Crystallization of fat in and outside milk fat globules
-Effect of processing and storage conditions

PhD thesis by
Patrizia Buldo
November 2012

Department of Food Science
Faculty of Science and Technology
Aarhus University
Main supervisor
Associate professor Lars Wiking

Assessment committee
“Considerate la vostra semenza: fatti non foste a viver come bruti, ma per seguir virtute e canoscenza"

Dante Alighieri, Inferno- Canto XXVI (vv. 112-120)
I. Preface

This PhD thesis is the results of a PhD study performed from November 2009 to November 2012 at The Department of Food Science, Aarhus University (Denmark). The project was financed by Arla Foods, The Ministry of Food, Agriculture and Fisheries (grant no. 3414-09-02406), The Danish Dairy Research Foundation, and FOOD Denmark. The PhD project was part of the research project “Fat Structure – Innovation of new types of butter and spreads” which is in collaboration with Arla Foods’ Strategic Innovation Centre and The Department of Food Science at Copenhagen University.

During the PhD study, a 3-months’ study abroad was conducted at The Department of Food Process Engineering and Applied Science, Dalhousie University (Halifax, Canada), with Professor Gianfranco Mazzanti, and one month was spent at The Department of Food Science, Copenhagen University.

Patrizia Buldo

Viborg, November 2012
## II. Table of contents

I. Preface ...................................................................................................................................................... 4

II. Table of contents......................................................................................................................................... 5

III. Abstract...................................................................................................................................................... 8

IV. Sammendrag (Abstract in Danish) ........................................................................................................... 10

V. List of papers ............................................................................................................................................ 12

VI. List of abbreviations and symbols .......................................................................................................... 13

VII. Background and aim ................................................................................................................................. 14

1. Introduction ............................................................................................................................................. 16

1.1 Milk Fat .................................................................................................................................................. 16

1.1.1 Lipid structure of milk fat.................................................................................................................. 16

1.1.2 Lipid composition of milk fat .......................................................................................................... 17

1.1.3 Lipid synthesis of milk fat ............................................................................................................... 19

1.2 Fat crystallization ................................................................................................................................... 20

1.2.1 Crystallization: nucleation and crystal growth .................................................................................. 20

1.2.2 Polymorphism of fat ....................................................................................................................... 22

1.2.3 Crystallization and polymorphism of milk fat ................................................................................. 25

1.2.4 Milk fat crystallization under processing conditions ........................................................................ 28

1.2.5 Effect of vegetable oil and minor components on crystallization of milk fat ................................. 29

1.2.6 Fractions of milk fat......................................................................................................................... 30

1.3 Physical destabilization of dairy emulsions............................................................................................ 31
1.3.1 Partial coalescence phenomenon in dairy products ................................................................. 32
1.4 Butter and butter blend manufacturing ......................................................................................... 33
  1.4.1 Thermal treatment of the cream ............................................................................................. 34
  1.4.2 Churning process of the cream ............................................................................................. 35
  1.4.3 Butter blends manufacturing ................................................................................................. 35
1.5 Microstructure of fat crystal network .......................................................................................... 36
  1.5.1 Microstructure of butter and butter blends ........................................................................... 36

2. Methods used for the study of crystallization, microstructure and textural properties of milk-fat systems ......................................................................................................................................................................................... 38
  2.1 Rheological measurements ......................................................................................................... 38
    2.1.1 Small deformation techniques- Oscillation tests ................................................................. 39
    2.1.2 Large deformation techniques ............................................................................................. 40
  2.2 Thermal analysis- Differential Scanning Calorimetry ............................................................... 41
  2.3 Solid fat content determination- Pulsed Nuclear Magnetic Resonance spectroscopy .......... 41
  2.4 Particle size measurements- Mastersizer ................................................................................ 42
  2.5 Polymorphisms and lamella stacking detection- x-ray diffraction ......................................... 43
  2.6 Microstructure analysis- Confocal Laser Scanning Microscopy ............................................. 43
  2.7 Multivariate data analysis- PCA and PLS ............................................................................... 45

3. Summary of included papers and manuscripts ........................................................................... 49
  3.1 Crystallization mechanisms in cream during ripening and initial butter churning - Paper I .... 49
  3.2 Effect of globular fat on crystallization and rheological properties of milk fat based emulsions - Paper II .................................................................................................................................................................................. 50
  3.3 Microstructure and textural properties of milk fat systems during temperature fluctuations - Paper III .................................................................................................................................................................................. 51
3.4 The Role of Mixing Temperature on Microstructure and Rheological Properties of Butter Blends - Paper IV

3.5 Multivariate data analysis for finding the relevant fatty acids contributing to the melting fractions of cream - Paper V

4. General Discussion

4.1 Effect of processing conditions and of addition of vegetable oil on crystallization and on rheological properties of butter and butter blends (Paper I; IV)

4.1.1 Effect of ripening time and churning of cream on crystallization and partial coalescence

4.1.2 Effect of mixing temperature and of addition of vegetable oil on microstructure and rheological properties of butter blends

4.2 Characterization of microstructure and textural properties of milk fat systems (Paper II; III)

4.2.1 Rheological techniques to identify the textural descriptors of milk fat systems

4.2.2 Microstructure elements responsible for the textural properties of milk fat systems

4.3 Crystallization of milk fat systems during storage and handling - effect on microstructure and on textural properties (Paper II; III)

4.3.1 Crystallization and microstructure formation in milk fat systems

4.3.2 Effect of storage time on microstructure and on textural properties of milk fat systems

4.3.3 Microstructure and textural changes occurring during handling of milk fat systems (Paper III)

4.4 Identification of the fatty acids responsible of the melting fractions of cream (Paper V)

5. Conclusions and Future Perspectives

6. References

Acknowledgments/ Agradecimentos/ Ringraziamenti
III. Abstract

The textural properties and the physical stability of butter and butter blends are affected by the
crystallization of milk fat occurring in and outside the milk fat globules. Knowledge on milk fat
crystallization during butter and butter blends manufacturing, thus on microstructure formation in
presence of milk fat globules, is fundamental to improve product quality, and to optimize production
processes.

In this PhD study crystallization mechanisms, microstructure characteristics and textural properties of
milk fat systems, have been studied during production process, storage time and temperature
fluctuations. The focus was on ripening and churning time, addition of vegetable oil, mixing
temperature, ratio of intact milk fat globules to anhydrous milk fat (AMF), and different emulsion
types.

Primary crystallization was studied by differential scanning calorimetry (DSC), pulsed nuclear
magnetic resonance (p-NMR) and x-ray diffraction. Secondary crystallization was studied by small
oscillation rheology. Partial coalescence of fat globules and butter grains aggregation occurring during
ripening and churning of the cream were studied by static light scattering and viscosity measurements.
The microstructure was studied by confocal laser scanning microscopy (CLSM), which images were
linked to the textural properties obtained by large deformation rheology.

At 10°C, ripening time longer than one hour did not affect the churning time, thus the time of butter
grains aggregation. The viscosity measurements performed with a rheometer cell were able to detect
the phase inversion of the emulsion. During churning the transition from $\alpha$ to $\beta'$ form was accelerated.
Yet, partial coalescence of milk fat globules was independent from the crystal polymorphism.

The crystallization onset temperature was delayed in presence of milk fat globules in the system, i.e.
butter grains. The study of the microstructure in milk fat systems showed that in butter grains and in
emulsions made of butter grains and AMF, microstructure stabilization occurred after one day of
storage, whereas in AMF system, it occurred after 14 days. This was probably due to a critical SFC in
the continuous phase that needed to stabilize the microstructure. Moreover, harder systems were
obtained by adding AMF to butter grains.
The microstructure of the system was the main factors influencing the textural properties of milk fat systems, whereas, the level of SFC, within the limits of 46-52%, did not contribute to the textural characteristics. In addition, this PhD study demonstrated that more accurate structural information is obtained by using several rheological techniques and correlating the results to microscopy images.

