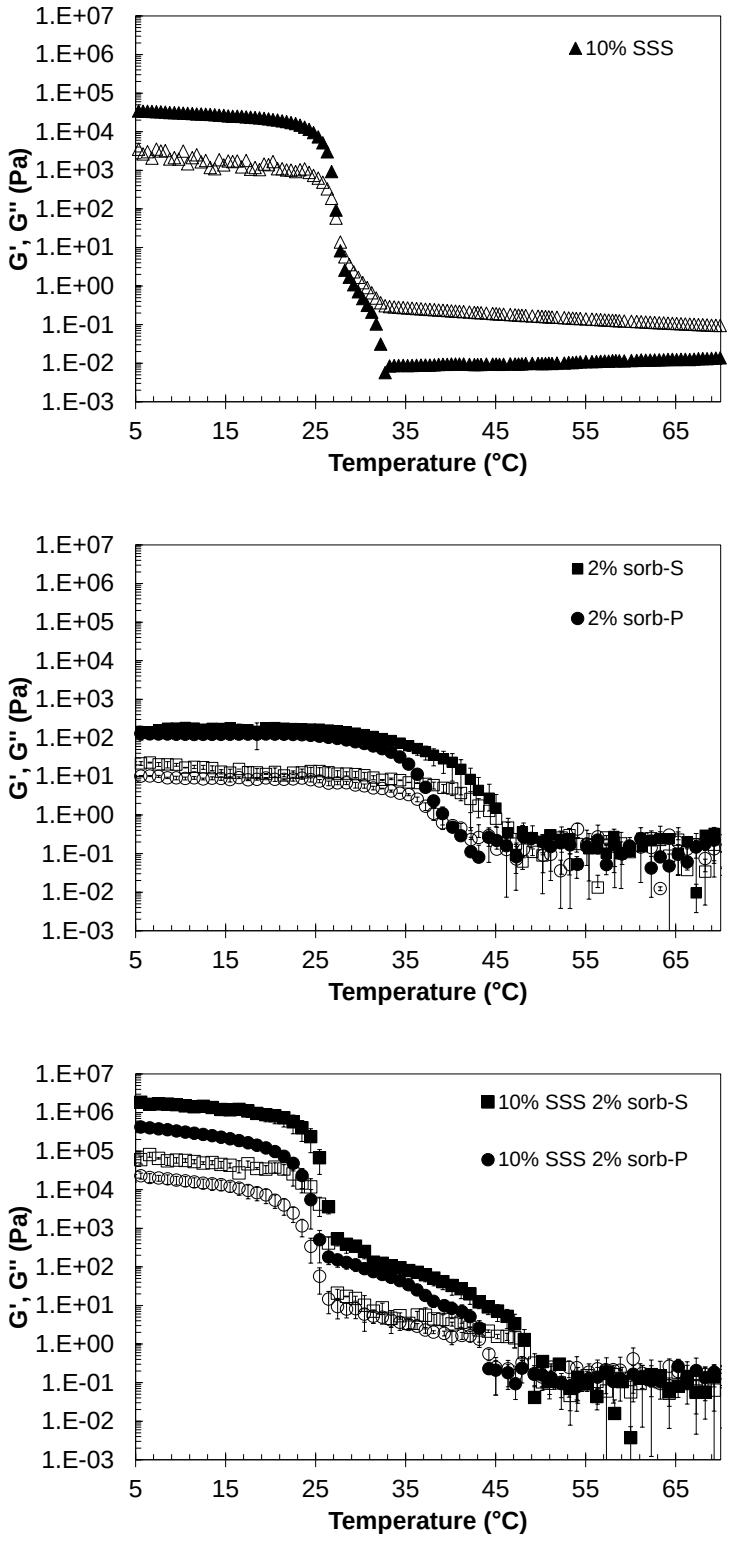

Figure 4.3. Evolution of the elastic modulus (closed symbols) and viscous modulus (empty symbols) upon cooling at 1 °C/min.

To explore the effect of sorb-S and sorb-P on SSS crystallisation, polarized light microscopy was used to visualise the microstructure of the fat network. Micrographs of 10 % SSS in the presence of 2 % emulsifier 24 h after cooling the samples from 80 °C to 5 °C at 1 °C/min are presented Figure 4.4.

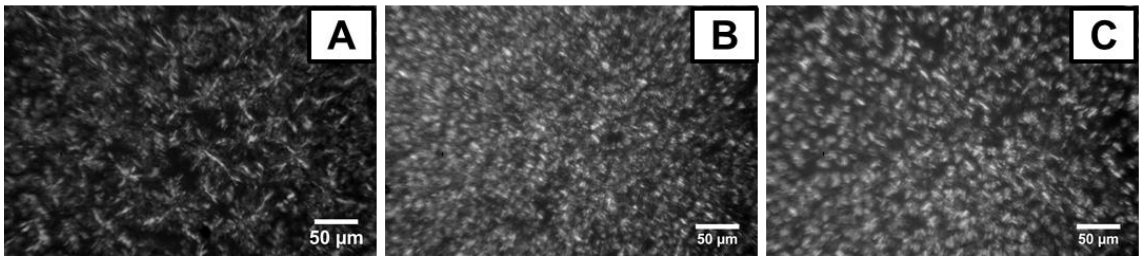

Figure 4.4. 10 % SSS (A), 10 % SSS with 2 % sorb-S (B) and 10 % SSS with 2 % sorb-P (C) after quiescent cooling at 1 °C/min and 24 h storage at 20 °C

The crystal morphology was spherical regardless of the addition of emulsifier. However, adding sorb-S or sorb-P resulted in lessening dramatically the crystal aggregate size as predicted according to the work presented in *3.4.3 Microstructure*. Therefore lower crystal stabilities with 2 % emulsifier observed by DSC could be related to a decrease in the crystal size. Sorb-S and sorb-P would have a surface effect on SSS crystal lattice and would create impurities which hindered crystal growth. This effect was even more predominant with sorb-S and this was in good agreement with a previous study of SSS and tripalmitin crystallisation in the presence of stearic emulsifiers; it has been shown the importance of the carbon chain length compatibility with the incorporation of the emulsifier onto the triglyceride crystal lattice and the effect on polymorphic transitions (Aronhime, 1988). Higher final G' and smaller fat clusters exhibited with sorb-S illustrated a significant effect of chain length compatibility on the fat network formation.

4.4.2 EFFECT OF SHEAR ON SSS CRYSTALLISATION

The effect of shear was studied until two different stages of SSS crystallisation; at the start of crystal growth (31 °C) and at the end of the fat network formation (20 °C). SSS crystallisation under shear was compared to quiescent crystallisation and G' evolution was analysed (Figure 4.5). Shearing until 20 °C, end of SSS crystallisation and fat network formation, caused a decrease in the final G' which was higher when increasing the shear rate to 50 and 500 s^{-1} (Figure 4.5.A). However shearing until the start of SSS crystal growth (31 °C) and before the fat network formation, displayed the opposite effect with higher G' when increasing the shear rate (Figure 4.5.B). Moreover this sharp increase in G' occurred from higher temperatures than upon a quiescent cooling. Therefore, shearing until the end of the crystallisation process disrupted the fat network and generated lower G' while limiting the shearing until the start of the fat network formation developed a stronger network by promoting secondary homogeneous nucleation. On one hand, shearing during nucleation maintained the local supercooling and induced crystal growth and on the other hand higher shear rates raised the overall bulk temperature and promoted partial melting of the crystals and the creation of more nucleation sites. Thus, the system strength could be controlled by shearing until specific stages of SSS crystallisation.

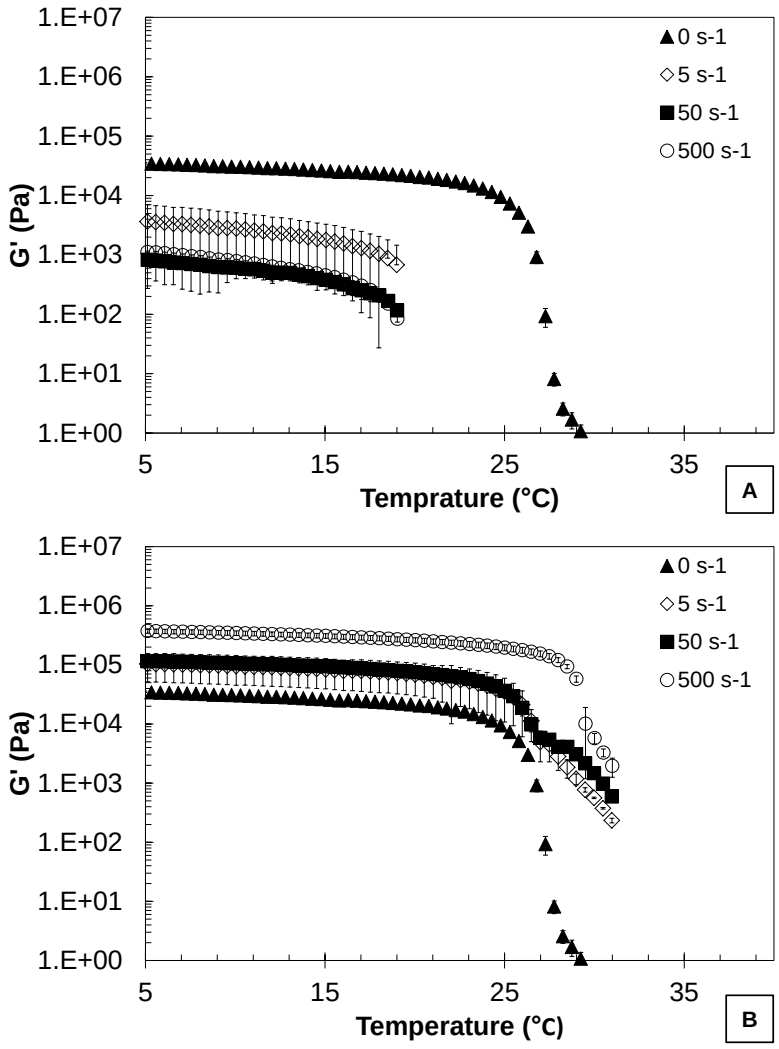

Figure 4.5. Elastic modulus upon quiescent cooling at 1 °C/min and after shearing 10 % SSS in sunflower oil until 20 °C (A) or 31 °C (B).

In addition, the resulting final crystalline morphology (Figure 4.6) revealed the impact of shearing until specific stages of SSS crystallisation. The application of shear until 20 °C created spherical fat clusters with a well-defined interface with the liquid oil and this effect was stronger when increasing the shear rate. This crystal morphology has already been observed by shearing 10 % fully hydrogenated canola oil until the end of crystallisation (Tran *et al.*, 2014) and by shearing a high melting fraction of milk fat in a low melting fraction (Herrera and Hartel, 2000a). Shearing until the beginning of the network formation generated more diffused crystals in the bulk thus homogeneous microstructure when shearing at 500 s^{-1}.

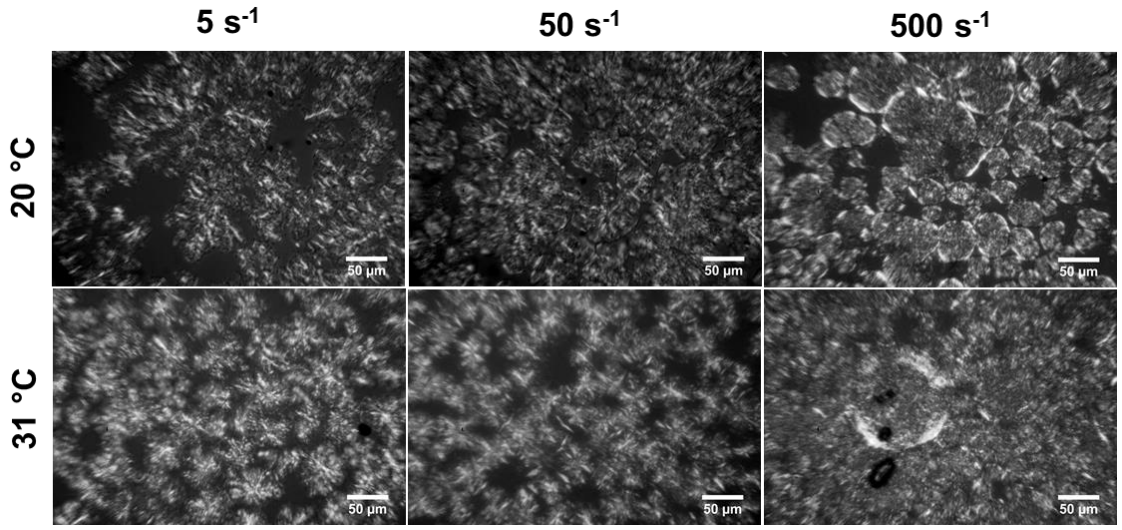

Figure 4.6. Micrographs of 10 % SSS after cooling under shear of 5 s^{-1}, 50 s^{-1} and 500 s^{-1} until 20 °C and 31 °C.

As a result, shearing SSS upon cooling until the end of the network formation lead to the formation of well-defined spherical crystal aggregates, especially at 500 s^{-1}, which developed weaker crystal networks. Besides, shearing before the fat network formation uniformly dispersed fat crystals in the bulk and produced higher G'.

4.4.3 INCREASING THE INTERACTION SSS-EMULSIFIER WITH SHEAR

Subsequently, shear was used as a precursor for SSS-emulsifier interactions during SSS crystallisation upon cooling. Therefore in order to increase the surface effect of SSS with the emulsifier, the samples were sheared until three different stages of crystallisation; 20 °C related to the end of the fat network formation, 31 °C to the cross-over of G' and G'' of 10 % SSS upon cooling, 39 °C to the end of the emulsifier aggregation and before SSS crystallisation.

The elastic modulus-temperature curves of 10 % SSS in the presence of 2 % sorb- S and sorb-P are presented Figure 4.7. Applying shear until the end of the fat network

formation showed a drastic reduction in G' of two to three orders of magnitude (Figure 4.7 A and D). However it has been reported higher sedimentation level thus an increase in crystal sintering by increasing the polarity of the crystal surface (Johansson and Bergenstahl, 1992a); therefore the effect of shear predominated on the effect of sorb-S and sorb-P and the network did not recover from the shear applied until 20 °C. When 10 % SSS and 2 % sorb-S were sheared until 31 °C (Figure 4.7 B), the final G' was lower with shear rates of 5 and 50 s^{-1}, while the network strength was similar when shearing at 500 s^{-1} to a quiescent cooling. Moreover high shear rates promoted the formation of stronger networks from higher temperatures. Figure 4.7.E. shows the effect of shearing until 31 °C on SSS crystallisation with 2 % sorb-P. Lower shear rates did not affect the evolution of G' while shearing at 500 s^{-1} generated higher G' from higher temperatures. Besides a slight different trend when adding sorb-S or sorb-P, high shear rates created the same evolution of G' regardless of the size of the emulsifier carbon chain length. On the other hand, shearing until the end of emulsifier aggregation and before SSS crystallisation lead to opposite trends (Figure 4.8.C and F); the creation of weaker networks in the presence of sorb-S and stronger networks with sorb-P. These results demonstrated that high shear would better disperse sorb-P aggregates in the bulk and increased the sintering between crystals hence rose the final G'. In addition, the longer carbon chain length of sorb-S developed stronger bonds between the emulsifier molecules and required higher shear rates to break-down and be efficiently spread in the bulk.

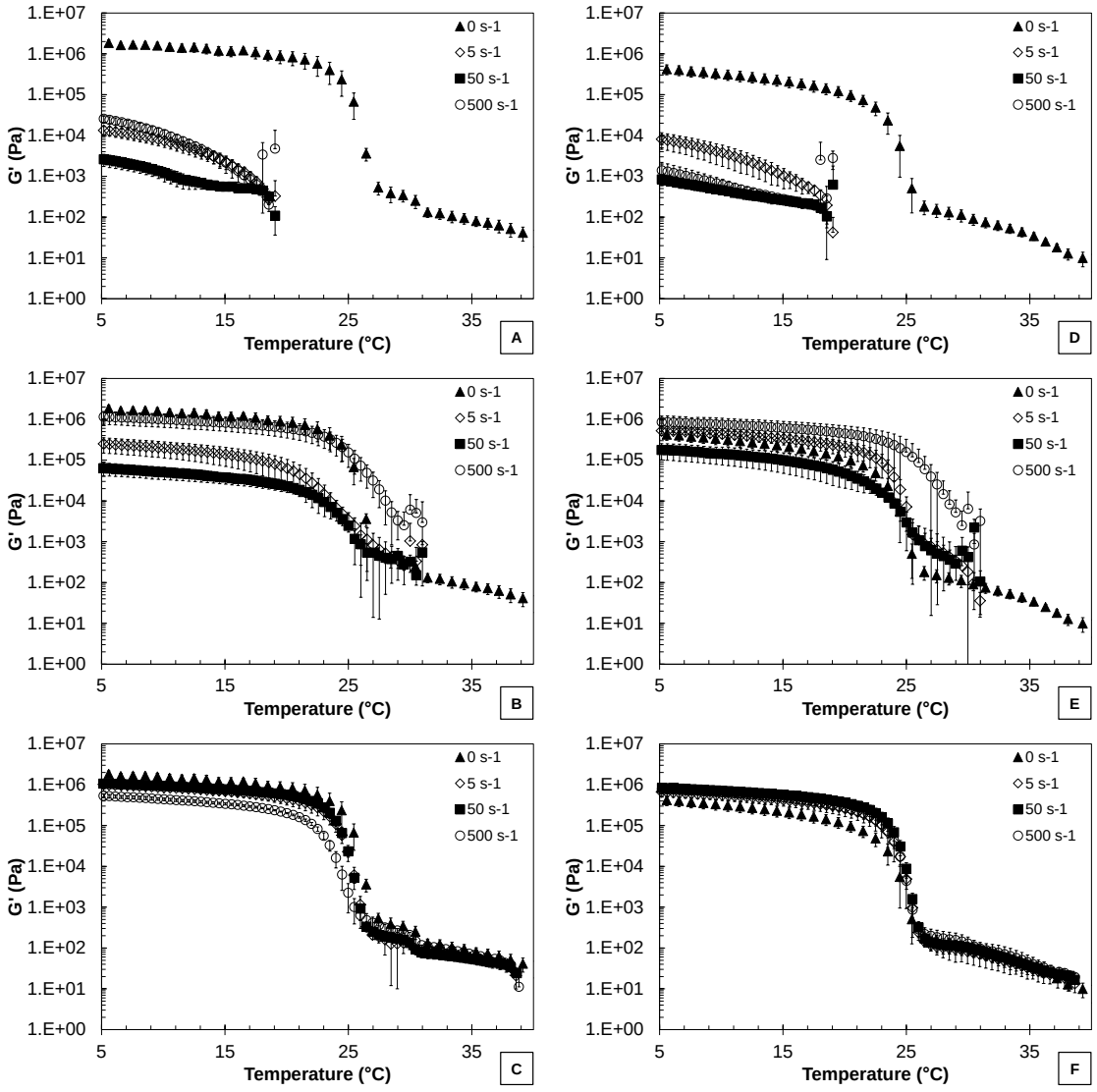

Figure 4.7. Elastic modulus upon quiescent cooling at 1 °C/min and after shearing 10 % SSS with 2 % sorb-S (A, B, C) or 2 % sorb-P (D, E, F) in sunflower oil until 20 °C (A, D), 31 °C (B, E) and 39 °C (C, F).

Concerning the crystal morphology of 10 % SSS with 2 % sorb-S (Figure 4.8) at the end of the crystallisation process, shearing until 20 °C created the same microstructure as in the absence of emulsifiers with well-defined spherical fat clusters. Applying shear until 31 °C generated smaller fat clusters more dispersed in the bulk. However the microstructure was less homogeneous than in the absence of emulsifier. Finally shearing during the aggregation

of sorb-S formed larger fat aggregates hence improving the emulsifier-emulsifier interactions and preventing a surface effect between SSS and emulsifiers.

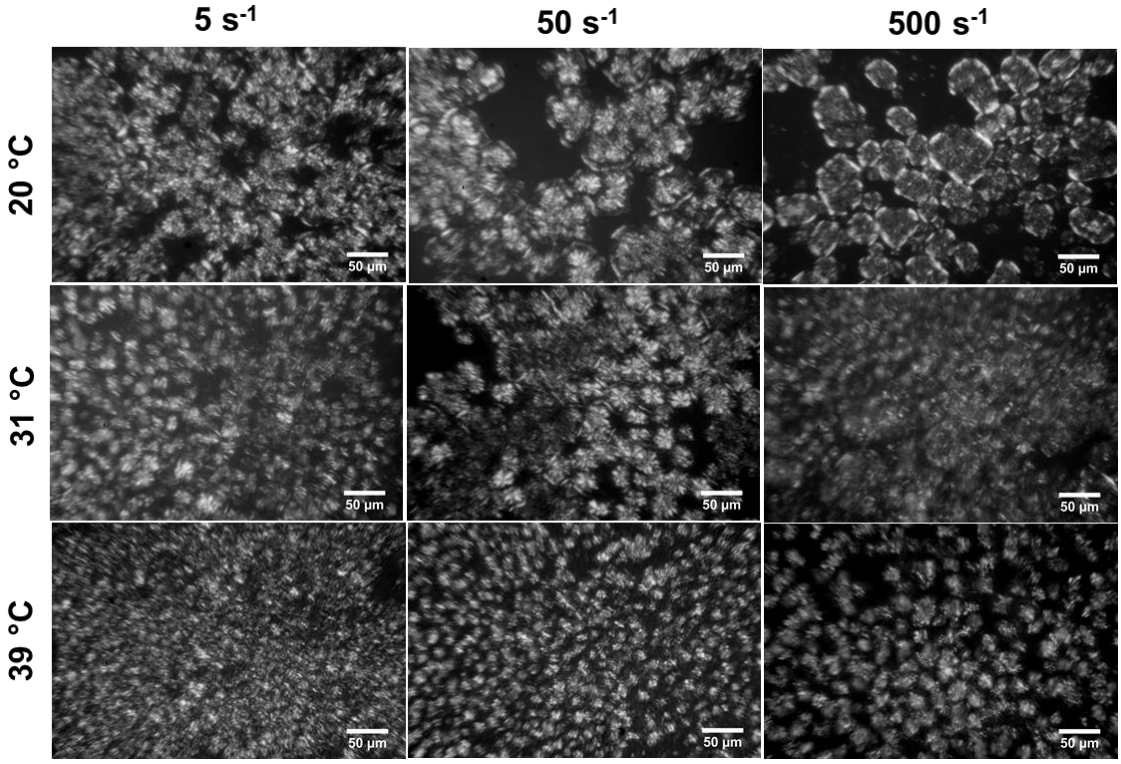

Figure 4.8. Micrographs of 10 % SSS with 2 % sorb-S after cooling under shear until 20 °C, 31 °C and 39°C. Shear rates of 5 s^{-1}, 50 s^{-1} and 500 $^{-1}$ were applied.

The effect of shear until 20 °C and 31 °C on the crystal morphology of 10 % SSS with 2 % sorb-P was comparable to the one with sorb-S (Figure 4.9). However different trends in the microstructure appeared with a decrease in the fat cluster size when increasing the shear rate until 39 °C. As a result, the microstructure and macrostructure of the system showed an opposite effect when changing the length of emulsifier carbon chain. Therefore smaller crystal aggregates were related to higher G' and larger crystal aggregates to lower G' in that case. Shear would disrupt more of sorb-P aggregates than sorb-S aggregates thus creating more nucleation sites and smaller clusters.