The presence of a crystal network within the fat globules did not contribute to the hardness and stiffness of the system. However, the elasticity of the system was strongly influenced by the presence of fat globules, as they conferred high deformation to the structure. On the contrary, the presence of a crystal network in the continuous phase, with or without fat globules in the system, such as butter and AMF, respectively, strongly contributed to the hardness and stiffness of the system. However, those systems were characterized by low structure deformation. Brittleness increased by increasing the volume fraction of fat crystals in the continuous phase, whereas it decreased by increasing the amount of fat globules.

Temperature fluctuations (from 5 to 25°C, and back to 5°C) did not affect the textural properties of emulsion systems made of intact fat globules. On the contrary, when both intact fat globules and bulk fat were present in the system, like in butter, an increase in hardness, stiffness, brittleness, and a decrease in elasticity followed temperature fluctuations. This was caused by flocculation of fat globules which led to a denser and stronger network. Similar behavior was observed in AMF system, however, in this system, the changes in textural properties during temperature fluctuations were minor and they were caused by formation of smaller spherulites and by a finer network.

The addition of rapeseed oil to butter and the high mixing temperature applied during butter blend manufacturing decreased the stiffness of the systems by solubilizing some of the triacylglycerols (TAG) present in the network, and further inhibiting the ability to rebuild the rigidity of the crystal network during subsequent storage.

The insights obtained during this PhD study demonstrated the effect of milk fat globules on crystallization mechanism, microstructure formation and textural properties of milk fat systems. The obtained results give new suggestions and approaches to improve quality of milk fat-based products and to optimize the production process.
IV. Sammendrag (Abstract in Danish)

Teksturegenskaber og den fysiske stabilitet af smør og blandingsprodukter påvirkes af mælkefedtkrystallisation i og uden for mælkefedtkugler. Viden om mælkefedtkrystallisation og mikrostruktur under fremstillingen af smør er afgørende for at forbedre produkternes kvalitet, og for at optimere produktionsprocesserne.

I dette PhD. studie er krystallisering mekanismer, mikrostruktur karakteristika og teskturegenskaber af mælkefedtsystemer blevet undersøgt under produktionsprocesser, gennem lagrinstid og ved temperatursvingninger. Fokus har været på flødens temperatursbehandling og kæringstid samt på tilsætning af vegetabilsk olie og dennes iblandingstemperatur. Ligeledes er påvirkningen fra forholdet mellem intakte mælkefedtkugler og vandfrit mælkefedt (AMF) i forskellige emulsion typer blevet undersøgt.


En modning af fløden på over en time havde ingen yderlige effekt på smørkorn dannelse og dermed kærnetid. Viskositetsmålingerne udført med et rheometer var i stand til at detektere fasevendingen i fløden. Kæring fremskynder overgangen fra $\alpha$ til $\beta'$ krystaller, men sammensmeltningen af mælkefedtkugler var uafhængig af krystal polymorfi.

Effekten af mælkefedtkugler på krystallisationen blev undersøgt i systemer, hvor smørkorn blev blandet i AMF. Krystallisationen af mælkefedt skete ved lavere temperatur i systemerne med tilstedevarsel af fedtkugler sammenlignet med AMF. Mikrostrukturen for smørkorn og systemet, hvor disse var blandet med AMF system stabiliserer sig i løbet af den det første døgn, mens det tog 14 dage i AMF før stabiliseringen fremkom. SFC øgedes også indtil dag 14, hvilket forklarer udviklingen i det krystallinske netværk. Hårdere systemer blev opnået ved tilsætning af AMF til smørkorn. Mikrostrukturen var den vigtigste faktor, der påvirked teksturen af mælkefedtsystemerne, mens
ændringer i SFC, i intervallet 46-52%, ikke bidrager til ændringer i tekturen. Derudover viste dette PhD. studie, at mest strukturel information opnås ved at anvende adskillige rheologiske teknikker og relaterer resultaterne til mikroskopibilleder.


Emulsionssystemer fremstillet af intakte mælkefedtkugler ændrede ikke teksturegenskaber ved temperatursvingninger (fra 5 til 25 °C og igen til 5 °C) gennem lagringen. Derimod øges hårdhed, stivhed og sprødhed af temperatursvingninger, når der er en kontinuerlig fedtfase i systemet, som i smør. Dette var blandt andet forårsaget af flokkulering af fedtkugler, der førte til et tættere og stærkere netværk uden om fedtkuglerne. Tilsvarende effekt blev fundet ved AMF, her var ændringerne dog mindre og skyldes at der blev dannet et finere og tættere krystallinsk netværk.


V. List of papers


VI. List of abbreviations and symbols

AMF  Anhydrous Milk Fat
CLSM  Confocal Laser Scanning Microscopy
DSC  Differential Scanning Calorimetry
FA  Fatty Acid
HMF  High Melting Fraction
LMF  Low Melting Fraction
LVR  Linear Viscoelastic Region
MFGM  Milk Fat Globule Membrane
MMF  Medium Melting Fraction
O/W  Oil in Water
PCA  Principal Component Analysis
PLS  Partial Least Squares
p-NMR  Pulsed Nuclear Magnetic Resonance
SFC  Solid Fat Content
TAG  Triacylglycerol
W/O  Water in Oil
G'  Elastic modulus
G''  Viscous modulus
G*  Complex modulus
γ  Strain
τ  Shear stress
ω  Frequency
VII. Background and aim

Butter has a unique flavor which makes it a valuable component in many food products. However, there is an increase in demand from consumers for healthy fat-based products, with a low level of saturated fatty acids, which are known to increase the risk of cardiovascular disease (Astrup et al., 2011). This can be achieved by blending milk fat with vegetable oils, so-called butter blends. Moreover, from an industrial point of view, production of butter blends is beneficial, as some vegetable oils, such as rapeseed oil or canola oil, are less expensive and more easily accessible than milk fat.

Butter and butter blends are plastic fats made of liquid and crystallized fat, with a very complex microstructure. The microstructure of butter and butter blends is formed by a continuous crystal network made of partially coalesced fat globules dispersed in liquid oil, and interrupted by intact fat globules, water and air droplets (Juriaanse & Heertje, 1988). The physical and chemical factors influencing milk fat crystallization are fatty acid composition, addition of vegetable oil, presence of milk fat globules, cooling rate, crystallization temperature and shear force. These regulate the formation, morphology and size distribution of crystals, thus the microstructure of milk fat-based products (ten Grotenhuis, 1999; Herrera & Hartel, 2000a; Campos et al., 2002; Lopez et al., 2002a; Martini et al., 2002a; Wright et al., 2005; Wiking et al., 2009a; Fredrick et al., 2011). Product microstructure is closely related to product quality as it influences its macroscopic properties, such as texture (Herrera & Hartel, 2000b; Herrera & Hartel, 2000c; Campos et al., 2002). These concepts are schematically illustrated in Figure 1.

The current knowledge of how physical and chemical factors affect crystallization and microstructure formation is limited, especially when the milk fat is present in both a continuous and an emulsified state, like in butter and butter blends (Lopez et al., 2001a; Lopez et al., 2002a; Fedotova & Lencki, 2010; Fredrick et al., 2011). It is important to improve the understanding of crystallization dynamics of milk fat in presence of emulsified milk fat, as milk fat is present in food as both dispersed and continuous phase.
The hypothesis of this study was that textural properties and physical destabilisation of milk fat-based products are due to crystal characteristics and microstructure (Figure 1). The overall aim of this PhD project is to provide insight in the crystallization mechanisms and their effect on physical, microstructure and textural properties of butter and butter blends. The focus is on fatty acid composition, presence of milk fat globules, addition of rapeseed oil and processing conditions such as ripening and churning of the cream, shear forces and mixing temperature of butter blends manufacturing. Moreover, storage time and temperature fluctuations stress of milk fat systems were studied.

A better understanding of the crystallization process and the microstructure changes in bulk and in emulsified state under process conditions and during handling and storage will improve quality of milk fat-based products, and it will contribute to the optimization of the production process.
1. Introduction

Milk lipids have the main responsibility for the textural properties of fat-based dairy products. The microstructure and textural properties of milk fat-based products are influenced by the crystallization of milk fat. Milk fat crystallization is affected by chemical and physical parameters, such as the fatty acid (FA) composition, presence of milk fat globules, temperature conditions and shear forces. The quality of the raw material and the parameters used during product manufacturing are fundamental for the crystallization process, thus for the final quality of the product.

1.1 Milk Fat

1.1.1 Lipid structure of milk fat

Milk fat is one of the most complex natural fats. It has a unique flavor and nutritional properties. The fat content in cow’s milk is in the range of 3 to 6% (MacGibbon & Taylor, 2006). Bovine milk fats are present in the form of spherical globules, the size of which is around 4 µm (Mulder & Walstra, 1974). Milk fat globules are composed by a triacylglycerol (TAG) core surrounded by a complex membrane, the milk fat globule membrane (MFGM). MFGM accounts for 2-6% of the total mass of the fat globule. Approximately 90% of the MFGM is composed by polar lipids and proteins, whereas the remaining part includes glycoproteins, cholesterol, enzymes and other bioactive components (Figure 2a) (Keenan & Mather, 2006; Dewettinck et al., 2008). The polar lipid of the MFGM includes: glycerophospholipids, such as phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine and phosphatidylinositol; and sphingolipids, such as sphingomyelin (Rombaut et al., 2007; Sanchez-Juanes et al, 2009). The main proteins found in the MFGM are: butyrophilin; mucin (MUC1, MUC15); xanthine oxidase/dehydrogenase; cluster of differentiation 36 (CD36); adipophilin; periodic acid Schiff glycoprotein (PAS 6/7, PAS III) and fatty acid-binding protein (Keenan & Mather, 2006; Dewettinck et al., 2008).

MFGM has a trilayer structure which thickness is in the range of 10-20 nm. The inner layer, which covers the TAGs core of the milk fat globule, is a monolayer composed of protein and polar lipids originating from the endoplasmatic reticulum, whereas the outer membrane is a bilayer membrane composed of polar lipids from the apical plasma membrane of the mammary epithelial cells (Figure 2a).
(Heid & Keenan, 2005). Recent studies suggest a new model for the lateral organization of the MFGM, which shows sphingomyelin in the rigid liquid-ordered domain surrounded by the liquid-disordered domain, which consists of a matrix of liquid glycerophospholipids (Figure 2) (Lopez et al. 2010; Lopez et al. 2011). The composition and structure of the MFGM can be altered by mechanical and thermal factors (Evers, 2004).

![Figure 2: Milk fat globule membrane. a) Schematic representation of the trilayer structure and composition of the milk fat globule membrane. b) Schematic three-dimensional representation of the organization of the polar lipid in the milk fat globule membrane. Images are not to scale. Adapted from Lopez et al., (2010) and Lopez et al., (2011). Reprint with permission of Elsevier Limited.](image)

1.1.2 Lipid composition of milk fat

The core of the milk fat globule is mainly composed of TAGs which account for 98.3% (w/w) of the total composition; however, the remaining 1.7% includes several other components (Walstra & Jenness, 1984). An overview of the main classes of milk fat is reported in Table 1.
Table 1: Main classes of lipids in milk. Adapted from Walstra & Jenness (1984)

<table>
<thead>
<tr>
<th>Lipid class</th>
<th>Amount (%, w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triacylglycerols</td>
<td>98.3</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>0.8</td>
</tr>
<tr>
<td>Diacylglycerols</td>
<td>0.3</td>
</tr>
<tr>
<td>Sterols</td>
<td>0.3</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>0.1</td>
</tr>
<tr>
<td>Monoacylglycerols</td>
<td>0.03</td>
</tr>
<tr>
<td>Others*</td>
<td>trace</td>
</tr>
</tbody>
</table>

*It includes: carotenoids, fat-soluble vitamins and flavour compounds

Approximately 400 fatty acids have been identified in milk, but only 14 of these FAs are present in amounts higher than 1% (Table 2) (Kaylegian & Lindsay, 1995). The predominant (70-75%) FAs present in milk are saturated FAs, with a carbon chain length varying from 4 to 18. The most abundant saturated FAs are palmitic acid (C16:0), stearic acid (C18:0) and myristic acid (C14:0). Among the unsaturated FAs, the most abundant are the cis-monoenoic acids, such as oleic acid (C18:1 cis9) and palmitoleic acid (C16:1 cis9), whereas the most abundant trans FAs are the C18:1 trans 11.

Table 2: Composition of the major fatty acids in milk fat. Adapted from Kaylegian & Lindsay (1995)

<table>
<thead>
<tr>
<th>Fatty acid Carbon number</th>
<th>Fatty acid common name</th>
<th>Average range (%, w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:0</td>
<td>Butyric</td>
<td>2-5</td>
</tr>
<tr>
<td>6:0</td>
<td>Caproic</td>
<td>1-5</td>
</tr>
<tr>
<td>8:0</td>
<td>Caprylic</td>
<td>1-3</td>
</tr>
<tr>
<td>10:0</td>
<td>Capric</td>
<td>2-4</td>
</tr>
<tr>
<td>12:0</td>
<td>Lauric</td>
<td>2-5</td>
</tr>
<tr>
<td>14:0</td>
<td>Myristic</td>
<td>8-14</td>
</tr>
<tr>
<td>15:0</td>
<td>Pentadecanoic</td>
<td>1-2</td>
</tr>
<tr>
<td>16:0</td>
<td>Palmitic</td>
<td>22-35</td>
</tr>
<tr>
<td>16:1</td>
<td>Palmitoleic</td>
<td>1-3</td>
</tr>
<tr>
<td>17:0</td>
<td>Margaric</td>
<td>0.5-1.5</td>
</tr>
<tr>
<td>18:0</td>
<td>Stearic</td>
<td>9-14</td>
</tr>
<tr>
<td>18:1</td>
<td>Oleic</td>
<td>20-30</td>
</tr>
<tr>
<td>18:2(^1)</td>
<td>Linoleic</td>
<td>1-3</td>
</tr>
<tr>
<td>18:3</td>
<td>Linolenic</td>
<td>0.5-2</td>
</tr>
</tbody>
</table>

\(^1\) Contains also about 3% of trans isomers (Jensen & Newberg, 1995)
In general, long chain FAs are almost entirely esterified at the *sn*-1 and *sn*-2 position of milk TAG, whereas the short chain FAs are mainly esterified at the *sn*-3. Medium chain FAs are mainly esterified at *sn*-2 (Parodi, 1979; Gresti et al., 1993; Jensen & Newberg, 1995; Kaylegian & Lindsay, 1995). An overview of the stereo-location of the milk FA in the TAG molecules is reported in Table 3.