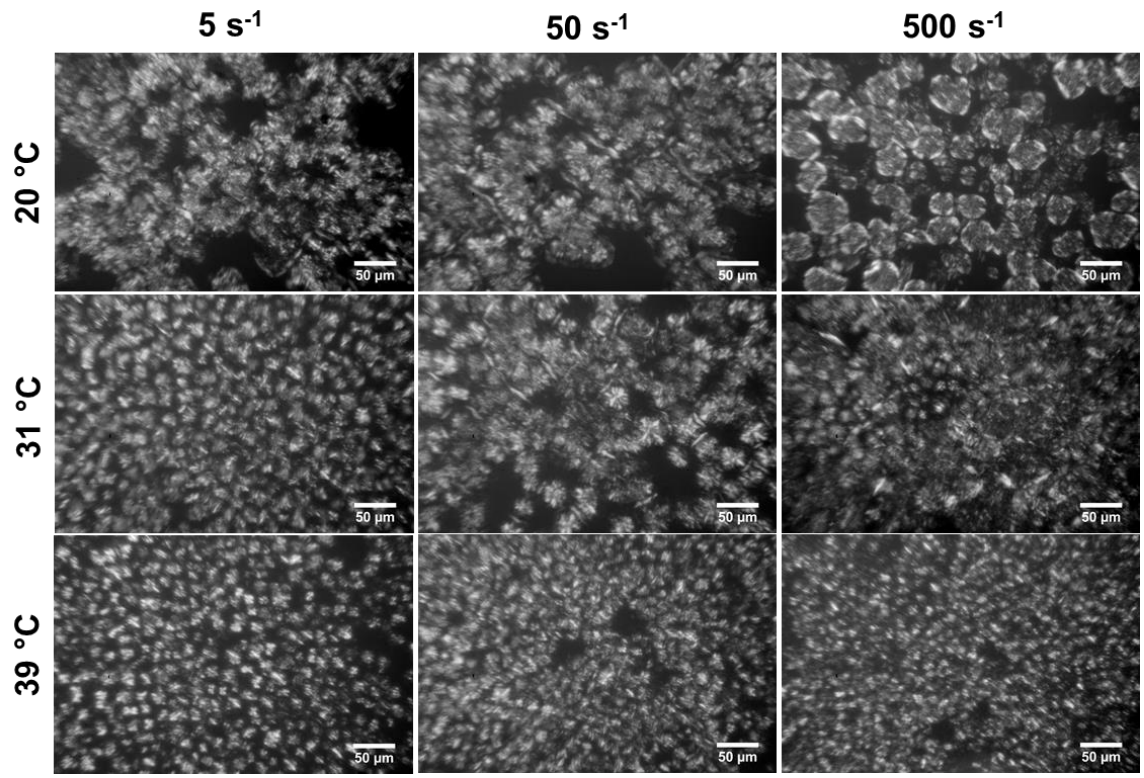

Figure 4.9. Micrographs of 10 % SSS with 2 % sorb-P after cooling under shear until 20 °C, 31 °C and 39°C. Shear rates of 5 s^{-1}, 50 s^{-1} and 500 $^{-1}$ were applied.

4.5 CONCLUSION

The effect of shear on different stages of SSS crystallisation and on the interaction of two types of emulsifier with SSS was studied using a cone and plate geometry and was analysed by low deformation rheology and polarized light microscopy.

Shearing until the end of crystallisation created well-defined spherical fat clusters with weaker networks regardless of the addition of emulsifiers. However shearing until the beginning of the network formation produced smaller clusters well-dispersed in the bulk by secondary nucleation and stronger network. Adding emulsifiers reduced the final G' with lower shear rates but higher shear rates predominated and promoted higher G'. This effect could be explained by the modification of the heterogeneous primary nucleation; shearing the

samples in the temperature range of the emulsifier aggregation showed an enhancement of sorb-S aggregation with the creation of larger aggregates under higher shear rates that lessened emulsifier-SSS interactions and developed weaker networks with lower shear rates. On the other hand, the smaller carbon chain length of sorb-P generated weaker emulsifier aggregation and higher effect of shear on its dispersion in the bulk hence an improvement of the primary heterogeneous nucleation with the creation of more nucleation sites and stronger fat networks.

This study has demonstrated for the first time the possibility to use shear as a precursor for triglyceride-emulsifier interaction by inducing secondary nucleation but also by modifying the properties of the primary heterogeneous nucleation generated by the addition of emulsifiers.

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5. FAT NETWORK PROPERTIES AFTER CONTROLLING NUCLEATION BY SEEDING TRISTEARIN WITH WAXES.

5.1 ABSTRACT

The nucleation of tristearin and its influence on the final textural properties of the system were controlled at a quiescent process by varying the cooling rate and adding carnauba wax (CW) and sunflower oil wax (SW). The thermal behaviour was analysed by Differential Scanning Calorimetry, the crystal size by light scattering and the microstructure by polarized light microscopy. Moreover the textural properties were studied upon cooling with rheology through applying low deformations which allowed the analysis of the evolution of the elastic modulus G'. The final hardness of the system was analysed by cone penetrometry. Waxes addition decreased the induction time for crystallisation and increasing the cooling rate in the temperature range of wax crystallisation decreased the fat crystal size and promoted a better dispersion of crystals in the bulk with SW; therefore the final hardness sharply increased in the presence of SW. his resulted in the promotion of secondary nucleation with SW and primary heterogeneous nucleation with CW. This study showed the possibility to control the nucleation step by adding waxes and to tailor the final textural properties of the product by changing the cooling rate.

5.2 INTRODUCTION

Consumers characterise food products mainly by their colour, flavour, shape and texture. A desirable texture can be achieved by controlling the microstructure of the final product and the microscale arrangement between an aqueous phase, an oil phase and an air phase (Jakubczyk and Niranjan, 2006, Guzey and McClements, 2006). For over thirty years, a focus has been directed on the control of the oil phase as it can have an impact on the texture and the properties of high fat content products like chocolate and margarine. These two

products differ in their amount and type of fat, therefore in their final texture and hardness. As a consequence, the fat network created influences differently the final textural properties of the product; chocolate is a hard product at room temperature compared to margarine and the main issue associated is the expulsion of oil from the fat network due to polymorphic transitions called fat blooming (Kinta and Hartel, 2010). This phenomenon can be avoided by controlling the polymorphic transitions, thus the type of polymorph created, either by varying the temperature of crystallisation or by seeding cocoa butter with the appropriate polymorph (Svanberg *et al.*, 2013, Hachiya *et al.*, 1989). On the other hand, margarine should be stored at lower temperatures compared to chocolate and should be easily spreadable with a smooth texture (Tanaka *et al.*, 2007); however as margarine is composed of a low amount of saturated fatty acids, its microstructure is very unstable at room temperature attributed to the melting of fat and generation of a granular structure due to the transition to the most stable polymorph with temperature change (Saadi *et al.*, 2012). So it has been shown that controlling the oil phase through addition of emulsifiers or by changing the type of triglycerides and seeding the system with the proper polymorph can allow for control of margarine microstructure and hence its texture.

Accordingly, these two examples show the challenge of the food industry for tailoring the material properties that controlled textural attributes such as hardness and spreadability by controlling fat crystallisation and consequently the formation of the fat network. It has been demonstrated that the microstructure influenced the fat network of the system which is informed by crystals sintering, the size and shape of the crystals and the type of polymorphs (Toro-Vazquez *et al.*, 2004, Marangoni and Narine, 2002, Johansson and Bergenstahl, 1995). These parameters can be controlled by changing the fat composition, through addition of additives and changing of the crystallisation process.

The formation of a fat network takes place first at the nanoscale and then at the micro and macroscales upon cooling as followed (Himawan *et al.*, 2006, Metin and Hartel, 2005). The starting point of crystallisation is when the melting temperature is reached. When cooling the system below the melting point, the driving force for crystallisation rises and the saturation increases. The onset of crystallisation begins when the system becomes supersaturated, corresponding to the formation of the first nuclei. These nuclei generate an interface for crystallisation and grow into platelets due to the incorporation of triglycerides in the crystal lattice. As the platelet growth on the interface of nuclei requires less energy for triglyceride aggregation than the creation of new nuclei (Kashchiev, 2000), the first nuclei generate crystal growth and the end of the nucleation step. The higher the number of nuclei in the bulk, the higher the interface for crystallisation, the faster the crystal growth thus the more homogeneous is the crystal aggregate size with smaller crystals. A way of controlling the number of nuclei and therefore the crystal size is to change the driving force for crystallisation by modifying the cooling rate. The study of the microstructure of crystallised high melting fraction of milk fat has shown that the presence of larger crystals with a broader range of sizes when decreasing the cooling rate from 5.5 °C/min to 0.1 °C/min (Martini *et al.*, 2002, Herrera and Hartel, 2000a). Moreover, the use of cryo-TEM for studying crystal size of fully hydrogenated canola oil at different scales has confirmed the previous results (Maleky *et al.*, 2012, Acevedo and Marangoni, 2010a, Acevedo and Marangoni, 2010b); higher cooling rate has presented a decrease of the crystal size at the micro and nano-scales.

Besides the control of nucleation by varying the cooling rate, two main types of nucleation are described; primary and secondary nucleation. The primary nucleation corresponds to the association of the first nuclei while the secondary nucleation relates to the formation of a second type of nuclei either by breaking mechanically the first crystals created

using shear (Acevedo *et al.*, 2012a, Sonwai and Mackley, 2006, Herrera and Hartel, 2000a) or by creating new nuclei as a result of the growth on primary crystals. This second type of nucleation is also referred to seeding. Furthermore fat crystals are arranged in different conformations called polymorphs like α, β' and β in their range of stability. High driving force for crystallisation promotes the formation of the α-form subsequent polymorphic transitions towards more stable forms resulting in better crystal stability. Finally crystals sinter together and can eventually form a fat network.

Seeding has been extensively used in the food industry as well as other industries such as pharmaceuticals where the fat crystal size distribution can be customized by adding specific seeds (in terms of size, number and polymorphs) to the system. Chung *et al.* (1999) studied the effect of the particle size distribution in relation to seeding and change in temperature profiles. It has been demonstrated that optimal seeding can provide a better control of the particle size distribution compared to optimizing the cooling rate only. Moreover Aamir *et al.* (2010b) has shown the requirement for an appropriate seed concentration and type in order to achieve the desired particle size distribution. Finally they have modelled the effect of seed preparation with respect to the final particle size distribution by changing the temperature profile in order to design the type and size of seeds produced (Aamir *et al.*, 2010a).

A novel approach for controlling the textural properties in low fat system has been the use of food-grade waxes (Hwang *et al.*, 2014) such as candellila, carnauba, sunflower oil and rice bran waxes which create an organogel (Martini *et al.*, 2015, Hwang *et al.*, 2014, Blake *et al.*, 2014, Chopin-Doroteo *et al.*, 2011, Toro-Vazquez *et al.*, 2009, Dassanayake *et al.*, 2009). However little interest has been given to the use of waxes for controlling fat crystallisation via

their seeding effect as reviewed by Martini *et al.* (2008) in their study of milk fat crystallisation with sunflower oil wax. It has been shown a decrease in the induction time for milk fat crystallisation and the production of smaller crystals.

Therefore the objective of this study was to elucidate how tailor the textural properties of a low saturated fat system could be tailored by controlling the nucleation step. Adding high melting molecules such as wax would promote secondary nucleation by seeding tristearin. Moreover changing the cooling rate in the temperature range of wax crystallisation would allow the design of nuclei number and therefore of fat cluster size distribution and thus the final hardness.