**Table 3: Stereo-location of milk fatty acids in triacylglycerol molecule.** Adapted from Kaylegian & Lindsay (1995) and Parodi (1979)

<table>
<thead>
<tr>
<th>Fatty acid Carbon number</th>
<th>Sn-1 (mol %)</th>
<th>Sn-2 (mol %)</th>
<th>Sn-3 (mol %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:0</td>
<td></td>
<td></td>
<td>98.1</td>
</tr>
<tr>
<td>6:0</td>
<td></td>
<td></td>
<td>93.2</td>
</tr>
<tr>
<td>8:0</td>
<td></td>
<td>43.5</td>
<td>52.2</td>
</tr>
<tr>
<td>10:0</td>
<td></td>
<td>51.4</td>
<td></td>
</tr>
<tr>
<td>12:0</td>
<td></td>
<td>59.8</td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td></td>
<td>62.2</td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>44.4</td>
<td>43.1</td>
<td></td>
</tr>
<tr>
<td>18:0</td>
<td>56.2</td>
<td></td>
<td>27.8</td>
</tr>
<tr>
<td>18:1</td>
<td>59.3</td>
<td></td>
<td>41.3</td>
</tr>
</tbody>
</table>

The most abundant TAGs in milk fat are those with a carbon number from 34 to 40 and from 48 to 52. They are C16-based and include C4-C16-C18:1, C4-C16-C16, C4-C14-C16, C14-C16-C18:1 and C16-C16-C18:1 (Gresti et al., 1993; van Aken et al., 1999). Fatty acid composition and the fat content of the milk fat globule can vary considerably between breeds of the cow, feeding regimes, seasons, stages of lactation and genetic variations. The FA composition and their position in the TAG molecule influence the crystallization behavior of milk fat, and thus the physiochemical properties and functionality of milk fat-based products. Moreover, the configuration of the double bond in the FA molecule is also important for the physiochemical properties of milk fat products. *Trans* configuration results in a more linear and rigid molecule with a higher melting point than the *cis* configuration, thus *trans* FAs are more similar to saturated FA than unsaturated.

1.1.3 Lipid synthesis of milk fat

Two main FA synthesis pathways are distinguished. *De novo* synthesis, which occurs inside the mammary epithelial cells, synthesize short and medium chain saturated fatty acids (C4 to C14) and part of C16:0. The precursors for the synthesis of these FAs are acetate and β-hydroxy butyrate, which are
products of the fermentation of carbohydrates in the rumen. The plasma lipid is the other pathway of milk FAs synthesis, and it includes long chain FAs (≥ C18 and some C16) which derive from the feed or from body fat mobilization. The precursors for the plasma lipid synthesis are TAGs rich in chylomicron and very low density lipoproteins (VLDL).

Unsaturated FAs from the diet are subjected to biohydrogenation by the microorganisms in the rumen, resulting mainly in stearic acid (C18:0) and small amounts of monounsaturated C18 FA (Harfoot & Hazlewood, 1988; Jenkins, 1993). However, incomplete biohydrogenation can occur in the rumen (Bauman et al., 1998). Part of the palmitic and stearic acids (C16:0 and C18:0) from the feed passes through the rumen unchanged, whereas part of C18:0 is converted to C18:1 by stearoyl-CoA desaturase in the mammary tissue (Grummer, 1991).

Several studies have focused on the effect of cow nutrition on milk FA composition, either as seasonal variation (i.e. pasture vs. indoor) or as diet variation and fat supplements (Wrenn et al., 1976; Grummer, 1991; Palmquist et al., 1993; Bayourthe et al., 2000; Chilliard et al., 2000; Baer et al., 2001; Couvreur et al., 2006; Havemose et al., 2006; Warntjes et al., 2008; Larsen et al., 2012). In general, a diet high in long chain FAs results in a higher concentration of C18:0 and C18:1 and in a decrease of de novo-synthesized FA (Grummer, 1991; Palmquist et al., 1993). Moreover, feeding of rumen-protected lipids increases the amount of polyunsaturated FAs in milk fat, mainly linoleic acid (C18:2) (Wrenn et al., 1976).

1.2 Fat crystallization

1.2.1 Crystallization: nucleation and crystal growth

Proper control of fat crystallization during food manufacturing leads to products with the desired physiochemical properties. Crystallization is a supramolecular process where randomly organized molecules in a fluid come together and form an ordered three-dimensional molecular structure: a crystal (Davey & Garside, 2000). The steps leading to crystallization are nucleation and crystal growth. Nucleation occurs either by supercooling the system or in a supersaturated system (Davey & Garside, 2000). In TAGs, three types of nucleation can take place. In the absence of catalytic impurities, such as solid interfaces or foreign particles, fat molecules undergo homogeneous nucleation if they are
sufficiently supercooled, whereas, heterogeneous nucleation takes place in systems that contain catalytic impurities, and it requires lower supercooling than homogenous nucleation (Mulder & Walstra, 1974). Heterogeneous and homogeneous nucleation mechanisms are referred to as primary nucleation, whereas if nucleation occurs in the presence of other crystals in the system, it is referred to as secondary nucleation (Walstra, 1998). Nucleation is favored when the change of free energy in the system, \( \Delta G \), is negative. The Gibbs equation (Equation 1) and Figure 3 better explain this concept.

\[
\Delta G = \Delta H - T\Delta S
\]  

(Equation 1)

where \( \Delta H \) is the change in enthalpy of the system, \( T \) is the temperature, and \( \Delta S \) is the change in entropy. Positive changes in enthalpy correspond to an endothermic process (melting), whereas negative changes correspond to an exothermic process (crystallization).

**Figure 3: Free Energy (\( \Delta G \)) diagram.** \( \Delta G \) changes as function of cluster size (radius) and supersaturation. Low supersaturation curve (a) and high supersaturation curve (b), and critical radius of the cluster (r*). Adapted from Davey & Garside (2000).

In Figure 3, the value of \( \Delta G \) indicates the energy barrier that needs to be overcome to form a stable nucleus when the liquid is below its melting temperature. In order for a nucleus to exist and consequently grow, its size must be above a critical radius (r*) where the \( \Delta G \) is lowered (Figure 3). The value of \( \Delta G \) and r* depends on the driving forces of nucleation, e.g. by increasing the saturation or the cooling rate (curve b in Figure 3), \( \Delta G \) and r* decrease (Davey & Garside, 2000).
Growth of crystal nuclei occurs by annexation of molecules on already existing crystals. As for the nucleation, crystal growth increases with supersaturation and supercooling. By increasing the viscosity of the melt, the growth rate is reduced as the diffusion of the molecules and the mass transfer of the latent heat are delayed. Both, the rate of nucleation and growth characterize the initial crystal size distribution. At high nucleation rates, many small crystals are formed, instead few large nuclei are formed at low rates (Campos et al., 2002). As more TAGs crystallize, they start to collide due to Brownian motion, and this leads to a decrease of the growth rate as interactions between crystals prevail (Himawan et al., 2006). Crystals in the network are held together by van der Waals forces (van den Tempel, 1961; Haighton, 1963). Post crystallization processes such as sintering, refers to the formation of strong solid bridges between crystals in the crystal networks, and secondary nucleation might occur (Johansson & Bergenståhl, 1995; Walstra, 1998). Thereafter, textural properties, such as hardness, stiffness and brittleness, are affected.

In presence of molecules with similar shape and configuration but different composition, mixed or compound crystals are formed (Mulder & Walstra, 1974; Walstra & van Beresteyn, 1975a; Walstra & van Beresteyn, 1975b; Fredrick et al., 2011). Compound crystals are characterized by a melting range, as change of temperature will cause melting of some TAGs and simultaneous crystallization of others TAGs (Fredrick et al., 2011). High cooling rate promotes formation of compound crystals as TAGs are forced into an unstable crystal structure (Martini et al., 2002a).

### 1.2.2 Polymorphism of fat

Crystal polymorphism refers to the ability of solid TAGs (Figure 4a) to form different crystalline structures. Most fats are characterized by three typical polymorphic forms: \( \alpha \), \( \beta' \) and \( \beta \); however, more polymorphic sub-forms have been identified. The spatial arrangement characteristics of the polymorphic forms are reported in Figure 4b. The polymorphic forms are described according to the crystal subcell structure, which characterizes the cross sectional packing modes of the zigzag hydrocarbon chain (Timms, 1984). The \( \alpha \) form corresponds to a hexagonal subcell structure (H), where the acyl chains do not form an angle of tilt; \( \beta' \) corresponds to an orthorhombic perpendicular subcell structure (O\( \perp \)), where the chains are perpendicular, and they form an angle of tilt between 50° and 70°;
β corresponds to a triclinic parallel subcell structure (Tı/) with the same angle of tilt as β’ but with a parallel chain orientation (Larsson, 1966; Himawan et al., 2006) (Figure 4b).

Figure 4: Triacylglycerol molecule (a); polymorphism (b) and lamella stacking (c) of fat crystals. Adapted from Sato, K., (1999).

The density, stability and melting point of the polymorphs increase from α to β (Timms, 1984). The α form is the least stable and can transform into β’ or β based on thermal treatment. The β’ form is metastable and is desirable in butter and margarines for its optimal crystal morphology and crystal network that in turn gives desired textural properties. The β form is the most stable and it is characterized by large and plate-like crystals, resulting in a poor crystal network and brittle structure in butter and margarine, although it is desirable for confectionary fat (Sato, 1999; Sato & Ueno, 2011). Based on the longitudinal stacking (thickness or d-spacing) of the acyl chain of TAGs molecules in lamellar structures, two configurations can be formed: 2L (double stacking) and 3L (triple stacking) (Figure 4c). The 2L is characterized by the acyl chain in the sn-2 position of the TAG at the side of the acyl chain of either sn-1 or sn-3 position, while in the 3L configurations, the acyl chain in sn-2 position is alone, and the acyl chain in the sn-1 and sn-3 position packs side by side (Figure 4c) (Himawan et al., 2006). The thickness of the lamellae depends on the length of the TAG and on the angle of tilt between the chain axis and the basal lamellar plane (Timms, 1984).

Both polymorphism and lamellar stacking of the crystals can be identified by measuring the short and long spacing, respectively, of x-ray diffraction patterns. Small angle scattering (SAXS; long spacing) gives information on the thickness of the TAG molecules, and the wide angle scattering (WAXS; short
spacing) gives information on the subcell structure or interchain distance. The α form is characterized by a short spacing line at 4.15 Å; the β’ form is characterized by two short spacing lines at 3.8 Å and 4.2 Å, and the β by a short spacing line at 4.6 Å among other peaks (Small & Hanahan, 1986; Larsson, 1994). The 2L and 3L stacking are characterized by short spacing chain length structures with a thickness of 40–50 Å and 55–75 Å, respectively. In general, TAGs with three saturated long chain FAs crystallize in 2L chain length packing, whereas 3L chain packing is obtained if TAGs contain FAs with different chain lengths and unsaturation.

Two types of polymorphism exist, enantiotropic and monotropic. Enantiotropic polymorphism is a reversible process and it is referred to a specific range of temperature and pressure where each polymorphic form exists in the most thermodynamically stable form (Aquilano & Sgualdino, 2001). Whereas, monotropic polymorphism is an irreversible process where one polymorphic form is always the most stable and it is formed by changing the surrounding parameters (temperature, pressure) (Aquilano & Sgualdino, 2001). In monotropic polymorphism, the most stable crystalline modifications are consecutively formed from α to β’ and then to β, when given sufficient time. Moreover, formation of a polymorph can also be melt-mediated, meaning that it can be formed directly from the melt solution (Himawan et al., 2006; Sato & Ueno, 2011). The polymorphic forms obtained when crystallization occurs from the melt are nucleation rate-dependent (Figure 5).

**Figure 5:** Schematic illustration of polymorphic formation dependent on crystallization and cooling rate, with the corresponding free energy. Tm indicates the melting temperature.
The crystallization rate decreases from $\alpha$ to $\beta$ and can be reached by applying the optimal cooling rate for each polymorphic form (Figure 5) (Sato & Ueno, 2011). Under low supercooling, the molecules will incorporate into the energetically most favorable sites, thus more stable polymorphic forms are likely to be formed, whereas at high supercooling the incorporation will be favored into less energetically favorable sites (Figure 5). However, it is not easy to predict the cooling rate for each polymorphic form as concurrent crystallization of more polymorphic forms may occur (Sato & Ueno, 2011).

1.2.3 Crystallization and polymorphism of milk fat

Milk fat in its natural state is present as water in oil (W/O) emulsion, e.g. in milk and cream, however, upon removal of the MFGM as well as the continuous water phase, only TAGs are present, thus anhydrous milk fat (AMF) is formed. AMF consists of 99.8% milk fat, and it is widely used in the food industry (e.g. in confectionary products) for its physiochemical properties (Bylund, 1995a). The differences in crystallization between the emulsified and bulk state have been explained by the nucleation theory (Walstra & Van Beresteyn, 1975a) (see chapter 1.2.1). Homogenous nucleation is more likely to occur within pure milk fat globules, whereas in the presence of catalytic impurities, heterogeneous nucleation occurs. In case of homogeneous nucleation in emulsified fat, a lower onset temperature of crystallization is expected (Vanapalli & Coupland, 2001). The crystal polymorphisms identified in milk fat are: $\gamma$, $\alpha$, $\beta'$ and $\beta$, moreover sub forms of the $\beta'$ form have recently been identified (ten Grotenhuis et al., 1999; Lopez et al., 2000; Lopez et al., 2001a; Lopez et al., 2001b).

Crystallization of milk fat leads to the formation of mixed or compound crystals as different TAGs are involved in one crystal (Mulder & Walstra, 1974; Walstra & van Beresteyn, 1975a; Walstra & van Beresteyn, 1975b). Several studies have been performed to characterize milk fat crystallization, both in bulk and emulsified states. Table 4 summarizes the main studies and highlight the most important findings.

Ten Grotenhuis et al. (1999) have studied the effects of different cooling rates on crystallization of AMF. By decreasing the cooling rate from -20°C/min to -0.5°C/min, the crystallization temperature increases from -15°C to 20°C. A higher crystallization temperature and a slow cooling rate lead to a more stable crystal polymorphism. At a cooling rate of -20°C/min, the $\gamma$ form is present, whereas at a
cooling rate of 0.5°C/min, β′ is formed. At a cooling rate in between these extremes, these polymorphic forms coexist. Under isothermal conditions, a different scenario has been observed. At isothermal temperatures in the range of -10 to 14°C, α and β′ coexist, and the time at which they crystallize decreases by increasing the crystallization temperature. However, between 17 and 20°C, only β′ is present, and the crystallization time increases.