5.3 MATERIAL AND METHODS

5.3.1 MATERIALS

Glyceryl tristearate (SSS) commonly named tristearin was purchased from Sigma-Aldrich (Sigma–Aldrich Company Ltd., Dorset, UK). CW was supplied by Alliance Boots PLC (UK) and SW by Cargill (USA). Sunflower oil was commercially purchased. The composition of SW and CW is shown Table 5.1.

10 % w/w SSS in sunflower oil was used as a reference, and different concentrations of wax were then added (0.5, 1 and 2 %). Samples were heated at 80 °C for 30 minutes to ensure a total dissolution.

Material	SW	CW
Ester content (%)	97 - 100	84 - 85
Free fatty acid (%)	0 - 1	3 - 3.5
Free fatty alcohol (%)	-	2 - 3
Hydrocarbons (%)	-	1.5 - 3
Resins/others (%)	0 - 3	6.5 - 10
Melting point (°C)	74 - 77	80 - 85
Carbon chain length	C36 - C48	C26 - C30

Table 5.1. Chemical and physical properties of SW and CW (Blake et al., 2014).

5.3.2 METHODS

5.3.2.1 THERMAL BEHAVIOUR

The thermal behaviour upon cooling and heating at 1 °C/min was analysed by Micro-DSC3 evo (Setaram Instrumentation, United Kingdom). Prior to the analysis, a calibration with indium at a scan rate of 1 °C/min was performed. 35-55 mg of sample was subjected to the following thermal program: isothermal at 100 °C for 5 minutes to remove the crystal memory, cooling at 1 °C/min to 5 °C, isothermal at 5 °C for 2 minutes, heating from 5 °C to 100 °C at 1 °C/min. An empty capsule was used as a reference. The melting and crystallisation temperatures as well as enthalpies were obtained from the thermograms.

5.3.2.2 RHEOLOGY

The analyses were performed using a Kinexus rheometer (Malvern, United Kingdom) and a cone-plate geometry with 40 mm diameter, with a cone angle of 4 ° and a gap of 0.15 mm. Samples were loaded at room temperature. The temperature was controlled by a Peltier system with a sensitivity of ± 0.2 °C. Oscillatory experiments were run within the LVR (*3.3.2.4 Rheology*).

In order to promote smaller crystals, samples were cooled at 10 °C/min from 80 °C to 45 °C corresponding to the range of wax crystallisation temperatures determined by DSC and then at 1 °C/min from 45 °C to 5 °C (Figure 5.1, process B). For production of larger wax seed crystals, samples were cooled at 1 °C/min from 80 °C to 5 °C (process A). Simultaneously, samples were subjected to low deformations at 1Hz and shear strain of 0.01 % and the evolution of the elastic and viscous moduli (G' and G'' respectively) was analysed during cooling. Two repetitions and one duplicate were performed.

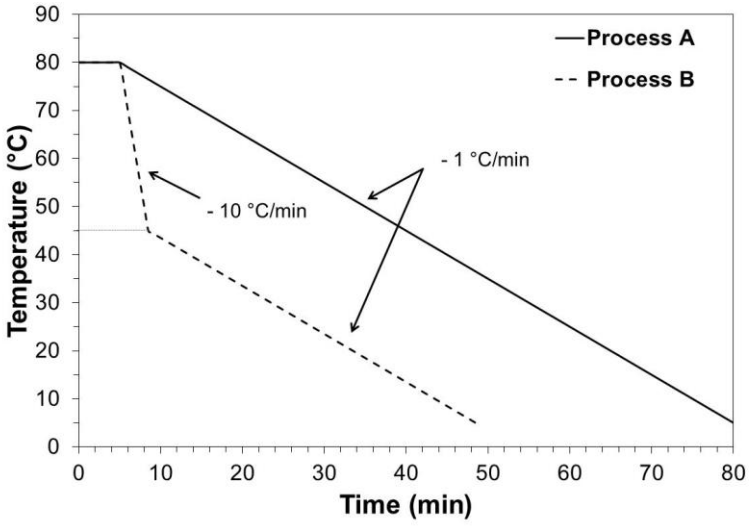

Figure 5.1. Process of crystallisation cooling at 1 °C/min from 80 to 5 °C (Process A) and cooling at 10 °C/min from 80 to 45 °C followed by cooling at 1 °C/min (Process B).

5.3.2.3 FAT CLUSTER SIZE

The fat cluster size was determined by the laser diffraction technique using a Mastersizer 2000 (Malvern, UK). The samples were dispersed in sunflower oil (refractive index of 1.467 (O'Brien, 2009b)) with 1650 rpm agitation speed. The fat aggregate size distribution was analysed and the average fat cluster size was represented by the equivalent mean volume diameter $D_{4,3}$, defined by Equation 1 (Leroux *et al.*, 2003):

$$D_{4,3} = \frac{\sum_i n_i \cdot d_i^4}{\sum_i n_i \cdot d_i^3} \quad (5.1)$$

where *n* is the number of fat cluster of diameter *d*.

Fat cluster size determination was performed 24 h after sample preparation at 20 °C and each analysis was repeated three times with one duplicate.

5.3.2.4 MICROSTRUCTURE

The samples were analysed by polarized light microscopy (Brunel microscopes Ltd, United Kingdom) and equipped with a camera (Canon EOS 1000D, Taiwan) after being cooled in the Rheometer and stabilised at 20 °C for 24 h (1.2.2.2 Rheology). A small amount of sample was placed on a glass slide and then covered with a glass cover slip. Each analysis was duplicated, and 20 images per sample were taken.

Micrographs were adjusted using ImageJ 1.49 (National Institutes of Health, Maryland, USA) by being processed in a 8-bit grayscale and adjusting the contrast and brightness.

5.3.2.5 HARDNESS

Fat blends were placed in a plastic pot of 48 mm diameter and 1cm height. Prior to crystallisation in a water bath, the samples were kept at 80 °C for 30 min in order to erase the crystal memory. Thereafter, the samples were subjected to two different processes; half of the samples was cooled at 2 °C/min from 80 °C to 15 °C and the other half was immersed in a

water bath at 40 °C and cooled until 15 °C at 2 °C/min. Subsequently, the samples were maintained for 24 h at 20 °C before analysis.

Hardness was studied by using a TA.XT.plus Texture Analyser (Stable Micro Systems Ltd., UK) with a 45 ° Perspex® cone. The cone penetrated the samples at a speed of 1 mm.s^{-1} for 5 seconds. Stress τ (Kg.mm^{-2}) and strain σ (%) were determined by the following equations (Shi *et al.*, 2005):

$$\tau = \frac{F}{A} \tag{5.2}$$

$$\sigma = \frac{h - H_0}{H_0} \tag{5.3}$$

where F is the compression force (N), A is the cross-sectional area of the sample (mm^2), and h is the height of the penetration (mm) and H_0 is the initial height (mm).

Five replicates were made and the maximum stress τ (Kg.mm^{-2}) during penetration was considered as the hardness of the system.

5.4 RESULTS AND DISCUSSION

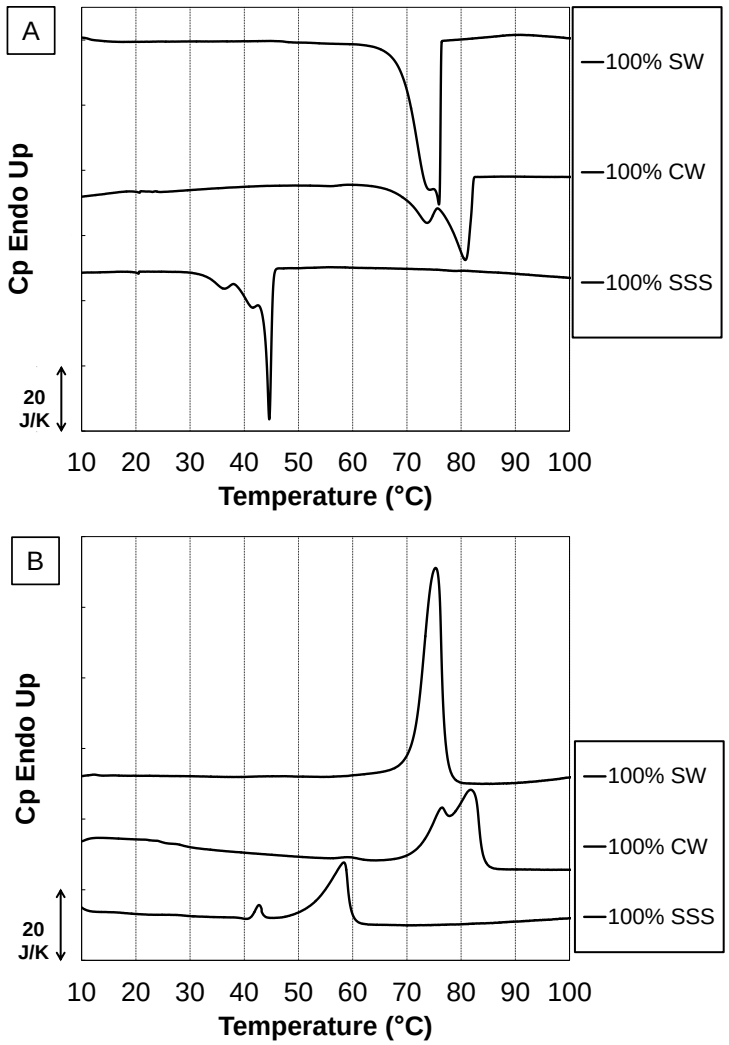

Figure 5.2. Thermal behaviour of 100 % SSS, CW and SW in sunflower oil upon cooling (A) and heating (B) at 1 °C/min.

The thermal behaviour of pure SSS, SW and CW was studied upon cooling and heating rate of 1 °C/min by DSC in order to analyse the crystallisation and melting temperatures (Figure 5.2). Crystallisation of SSS, SW and CW occurred at 45.5 °C 76.3 °C and 82.3 °C respectively (Table 5.2). Pure SSS crystallisation was characterised by a three-step crystallisation process while both waxes crystallised in two steps; the temperature range was narrow for SW and broad for CW with two distinct crystallisation peaks. The melting

profile was analysed in order to study the crystal stability. Both waxes melted in the same temperature range however one peak for SW indicated the presence of a single type of crystal while a two- endothermic peak associated with the melting of CW revealed the formation of multiple crystals. Moreover SW and CW showed a higher melting temperature than SSS that melted at 58.5 °C. These results are in good agreement with the single-component analysed in SW, the multi-component of CW (Table 5.1) and with the study of SW crystallisation by Martini and Anon (2003). Higher crystallisation temperatures and the difference in wax composition indicated the possibility to use both waxes as seeds for SSS crystallisation which would lead to diverse behaviours.