Lopez et al. (2002a) have studied the crystallization of AMF and cream at 4°C isothermal condition, after quenching AMF from 60 to 4°C. They observed that the main difference between cream and AMF is that AMF undergoes a faster polymorphic transformation to the most stable crystal form. Contrary to ten Grotenhuis et al., (1999), they observed formation of β crystal in AMF after 30 minutes of isothermal crystallization at 4°C. The β form is also formed in cream after 135 hours of isothermal crystallization, together with sub forms of β′ and small amounts of α. Thus, polymorphic evolution is delayed in emulsified systems because of lack of catalytic impurities (Lopez et al., 2002a). In previous studies (Lopez et al., 2000; Lopez et al., 2001b), similar findings have been observed at different experimental conditions. By quenching cream and AMF to -8°C, a 2L and 3L lamella stacking and the corresponding α form are observed in both samples (Lopez et al., 2000; Lopez et al., 2001b). By heating the samples from -8°C to 50°C at 2°C/min, the 3L disappears at 11°C and 13°C for AMF and cream, respectively. At these temperatures, only the 2L lamella stacking is present. The β′ form is formed at lower temperatures for AMF when compared with cream (2.4°C vs. 5°C). These two forms coexist for both AMF and cream up to 17°C (Lopez et al., 2000; Lopez et al., 2001b).

By reducing the cooling rate to 0.1°C/min and 0.15°C/min for AMF and cream, respectively, Lopez et al. (2001a; 2001c) observed different polymorph crystallizations in cream and AMF. By cooling AMF from 50 to -15°C, the initial nucleation occurs at 24°C and correspond to a 2L stacking and to a β′ polymorphic form. At lower temperatures, 13°C, crystallization of the unstable α form occurs, which coexist with the β′ down to -15°C (Lopez et al., 2001c). In cream, during a similar cooling process, the onset crystallization temperature occurs at a lower temperature, 20°C, than in AMF (Lopez et al., 2001a). Moreover, nucleation occurs in the α form, and later β′ is formed (Lopez et al., 2001a). These differences in crystallization between cream and AMF are explained by lack of nucleation sites within the fat globules, with the cooling rate not being sufficiently slow to lead to nucleation of the stable form (Figure 5) (Lopez et al., 2001a).
Table 4: Crystallization of milk fat in cream and AMF. The effect of cooling/heating rate in a temperature range or of isothermal temperature (Iso. T) on crystallization temperature (C_T), time of crystallization (C_t), polymorphism and lamella stacking formed in cream and AMF.

<table>
<thead>
<tr>
<th>Sample</th>
<th>T range (°C)</th>
<th>Cooling/ heating rate (°C/min)</th>
<th>Iso. T (°C)</th>
<th>C_T (°C)†</th>
<th>C_t (min) †</th>
<th>Polymorphism</th>
<th>Lamella stacking</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMF</td>
<td>70 to -65</td>
<td>-20</td>
<td>-15</td>
<td>α+ γ</td>
<td>β+α</td>
<td>ten Grotenhuis et al., 1999*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-10</td>
<td>15; -13</td>
<td>α+ γ</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-5</td>
<td>16; -12</td>
<td>α+ γ</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-3.33</td>
<td>16; -9</td>
<td>α+ γ</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-2.5</td>
<td>16; -9</td>
<td>α+ γ</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1.67</td>
<td>18</td>
<td>α</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-1</td>
<td>17</td>
<td>β</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.5</td>
<td>20</td>
<td>β</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMF</td>
<td>70 to -65</td>
<td>-10</td>
<td>-8</td>
<td>α</td>
<td>β+α</td>
<td>ten Grotenhuis et al., 1999*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>5</td>
<td>α</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>11</td>
<td>β+α</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>13</td>
<td>β+α</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>17</td>
<td>14</td>
<td>β+α</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cream</td>
<td>-8 to 50</td>
<td>+2</td>
<td>-8</td>
<td>α</td>
<td>β+α</td>
<td>Lopez et al., 2000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>13</td>
<td>α+ β'</td>
<td>β'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>17</td>
<td>α+ β'</td>
<td>β'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMF</td>
<td>-8 to 50</td>
<td>+2</td>
<td>-8</td>
<td>α</td>
<td>β+α</td>
<td>Lopez et al., 2001b</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.4</td>
<td>11</td>
<td>α</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>11</td>
<td>13</td>
<td>β+α</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMF</td>
<td>50 to -15</td>
<td>-0.1</td>
<td>24</td>
<td>β+α</td>
<td>β+α</td>
<td>Lopez et al., 2001c</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>13</td>
<td>β+α</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cream</td>
<td>55 to -8</td>
<td>-0.15</td>
<td>18</td>
<td>α</td>
<td>β+α</td>
<td>Lopez et al., 2001a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>18</td>
<td>α+ β'</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cream</td>
<td>55 to -8</td>
<td>-0.15</td>
<td>18</td>
<td>α</td>
<td>β+α</td>
<td>Lopez et al., 2001a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>18</td>
<td>α+ β'</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cream</td>
<td>60 to 4</td>
<td>4</td>
<td>1</td>
<td>α</td>
<td>β+α</td>
<td>Lopez et al., 2002a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>30</td>
<td>α</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>135h</td>
<td>150h</td>
<td>α</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMF</td>
<td>60 to 4</td>
<td>4</td>
<td>4</td>
<td>α</td>
<td>β+α</td>
<td>Lopez et al., 2002a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>30</td>
<td>α</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>150h</td>
<td>150h</td>
<td>α</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cream</td>
<td>60 to -10</td>
<td>-1</td>
<td>18</td>
<td>α</td>
<td>β+α</td>
<td>Lopez et al., 2002b</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>18&lt;T&gt;2</td>
<td>18</td>
<td>α</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMF</td>
<td>40 to 5</td>
<td>0.1</td>
<td>5</td>
<td>β+α</td>
<td>β+α</td>
<td>Campos et al., 2002</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>5</td>
<td>β+α</td>
<td>β+α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cream/ AMF</td>
<td>25</td>
<td>5</td>
<td>α</td>
<td>β+α</td>
<td>β+α</td>
<td>Fredrick et al., 2011</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

†: temperature and time refer to the first appearance of the polymorphic form

cα: Indicates that the α polymorphic form was formed already during cooling
In a recent study, Fredrick et al. (2011) observed the same crystallization mechanism between AMF and cream cooled at 25°C/min to 5°C. In both cases, the first crystal to be formed is the $\alpha$, and then the $\beta'$ form increases at the expense of $\alpha$.

Thereafter, as shown in Figure 5, the cooling rate is the fundamental factor for the final crystal polymorphism. Moreover, AMF crystallizes into a more stable polymorphic form due to the low amount of free energy in the system (Figure 5). In general, independently of the time and temperature, crystals in emulsions are smaller with a less organized structure compared to milk fat crystals in bulk fat (Lopez et al., 2001a; Lopez et al., 2001b). Although differences have been reported in the crystallization mechanisms between milk fat in bulk and in emulsion (Table 4), the polymorphic crystal forms, and the physical properties of the final crystallized products are shown to be similar (Lopez et al., 20001a; Lopez et al., 2001b; Fedotova & Lencki, 2010).

### 1.2.4 Milk fat crystallization under processing conditions

Processing conditions such as cooling rate, agitation rate and crystallization temperature influence milk fat crystallization (Figure 1). They affect the formation, morphology and size distribution of crystals, and thus the microstructure and texture of butter and butter blends. In general, at slow cooling rates, large crystals with a broad size distribution are formed, whereas many small and unstable crystals are present at fast cooling rates (Herrera & Hartel, 2000a; Campos et al., 2002; Martini et al., 2002a; Wiking et al., 2009a). AMF cooled at a fast rate (10°C/min) forms crystals with areas below 250 µm², while a slow cooling rate (0.1°C/min) leads to a crystal area range from 50 to 950 µm² (Wiking et al., 2009a). Concerning the level of SFC, high levels of SFC is present in samples crystallized at a high cooling rate (Herrera & Hartel, 2000a; Campos et al. 2002; Kaufmann et al., 2012a). A fast cooling lead to the incorporation of different TAGs within the crystal structure and promotes the formation of $\alpha$ form (Campos et al., 2002). On the contrary, at a slow cooling rate, TAGs have the time to arrange together in a more stable crystal as $\beta'$ (Campos et al., 2002; Wiking et al., 2009a). However, a delay in crystal growth was observed for slowly cooled emulsions (Tippetts & Martini, 2009).

Presence of mechanical agitation also influences the crystallization of milk fat. By increasing agitation rate, formation of more and smaller initial crystals is favored (Herrera & Hartel, 2000a; Herrera & Hartel, 2000b; Martini et al., 2002b; Kaufmann et al., 2012b). Incorporation of air during agitation may
facilitate the nucleation step (Herrera & Hartel, 2000a). Agitation forces can also break the already formed crystals (Kloek et al., 2005), the fragments of which might act as nucleation sites and favor secondary nucleation, resulting in a comparatively higher amount of crystals. However, this process is dependent on agitation rate, time and FA composition as different effects on structure formation have been reported in literature. Herrera & Hartel (2000c) observed a decrease in elastic, viscous and complex modulus by applying mechanical agitation during crystallization of milk fat fraction blends. By increasing the shear rate from 50 to 100 rpm, a drastic decrease in the elastic and complex modulus is observed, whereas from 100 to 200 rpm the decrease in moduli is smaller. This effect is more evident at high cooling rates (Herrera & Hartel, 2000c). Kaufmann et al. (2012b) demonstrated that an intermediate shear (50 s⁻¹), applied during crystallization of blends made of AMF and rapeseed oil, leads to a high complex modulus of the blends, whereas high shear rate (500 s⁻¹) results in a low complex modulus. However, no shear effect is reported on the final network of AMFs and blends made with 10% of rapeseed oil (Kaufmann et al., 2012b). In addition, a fast transition from α to β’ is observed in presence of shear, however, the onset time of α form is not affected by shear (Mazzanti et al., 2009). The composition of the α form is different between milk fat crystallized under static conditions and under shear, indicating a lower selectivity under shear (Mazzanti et al., 2009).

1.2.5 Effect of vegetable oil and minor components on crystallization of milk fat

There is an increase in the demand from the consumers for healthy fat-based products, with a low level of saturated FAs, which are known to increase the risk of cardiovascular disease (Astrup et al., 2011). This can be achieved by blending milk fat with vegetable oils, the so-called spreadable or butter blends. Butter blends have better spreadability at refrigerator temperature. The crystallization mechanisms of the butter blends differ from AMF. In general, addition of vegetable oil to AMF causes a decrease in SFC and melting points (Wright et al., 2005). Wright et al. (2005) observed a delay in crystallization onset, lower crystallization temperature and different polymorphic forms by blending canola oil with AMF in different concentrations. The decrease in SFC and melting point in butter blends is caused by dilution of milk fat and by the solubilization of some of the solid TAGs into the oil phase (Wright et al., 2000b; Wright et al., 2005; Kaufmann et al., 2012a). By increasing canola oil concentration, the crystal clusters become smaller and more uniform (Wright et al., 2005). Moreover, the presence of liquid oil influences polymorphic behavior, as more stable crystals are formed with increasing levels of liquid oil.
(Timms, 1980; Wright et al., 2005). In a recent study, Kaufmann et al. (2012a) have demonstrated that addition of rapeseed oil to AMF at a slow cooling rate did not affect melting behavior, whereas stress at fracture is drastically decreased by adding 10% of rapeseed oil. Contrary to this, at a high cooling rate, the melting temperature decreased with increasing amounts of rapeseed oil, and stress at fracture decreases almost linearly. Fast cooling leads to the formation of mixed crystals which can easily be solubilized by addition of vegetable oil (Wright et al., 2000b). On the other hand, slow cooling rate leads to crystallization of more stable crystals.

Minor components, such as phospholipids and diacylglycerides, have a significant effect on the crystallization behavior of TAGs. In general, diacylglycerides and phospholipids delay the crystallization onset of milk fat and crystal growth, which might be caused by their absorption on the crystals (Wright et al., 2000a; Vanhoutte et al., 2002a; Vanhoutte et al., 2002b; Wiking et al. 2009b). Addition of phospholipids to butter increases the spherulites size (Fedotova & Lencki, 2008) and the hardness of the product, moreover the polymorphic transition to a more stable form is facilitates (Fedotova & Lencki, 2010). However, minor milk fat components do not alter the thermodynamic properties of the system, such as thermal behavior and level of SFC (Wright et al., 2000a; Fedotova & Lencki, 2010).

1.2.6 Fractions of milk fat

The thermal behavior of milk fat is very complex because of the broad range of TAG composition. This diversity in TAG composition induces a wide melting range of milk fat, from -40°C to 40°C. The melting profile of milk fat is characterized by three melting fractions. The low melting fraction (LMF) melts in the range of -25°C to 10°C, the medium melting fraction (MMF) from 10°C to 19°C and the high melting fraction (HMF) above 20°C (Deffense, 1993). The TAGs of the LMF contain one long chain saturated FA and two short chain or cis unsaturated FAs and account for the 55% (w/w) of the total milk fat; whereas those of the MMF contain two long chain saturated FAs and one short chain or cis unsaturated FA and account for the 35% (w/w) of the total milk fat; the TAGs of the HMF contain mainly long chain saturated FAs which represent approximately the 10% (w/w) of the total milk fat (Timms, 1980; Marangoni & Lencki, 1998).
By changing the FA composition of milk fat, thus the TAG composition, a different fractionation is expected. In general, the melting point of the fat decreases with decreasing chain length and increasing degree of unsaturation of the FA in milk. The milk fat melting point decreases by increasing the concentration of short chain fatty acids and long chain unsaturated fatty acids and by decreasing long chain saturated fatty acid (Kaylegian & Lindsay, 1995). The melting point of butter oil increases by increasing the amount of C14:0 and C16:0, whereas it decreases by increasing C18:2 (Ortiz-Gonzalez et al., 2007). However, literature is scarce on the correlation between FAs and melting fraction of milk fat.

1.3 Physical destabilization of dairy emulsions

Many dairy products exist as emulsion, either oil in water (O/W), such as milk and cream, or as W/O, such as butter and butter blends. Food emulsions are thermodynamically unstable systems and the destabilization processes are (1) creaming or settling, caused by the difference in density between the two phases; (2) flocculation, referred to the aggregation of particles due to weak attractive forces; (3) Ostwald ripening, refers to the growth of large globules at the expense of small globules; (4) coalescence, the merging of two globules into one globule; (5) partial coalescence, the merging of two or more globules, which due to presence of crystals retain the original shape of the fat globule despite their aggregation; (5) phase inversions (Walstra, 1987). Emulsifiers stabilize O/W emulsions by lowering the surface tension between the oil and the water and form an interfacial layer around the globules (Dickinson, 1992). Moreover, emulsion stability is influenced by the presence of fat crystals and by the contact angle that they form with the continuous or dispersed phase (Darling, 1982). The contact angle is related to the surface tension of the interfaces by Young’s equation (Equation 2):

$$\gamma_{(o/w)} \cos \theta = \gamma_{(o/s)} - \gamma_{(w/s)}$$

Equation 2

where \(\theta\) is the contact angle measured through the water phase, and \(\gamma_{(o/w)}, \gamma_{(o/s)}\) and \(\gamma_{(w/s)}\) are the surface tensions of the oil/water, oil/solid and water/solid interfaces, respectively.
1.3.1 Partial coalescence phenomenon in dairy products

Partial coalescence is a fundamental phenomenon for the production of many dairy products, such as butter, ice cream and whipped cream. Partial coalescence occurs when fat crystals of an emulsion globule pierce and bind another globule. This occurs when the distance between particles decreases, and when the contact angle ($\theta$; Equation 2) is close to 90°, which is expected to destabilize the O/W emulsion and stabilize the W/O emulsion (Boode & Walstra, 1993; Johansson et al., 1995). The process is called partial coalescence as the fat globules retain some of their original shape but are linked by a semi-solid connection. The semi-solid connection keeps the original shape of the globules and does not lead the globule to coalescence. As partial coalescence proceeds, a crystal network made of aggregated fat globules will be formed, and it will be wetted by the surrounding liquid oil (Boode et al., 1993). In this system, the contact angle is above 90°, and the emulsion stabilization effect is hindered, leading to a phase inversion of the emulsion.