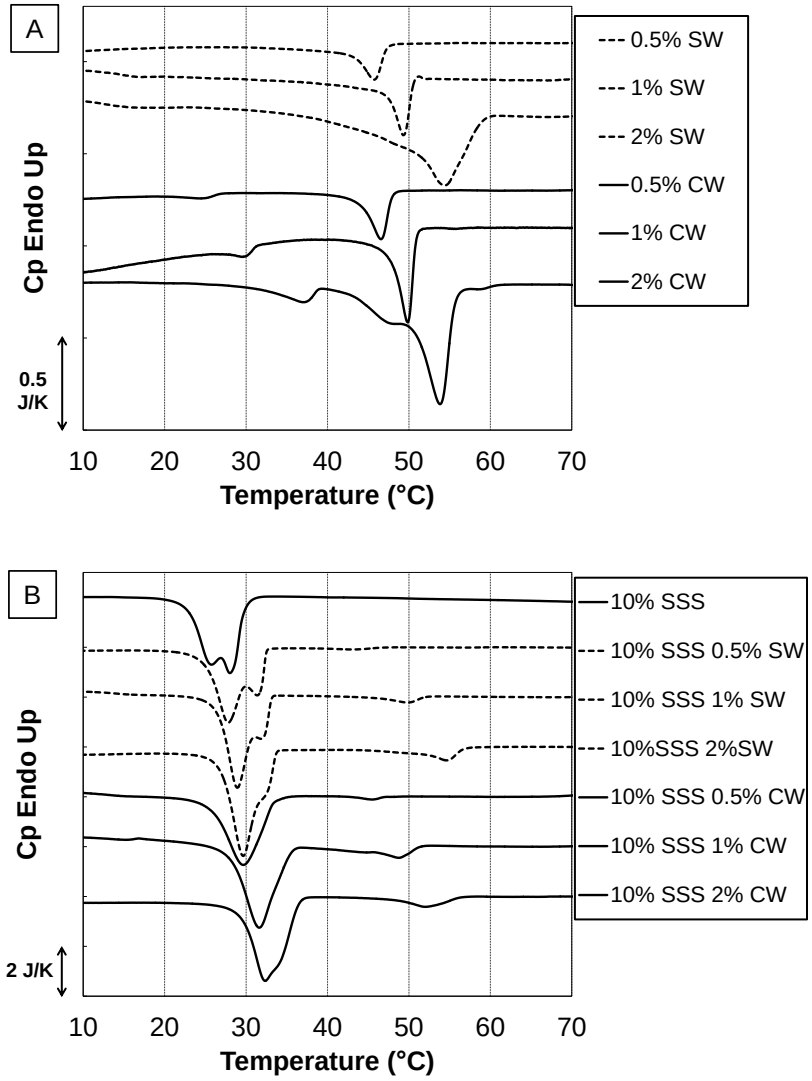

Figure 5.3. Thermal behaviour of 0.5 % to 2 % CW and SW in sunflower oil (A) and in presence of 10 % SSS (B) upon cooling at 1 °C/min.

	Peak number 1		Peak number 2	
	T_{onset} (°C)	Enthalpy (J/g)	T_{onset} (°C)	Enthalpy (J/g)
10% SSS	-	-	30.0 ± 0.1	-12.2 ± 0.3
100% SSS			45.5 ± 0.2	-125.3 ± 2.1
0.5% SW	47.6 ± 0.4	-0.6 ± 0.2	-	-
1% SW	50.6 ± 0.1	-0.7 ± 0.2	-	-
2% SW	57.6 ± 1.3	-3.1 ± 0.5	-	-
100% SW	76.3 ± 0.1	-261.3 ± 5.2	-	-
10% SSS 0.5% SW	45.8 ± 0.7	-0.3 ± 0.1	32.4 ± 0.1	-14.3 ± 0.2
10% SSS 1% SW	52.1 ± 0.3	-0.9 ± 0.1	33.2 ± 0.4	-15.1 ± 1.0
10% SSS 2% SW	56.8 ± 0.8	-2.5 ± 0.2	33.8 ± 0.2	-16.1 ± 0.5
0.5% CW	47.6 ± 0.5	-0.6 ± 0.1	-	-
1% CW	50.6 ± 0.3	-1.2 ± 0.1	31.2 ± 0.1	0.1 ± 0.1
2% CW	54.7 ± 1.0	-2.9 ± 0.3	37.7 ± 1.3	-0.3 ± 0.1
100% CW	82.3 ± 0.1	-180.9 ± 1.0	-	-
10% SSS 0.5% CW	46.9 ± 0.1	-0.4 ± 0.1	33.4 ± 0.1	-13.6 ± 0.1
10% SSS 1% CW	50.7 ± 0.4	-1.0 ± 0.1	35.0 ± 0.4	-14.3 ± 0.3
10% SSS 2% CW	56.5 ± 0.8	2.0 ± 0.4	36.7 ± 0.4	-15.6 ± 0.2

Table 5.2. Temperature at the onset of crystallisation and enthalpy of crystallisation.

	T_{peak} (°C)	Enthalpy (J/g)
10% SSS	48.7 ± 0.1	14.7 ± 0.1
10% SSS 0.5% SW	47.0 ± 0.1	14.2 ± 0.1
10% SSS 1% SW	46.8 ± 0.1	12.8 ± 0.4
10% SSS 2% SW	47.1 ± 0.4	14.0 ± 1.0
10% SSS 0.5% CW	46.9 ± 0.1	13.2 ± 0.1
10% SSS 1% CW	47.1 ± 0.2	13.3 ± 1.2
10% SSS 2% CW	47.3 ± 0.1	13.0 ± 0.1

Table 5.3. Temperature at the onset of crystallisation and enthalpy of melting.

The comparison of the crystallisation profiles of CW and SW in sunflower oil and when 10 % SSS was added are presented Figure 5.3. The crystallisation of 10 % SSS in the presence of low wax concentration resulted in earlier onset of SSS crystallisation which was

more pronounced with CW (Table 5.2). Furthermore, the process of SSS crystallisation was modified by wax; sharper second peak occurred when increasing SW concentration whereas broader single-peak of crystallisation appeared with 0.5 and 1 % CW and a two-step crystallisation with 2 % CW. Besides, the energy released by SSS crystallisation rose when increasing both wax concentrations although the enthalpy of wax crystallisation was comparable in the presence or absence of SSS. Therefore those waxes were used as a template for SSS crystallisation by acting like seeds. This complied with the effect of monoglycerides on tripalmitin crystallisation showing higher release of energy when seeds were added to the system (Basso *et al.*, 2010). In addition, the melting behaviour indicated comparable absorption of energy but lower melting temperature when adding wax to the system (Table 5.3). Blake *et al.* (2014) studied the polymorphs resulting from SW and CW crystallisation by powder X-ray diffraction and observed only the β'-form. As a result, the β' arrangement of SW and CW molecules was a template for SSS triglycerides and would create less stable crystals than in the absence of wax. Thus SW and CW seemed to influence SSS crystallisation with regards to the nucleation and the stability of crystals with both waxes exhibiting different behaviours according to the variation in their composition.

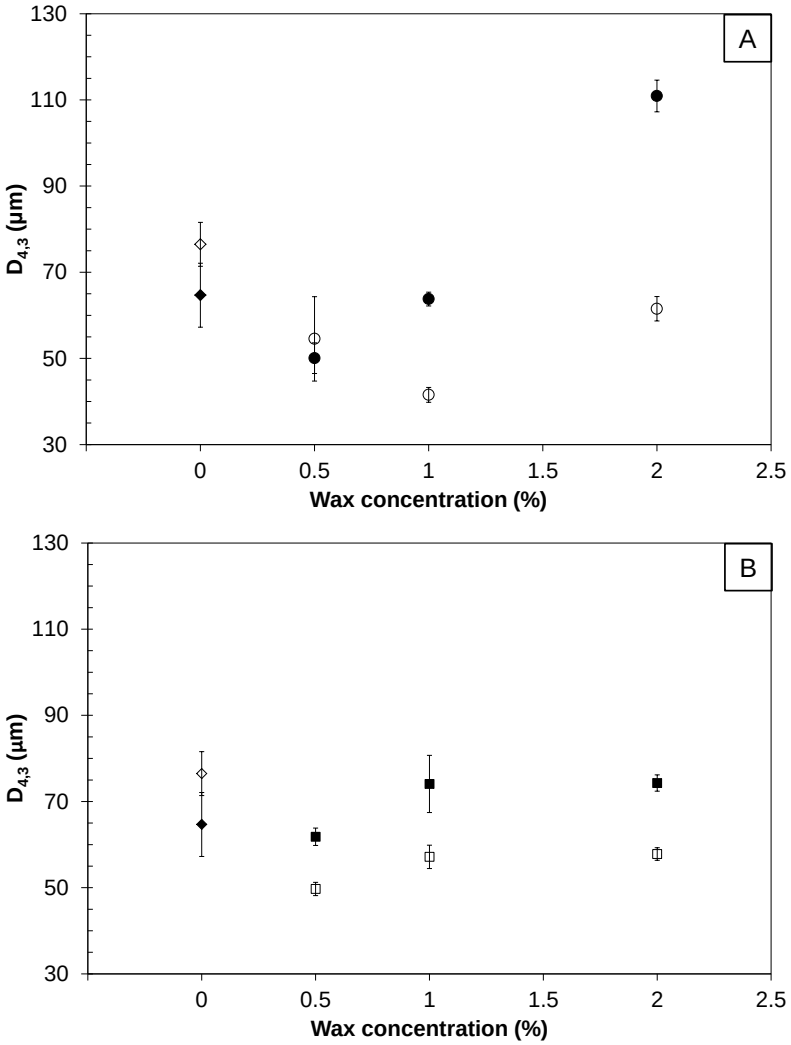

Figure 5.4. Crystal size of 10 % SSS and in the presence of SW (A) and CW (B) after process A (close symbols) or process B (open symbols).

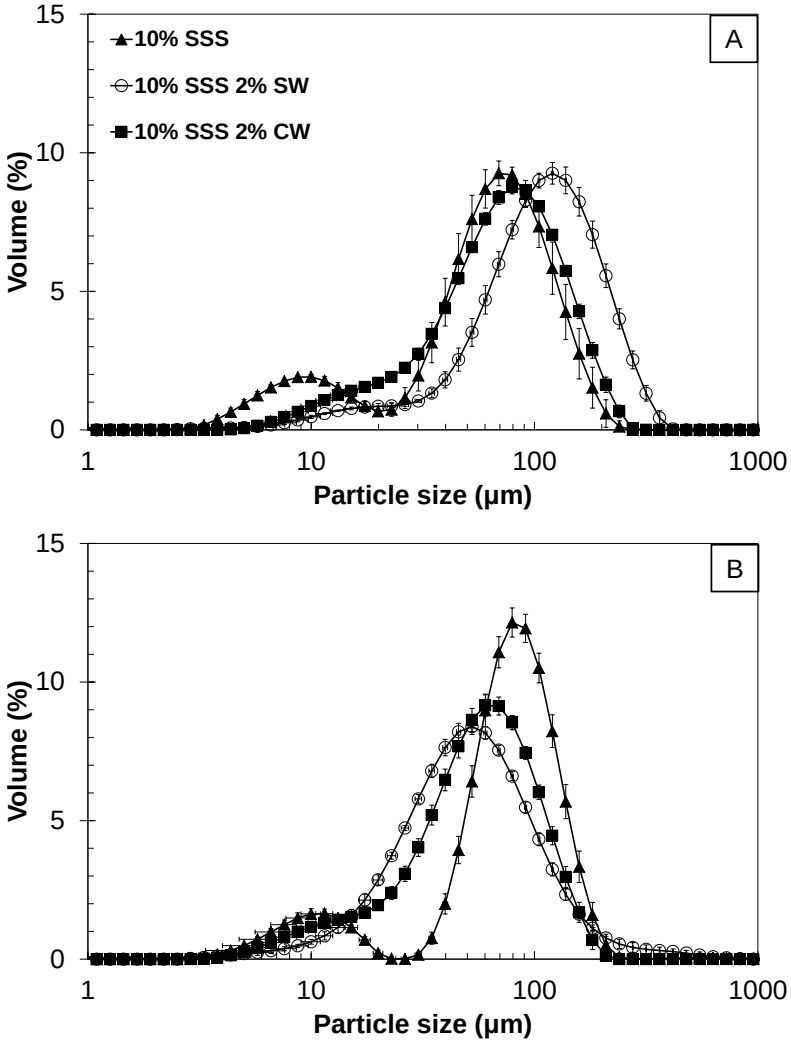

Figure 5.5. Particle size distribution of 10 % SSS with out and with 2 % SW and CW after process A (A) and process B (B).

Due to the consequent thermal profile of SSS with SW and CW analysed by DSC, the samples were submitted to specific cooling conditions to increase the amount of nucleation sites and therefore change the size of fat crystals. In order to change the amount of wax nucleation sites, two processes have been applied (Figure 5.1); process B ensure the formation of more wax nuclei by using fast cooling rate in the temperature range of wax crystallisation. The crystal size was studied by light scattering 24 h after the sample production (Figure 5.4). Changing the cooling rate above 45 °C generated the same size of fat clusters in the absence

of wax. However fat cluster size was lower after process B when adding SW or CW to the system. Figure 5.5 shows bimodal size distribution in the absence of wax and a mono-modal distribution with waxes after the two processes of crystallisation. Therefore seeding with SW and CW presented the possibility to tailor the particle size distribution and to homogenise the crystal size as already demonstrated by Doki *et al.* (2001). Consequently, light scattering could be used for determining the effect of cooling rate on fat crystal size as changing it generated more nucleation sites in the system and thus the formation of smaller fat clusters.

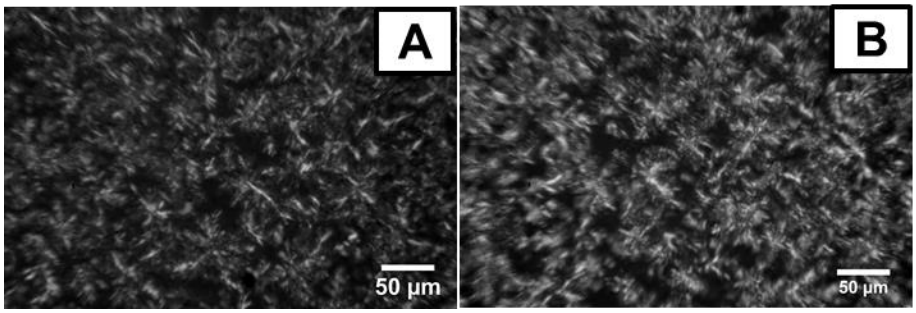

Figure 5.6. 10 % SSS after process A (A) and B (B).

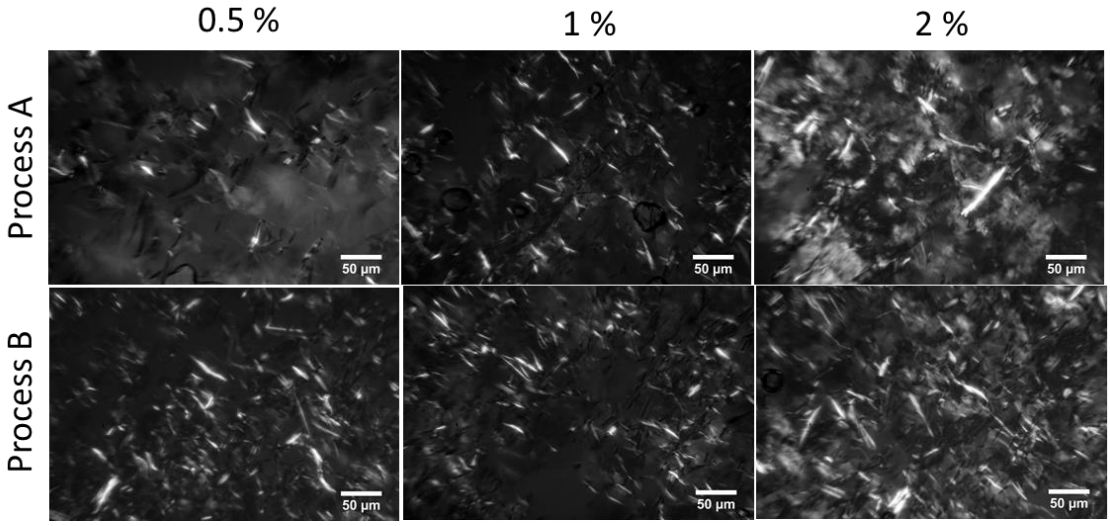

Figure 5.7. 0.5 %, 1 % and 2 % SW in sunflower oil after process A or B.

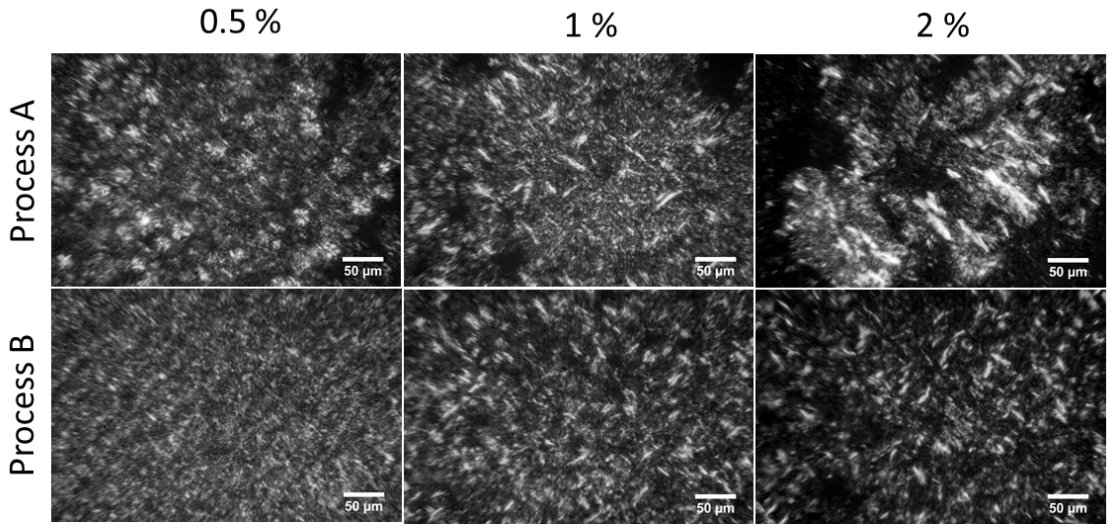

Figure 5.8. 10 % SSS in sunflower oil with 0.5 %, 1 % and 2 % SW after process A or B.

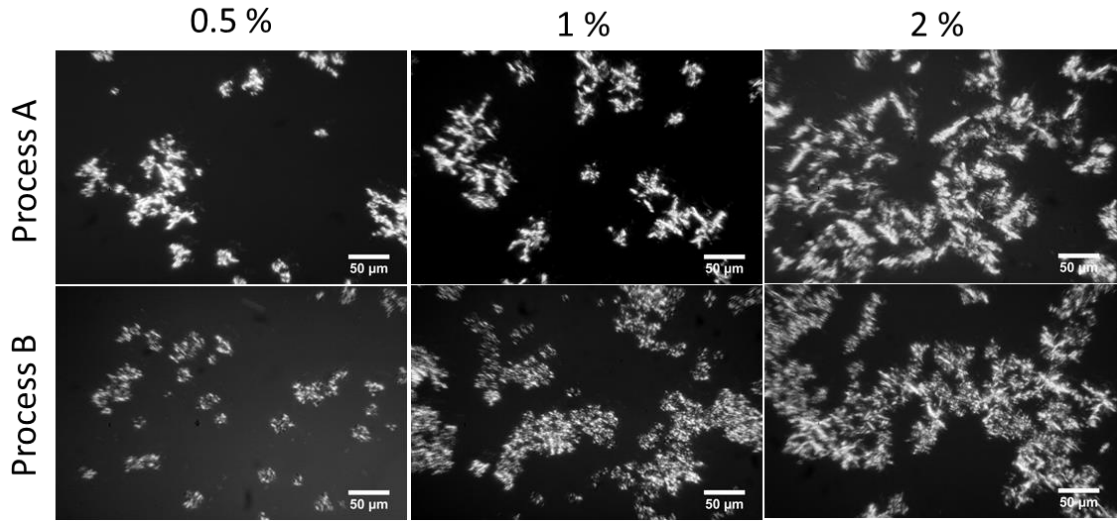

Figure 5.9. 0.5 %, 1 % and 2 % CW in sunflower oil after process A or B.

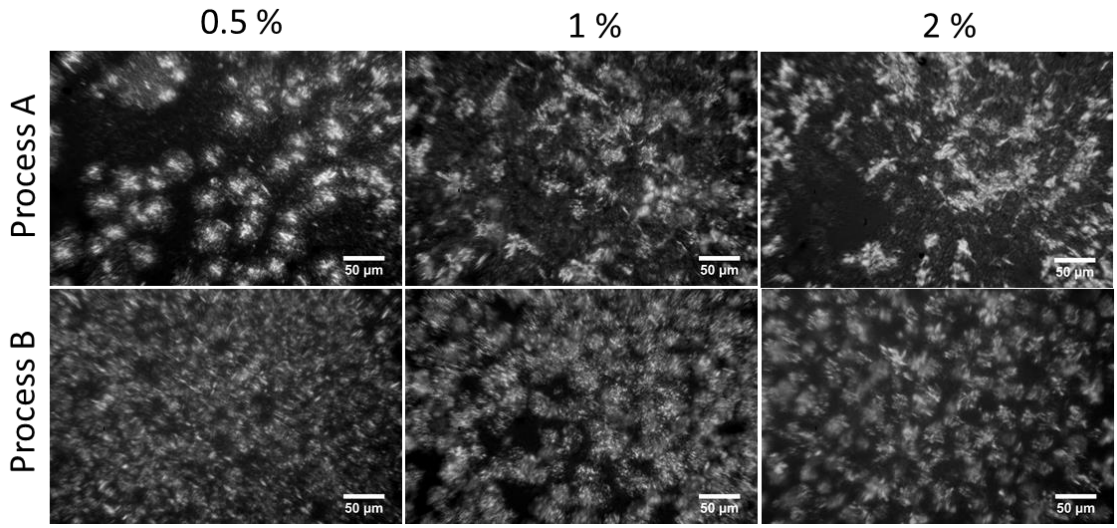

Figure 5.10. 10 % SSS in sunflower oil with 0.5 %, 1 % and 2 % CW after process A or B.

To explore the influence of tailoring the number of nucleation sites by changing the cooling rate, polarized light microscopy was used and the microstructure of SSS crystallised in the absence and presence of waxes subjected to different cooling rates was studied. Figure 5.6 shows the microstructure of 10 % SSS in sunflower oil 24 h after the end of crystallisation according to the two processes involved in this study. No significant difference in fat crystal shape and dispersion appeared on the micrograph. This observation was consistent with the absence of any change in fat crystal size as analysed by light scattering (Figure 5.4). When changing the cooling rate of SW and CW crystallisation in sunflower oil, various behaviours were observed on the micrograph; SW presented a needle-like shape as already shown by Martini and Anon (2003) and Martini *et al.* (2015) and increasing the cooling rate to 10 °C/min did not show any change in the dispersion of wax crystals (Figure 5.7). However the change of crystallisation process exhibited a change in CW dispersion especially with 2 % CW; crystals were more spherical than SW crystals and increasing the cooling rate exhibited less dense crystals which were more dispersed into the bulk (Figure 5.9). Further, although no

significant change occurred when varying the cooling rate of SW in sunflower oil, crystallising 10 % SSS with SW in sunflower oil at 1 °C/min lead to the formation of larger fat clusters and brighter hence denser nuclei of fat cluster corresponding to wax seed crystals (Figure 5.8). This phenomenon was more pronounced in presence of CW where SSS seemed to crystallise on the top of wax crystals (Figure 5.10). Finally changing the process of crystallisation in order to generate more nucleation sites resulted in a better dispersion of fat crystals into the bulk with SW and CW revealing more homogeneous microstructures in terms of crystal density.

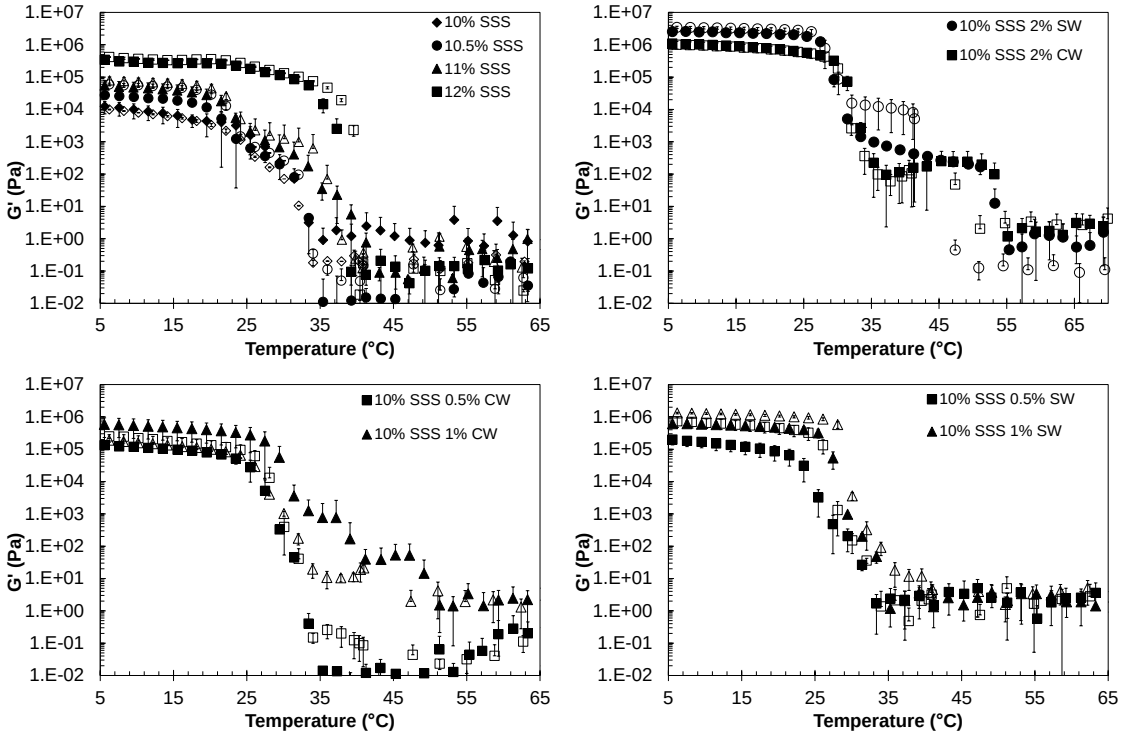

Figure 5.11. Evolution of the elastic modulus G' of 10 % SSS in sunflower oil and in the presence of 2 % SW or 2 % CW upon process A (close symbols) or B (open symbols).

The crystal growth and sintering were studied by analysing the evolution of the elastic modulus G' upon cooling (Figure 5.11). Changing the cooling rate above 45 °C did not influence the evolution of G' through increasing SSS concentration in the absence of wax.

This result was in good agreement with those reported by light scattering and polarized light microscopy confirming the absence of any effect in cooling rate above 45 °C. On the other hand, adding 2 % wax to SSS generated a distinct two-step crystallisation corresponding first to wax and then to SSS crystallisations. Applying faster cooling rate lead to the formation of stronger networks within the temperature of SW crystallisation, while no change in G' evolution occurred with CW. SW would be more efficient structuring component so required lower concentrations for gelling in the oil than CW (Blake *et al.*, 2014). In this respect, increasing the cooling rate in order to control the crystal size hence the number of nucleation sites, was more effective with SW as its single-component particularity would enhance the crystal sintering. SW and CW did not display any changes in the G' evolution and this could be explained by lower structuring efficiency with lower wax concentration. Nonetheless the rate of increase in G' in the range of SSS crystallisation was not modified by the early formation of stronger network corresponding to 2 % SW crystallisation upon faster cooling rate. This implied SSS crystal growth was already very fast so no significant change could be noticed by analysing G' evolution.

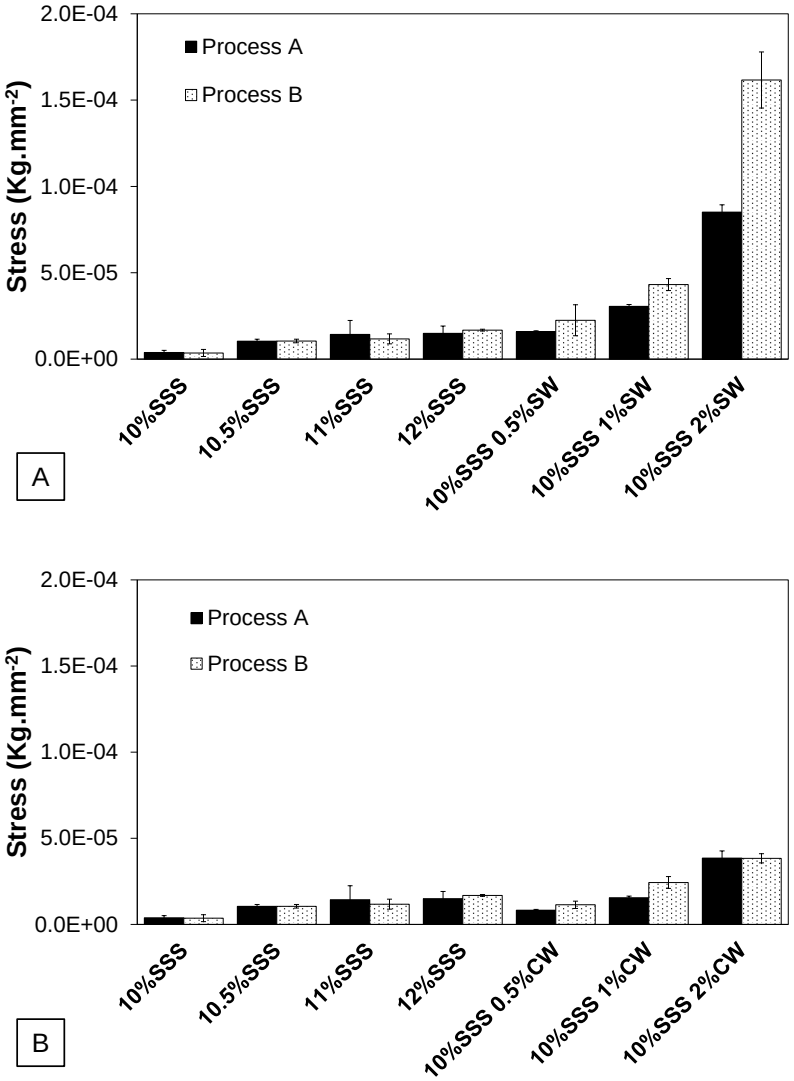

Figure 5.12. Stress at 20 °C, 24 h after process A or B; SSS in sunflower oil and in the presence of SW (A) and CW (B).

Hardness was represented by the maximum stress applied to the fat blends after a cone penetration of 5 mm. Figure 5.12.A shows the maximum stress for 10 to 12 % SSS and for 10 % SSS in the presence of SW. The stress increased slightly when increasing the concentration of SSS from 10 to 12 %, but it did not exhibit any significant differences when changing the cooling rate above 40 °C. The presence of similar final hardness regardless of the cooling rate applied above 40 °C was consistent with previous calorimetric analyses as SSS crystallised at least 10 °C below 40 °C for cooling rates of 1 °C/min and 10 °C/min. On the other hand,

adding crystallised material such as 0.5, 1 and 2 % SW to 10 % SSS sharply raised the final hardness at any given cooling rate. Furthermore, the final hardness was also influenced by the cooling rate applied above 40 °C revealing a significant increase in hardness with a cooling rate of 10 °C/min. Thus adding SW did not increase the hardness of the network only by strengthening the interaction between fat crystals, but also by changing the organisation of the fat network according to the driving force for crystallisation in their crystallisation temperature range as observed previously by polarized light microscopy.

Adding 0.5, 1 and 2 % of a multi-component material such as CW resulted in a slight increase in the final hardness and no significant differences occurred when changing the cooling rate above 40 °C (Figure 5.12.B.). These results are in good agreement with the evolution of G' upon cooling where the cooling rate had no influence. Accordingly, modifying the cooling rate in the range of CW crystallisation did not impact the final strength of the network and thus the interaction between fat aggregates. Therefore, increasing the number of CW nucleation sites by applying different cooling rates did not enhance their interaction with SSS; as a result. CW would act like a foreign surface for SSS crystallisation but did not interact with SSS promoting primary heterogeneous nucleation instead of second nucleation with SW.

Consequently SSS interacted better with SW than CW and this interaction could be controlled by applying different processes of crystallisation. As a result the type of wax used was important to determine the interaction between fat aggregates and thus the final hardness.

5.5 CONCLUSION

This study has characterised the thermal behaviour, microstructure, evolution of the elastic modulus and final hardness of SSS in sunflower oil in the presence of two types of wax that mostly differed by their multi (CW) and single-component (SW) nature. Lower concentrations of SW and CW could crystallise at higher temperature than SSS and changed the process of SSS crystallisation. Indeed, the induction time for crystallisation could be controlled especially with the addition of CW. Moreover changing the cooling rate in the range of wax crystallisation lead to a better control of SSS crystallisation. Besides the kinetics of crystallisation, the final crystal aggregate size decreased when changing the driving force for SW crystallisation and smaller fat aggregates and more dispersed crystals could be observed in the bulk by polarized light microscopy. As a result, the evolution of the elastic modulus varied when applying different cooling rates with SW and the final hardness increased sharply with higher driving force for crystallisation i.e. the creation of smaller fat crystal aggregates. However SW imparted a significant effect on the final fat network and changing the cooling rates appeared to control better the interaction between SW and SSS. On the other hand, CW decreased the induction time for SSS crystallisation but did not impact the final hardness; CW did not interact with SSS but acted as a foreign surface for SSS crystallisation by promoting primary heterogeneous nucleation, while SW could interact with SSS and generated secondary nucleation. Finally a comparison with the addition of the same amount of crystallised material with SSS demonstrated better efficiency of the addition of SW to SSS on the fat crystal network hardness.

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6. GENERAL DISCUSSION

This thesis has improved the understanding of fat crystallisation and its impact on textural properties by changing the process and formulation of low tristearin content systems.

Firstly this has been achieved by investigating the effect of the head group size of emulsifiers compatible with tristearin with regards of their carbon chain length. The thermal behaviour, polymorphic transitions, evolution of the solid fat content, evolution of the viscoelastic properties and microstructure have been studied with respect to the concentration of emulsifiers.

Then shear has been used as a tool for increasing the interaction between tristearin and emulsifiers when applied until specific stages of crystallisation. The evolution of viscoelastic properties and final microstructure have been elucidated as a function of the carbon chain length of emulsifiers.

Lastly the use of waxes as seeding agents for tristearin has been studied with regards of the type of wax and the process of crystallisation. Sunflower oil wax, a single component, and carnauba wax, a multiple component, have been crystallised upon different cooling rates in their crystallisation temperature range in order to evaluate the impact of their nucleation on tristearin crystallisation. The thermal behaviour, particle size, viscoelastic properties and final hardness of the network have been assessed.

6.1 SSS CRYSTALLISATION IN THE PRESENCE OF EMULSIFIERS

- **High HLB emulsifiers (Tween 60) generated a second phase in the bulk**

Neither the thermal behaviour, microstructure, SFC evolution or G' evolution have shown significant differences when Tween 60 (0.5, 1 and 2 %) was added to 10 % SSS

compared to 10 % SSS in sunflower oil. Therefore as Tween 60 is highly hydrophilic, it has been evidenced its aggregation in a second part of the bulk and the absence of interaction with SSS. This contradicted some studies showing a shorter induction time for crystallisation when adding Tween 60 to tripalmitin (Basso *et al.*, 2010). However it could be interesting to check its influence when added in water-in-oil emulsion where its organisation at the droplet interface could impact SSS crystallisation.

- **Span 60 or low MG concentrations co-crystallised with SSS.**

MG and Span 60 were widely studied with regards to their effect on the thermal behaviour of tristearin (Johansson and Bergenstahl, 1992a, Garti *et al.*, 1986, Lee and De Man, 1984), but few studies were reported on the network formation. Lower MG concentrations (i.e. 0.5 and 1 %) and all Span 60 concentrations used in this study have exhibited slight increase in SSS crystallisation temperature only. Moreover the melting temperature decreased in their presence revealing lower crystal stability thus polymorphic transitions hindering. It has also been shown an increase in the elastic modulus in their presence compared to 10 % SSS in sunflower oil due to better crystal sintering. As a result, Span 60 and low MG concentrations influenced TAG packing by creating impurities in the crystal lattice that would hinder polymorphic transitions, but also favour the crystal sintering with the addition of polar bonds in the fat network; those characteristics have been attributed to the co-crystallisation between SSS and those emulsifiers at specific concentrations.

- **Higher MG crystallised with SSS as a mixed-compound**

This can be attributed to earlier MG crystallisation compared to 10 % SSS when the concentration reaches 2 %; SSS crystallised at higher temperature as displayed by the thermal

behaviour and the evolution of SFC. Thus higher MG concentrations promoted secondary nucleation with the formation of seed crystals in the bulk allowing SSS crystallisation at the MG crystal interface. G' has also presented an increase at higher temperatures at both cooling rates of 1 and 10 °C/min with the possibility to achieve higher final G' than 12 % SSS. Therefore the crystal sintering has been even stronger and this could be due to the addition of glycerol-glycerol interactions.

6.2 THE EFFECT OF SHEAR DURING SSS CRYSTALLISATION WITH EMULSIFIERS

The effect of shear is a recent field of investigation, and the focus has been given mostly to its effect on polymorphic transitions and nanostructure of crystals in order to prevent blooming in chocolate (Shi and Maleky, 2015, Campos and Marangoni, 2014, Svanberg *et al.*, 2013, Acevedo *et al.*, 2012a, Mazzanti *et al.*, 2011). Little interest has been given to shear in the presence of emulsifiers at the macroscale.

- **Shearing until the beginning of SSS crystallisation increased the network strength**

Shearing until the nucleation of 10 % SSS was used to break down the first crystals created by inducing an increase in temperature which re-melted the crystals, thus promoting more nucleation sites. This has revealed an increase in the final G' in the range of shear rates used in this study and the generation of smaller crystals more dispersed in the bulk.

- **Shearing until the end of the network formation promoted a weak network**

The microstructure of 10 % SSS at the end of crystallisation has showed the presence of spherical and dense crystals in sunflower oil. Moreover the final G' decreased of an order of magnitude and would exhibit weak interactions between spherical crystals.

- **The carbon chain compatibility of emulsifiers with SSS determined the strength of the network when applying shear**

Shearing upon cooling until the temperature of emulsifier aggregation or until SSS crystallisation has shown an impact on the final G'; shearing with sorbitol monopalmitate has presented an increase in G' while a decrease in G' was shown with sorbitol monostearate. Therefore those two trends could be attributed to the better ability of sorbitol monostearate to aggregate when applying shear as the carbon chain is longer. As a consequence, the emulsifier was less dispersed in the bulk, as better aggregated, and promoted lower foreign interface for SSS crystallisation; shear has increased the emulsifier-emulsifier interactions thus decreasing the possibility of interacting with SSS. Shearing in the presence of emulsifiers with shorter carbon chain length would prevent their aggregation, dispersing them better in the bulk, favouring their interaction with SSS and increasing the strength of the network.

However the final properties of the network when applying shear until the end of SSS crystallisation was dominated by the shear rate over the presence of emulsifiers; the microstructure and the evolution of G' has shown the same trend as in the absence of emulsifiers.

6.3 FAT SYSTEM PROPERTIES AFTER SEEDING TRISTEARIN WITH WAX

Previously, wax have been used as a replacer of saturated fat and their ability to create an organogel and structure the oil has been investigated to a large extent (Martini *et al.*, 2015, Hwang *et al.*, 2014, Blake *et al.*, 2014, Hwang *et al.*, 2013, Mellema, 2009, Attama and Mueller-Goymann, 2008). Therefore, this thesis had for objective to understand how wax could be used in order to tailor tristearin crystallisation as foreign crystals in the system.

- **Waxes decreased the induction time for SSS crystallisation**

By both crystallising 20 °C above SSS, CW and SW have shown an increase in the crystallisation temperature of SSS and CW was the most efficient. These waxes differed by their multi-component (CW) and single-component (SW) nature and CW has been the most efficient to reduce SSS induction time.

- **The addition of SW increased the network strength**

The network strength has been investigated in terms of hardness by cone penetrometry and the addition of SW has exhibited the highest increase in hardness compared to the addition of the same amount of SSS (10 % SSS with 0.5, 1 or 2 % SW and 10.5, 11 or 12 % SSS). CW showed minor influence on hardness but 2 % CW has shown a significant increase. As a consequence, it has been presented that SW interacts with SSS and its seeding effect generated stronger network. However CW did not seem to interact with SSS thus very slight differences on the network formation; this demonstrated that CW could be used as a foreign surface to promote primary heterogeneous nucleation while SW could interact with SSS and change the strength of the fat network thus promoting secondary nucleation.

- **Varying the cooling rate in the range of wax crystallisation influenced SSS crystallisation**

When increasing the cooling rate in the temperature range of wax crystallisation, it has been shown more dispersed crystals in the bulk with smaller sizes and significant increase in hardness especially with SW. Therefore changing the cooling rate has resulted in modifying nucleation by increasing the number of nuclei. Furthermore SW had higher effect and this would be due to first, its single-component nature that enhanced the crystal sintering attributed to its needle-like microstructure, but also to its interaction with SSS.

6.4 FUTURE RECOMMENDATIONS

- **Improving the understanding of additive-SSS interactions and their effect on the polymorphs formed**

This study has used DSC techniques in order to compare the crystal stabilities and the presence of polymorphic transitions when glycerol monostearate, Span 60 or Tween 60 were added to SSS. Even though SSS polymorphs formed in the presence of these emulsifiers been widely studied by using X-ray analysis (Kalnin *et al.*, 2004, Garti and Zour, 1997, Elisabettini *et al.*, 1996, Shelef and Garti, 1988), it could be interesting to gain more knowledge on the crystal structure when changing the process by shearing or changing the cooling rate and by adding waxes. Combined with DSC, X-ray has been revealed to be a very good tool to have a better understanding of TAG arrangement in the crystal lattice. As such, the different behaviours observed between CW and SW could be better determined at the molecular scale.

- **Analysing crystallisation kinetics and induction time**

This study has determined the effect of formulation and process mostly on the thermal behaviour, microstructure and fat network strength. However to complete this study it could be relevant to investigate their effect on the induction time, crystallisation rate and crystal growth. A method described previously (*2.1.5 Crystal Growth*) is the use of the Avrami model and the application of isothermal crystallisation. Therefore crystallisation rate and crystal growth could be determined by the parameter *k* and *n* respectively. The induction time for crystallisation could be analysed by using light scattering or polarised light microscopy with temperature controlled system (Cerdeira *et al.*, 2004, Toro-Vazquez *et al.*, 2002, Wright *et al.*, 2000b, Herrera *et al.*, 1998).

- **Understanding the strength of fat network**

The strength of the fat network has been measured by low and large deformations and the elastic modulus and hardness were analysed respectively. However those parameters only allowed the determination of the network strength but did not fully explained the differences in structure. As a result a technique used recently like the study of the oil binding capacity of the system could be pertinent to better understand the ability of crystals to entrap the oil in the network and improve the final texture

- **Measuring the crystal size**

The diffusing light scattering by using the Mastersizer allows the determination of spherical particles flowing in liquid oil; this implies the creation of shear that could release heat, break-down the crystals and shape them according to the flow. In addition, the crystal shape is rarely spherical and rather dendritic and unshaped. Therefore this crystal size

measurement was interesting as a comparative study between samples but did not allow accurate determination of the crystal size.

A new way of measuring crystal size is the use of cryo-TEM (Maleky *et al.*, 2012, Acevedo *et al.*, 2012b, Acevedo *et al.*, 2012a); this results in freezing the crystals in a solvent and measuring the nano and meso-crystals by tomography.

- **Decreasing the concentration of emulsifier by optimising the nucleation process**

Finally this study has shown the possibility to promote heterogeneous nucleation by adding emulsifiers which did not crystallise in the concentrations used or by adding waxes that did not interact with SSS. The optimization of the interaction between SSS and emulsifiers has been developed by using emulsifiers with lower carbon chain and shearing upon cooling until specific stages of SSS crystallisation.

Another way of optimizing the nucleation process could be the use of sonication which adds bubbles as a foreign interface for crystallisation and would promote crystallisation at higher temperature (Liu *et al.*, 2015, Ban *et al.*, 2014). Furthermore sonication could be combined to the presence of emulsifiers and increase the interaction between SSS and the emulsifiers in order to develop a stronger network.

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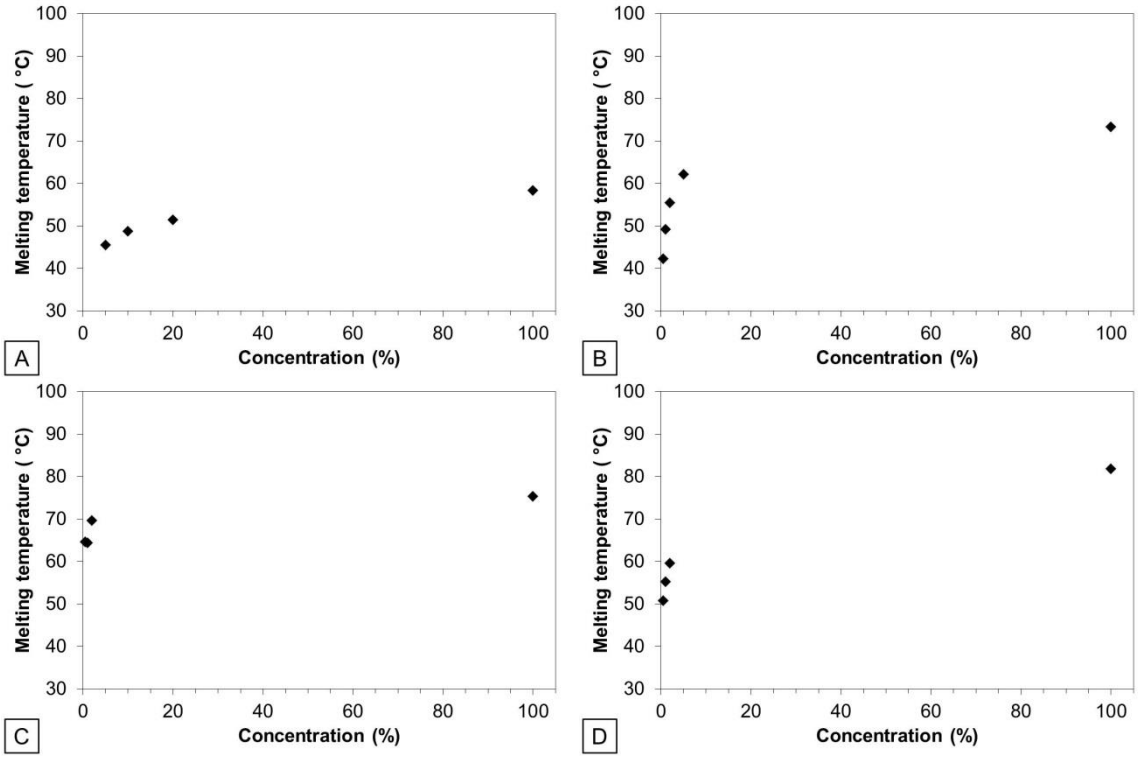
APPENDIX: Solubility



Solubility of SSS (A), MG (B), CW (C) and SW (D).