In food systems, partial coalescence is usually initiated by shear, as it decreases the distance between fat globules, thus it is more likely that fat globules collide (Goff, 1997; van Boekel and Walstra, 1981). Moreover, air also plays an important role in partial coalescence, as it accelerates the process. This is observed during whipping of cream, in the churning process of butter manufacturing and during manufacturing of ice cream. Continuous formation and breaking of air droplets, as occurring during whipping and churning process, induce partial coalescence mainly by the surface-mediated mechanism (Hotrum, 2004). This mechanism takes place in the absorption of fat globule at the air/water interface and subsequent flocculation and partial coalescence of fat globules, with concomitant release of liquid oil to the interfaces (Mulder & Walstra, 1974; Darling, 1982; Hotrum, 2004). At this stage, the surface-mediated mechanism dominates over the shear force (Hotrum, 2004). Partial coalescence is arrested by the resistance from interactions of solid fat crystals. In general, the extent and rate of partial coalescence determines the textural properties of the product, such as butter, ice cream and whipped cream. Moreover, upon tempering a stable emulsion partial coalescence may occur during recrystallization of the melted TAGs, leading to product consolidation (Drelon et al., 2006; Gravier et al., 2006).
1.4 Butter and butter blend manufacturing

Butter is defined as the product obtained from milk fat and must contain milk fat in the range of 80 to 90%, maximum 16% of water content and maximum 2% of non-fat milk-material (FAO/WHO, 2006; EU, 2007). Over the years, several processes have been used for butter production (Wilbey, 2009). The traditional manufacture method is the batch butter-making. However, the most common method used nowadays is the Fritz process performed by continuous butter-making equipment (Figure 6).

![Figure 6: Section of continuous butter-making equipment. Sectional description: 1) Churning cylinder; 2) Separation section; 3) Drying section; 4) Working section. Adapted from Bylund, G.; Dairy processing handbook (1995b). Reproduced with permission of Tetra Pak A/B, Lund, Sweden.](image)

The Fritz process involves concentration of the cream to 40% fat via centrifugation followed by a thermal treatment of the cream and a subsequent intense churning (Figure 6, section 1). The latter leads to phase inversion of the emulsion with formation of butter grains and separation of the water phase (Figure 6, section 2 and 3), the buttermilk. The aggregated butter grains go through a working section (Figure 6, section 4) with an Archimedean screw. In the working section, the surplus of water is eliminated, the moisture droplets will be homogenously distributed in the continuous liquid phase and consolidation of butter occurs. However, the crystallization process, thus structure stabilization of butter, is finalized in the first two weeks from manufacturing.
Alternative technologies exist for continuous butter making. To the methods, in which a 40% crystallized cream is churned, the “low-fat route” technology belongs, which includes the Fritz process, Senn method, Rohrwasser, and Westphalia Separator process (Wiechers & DeGoede, 1950). An alternative technology consists of the use of high fat cream, 80%, which is standardized and then cooled and crystallized under shear. Examples of this technology are the Alfa method, the Cherry-Burrell, the Creamery Package, the Kraft, and the New-way process (Wilbey, 2009). Moreover, a technology which is mainly used for recombined butter (and margarine) consists in the mixing of milk fat, water phase, and emulsifier followed by emulsification, cooling, and an intensive mechanical working.

1.4.1 Thermal treatment of the cream

A crucial step during the manufacturing process of butter is the thermal treatment of the cream, as it influences the structure of the final product. After pasteurization of the cream, a specific thermal treatment of 12-15 hours is performed. The thermal treatment is based on the FA composition and iodine value (refers to the amount of unsaturation in FAs) of the initial cream. In general, low temperatures are used for cream with a high iodine value (i.e. summer cream) and vice versa. The Swedish method or Alnarp “6–12–6” method, also referred to as “cold-warm-cold” processes, is one of the thermal treatments of the cream (Samuelsson & Petersson, 1937). The “cold-warm-cold” processes result in butter with high contents of liquid fat, thus to a soft final product. The temperature of the warm step is set at the end of the low melting range to separate this fraction from the TAGs with high melting temperature. Consequently, compound crystals will melt, leaving only pure HMF crystals. During the subsequent cold step, the temperature is set higher than the crystallization temperature of the LMF, therefore, more pure crystals will be formed, and the LMF will be available for the continuous phase of butter, thus the butter will be softer (Frede & Buchheim, 1994). In the case of summer cream, which has a higher iodine value, the thermal treatment is designed to form a firmer butter. In general, a warm step will be followed by two cold steps, “warm-cold-cold”. During the warm step, the HMF is melted, and the temperature of the cold step is set lower than the crystallization temperature of the LMF. The subsequent cold step has a slightly higher temperature than the previous step which allows the melting of part of the LMF (Frede & Buchheim, 1994).
1.4.2 Churning process of the cream

Churning is performed together with air, usually 1/3 of the churning cylinder is filled with cream. The air incorporates in the cream and, due to the presence of proteins, formation of foam occurs. During churning, some of the fat crystals present within the globules protrude from the milk fat globule membrane and liquid oil is released form the globules. The liquid oil will be attracted at the air bubbles interface and they will form aggregates together with intact and partially disrupted milk fat globules. At the bubbles interface, flocculation of fat globules occurs and due to the presence of crystallized fat inside the fat globules, partial coalescence takes place (Mulder & Walstra, 1974; Darling, 1982). Consequently, more liquid oil is released from the globules, and it is spread around the fat globules and the air droplets. The process will continue until a network made of aggregated fat globules and air bubbles, where the liquid phase is entrapped, is formed. During churning, the air bubbles interface becomes more concentrated, this proceeds until the fat globule aggregates become too large and too few to enclose the air bubbles. The air bubbles start to coalesce, leading to an unstable foam, thus to formation of butter grains which will grow until a sudden inversion of phase.

1.4.3 Butter blends manufacturing

Butter blends are generally defined as the products obtained by blending vegetable oil with milk fat. However, according the European Union standards for dairy-based spreadable fats, blend is defined as the product with a fat content between 80 and 90%, which can be both vegetable and/or animal fats (EU, 2007). Whereas, “three-quarter fat butter”, corresponding to the “smør 60” in Danish, must contain between 60 and 62% milk fat (EU, 2007). The most used vegetable oils are rapeseed oil, canola oil, and soya bean oil, however sunflower oil, olive oil or cotton seed oils have also been used. Several technology processes are used to manufacture butter blends, some of these include the churning method, the margarine process and the inversion of the cream method (Mortensen, 2009). In general, for batch churning process, vegetable oil can be added to the cream either in the storage tank or right before churning. Whereas for continuous churning process, vegetable oil is either added to cream in the storage tank or in the pipeline through the churn section (Mortensen, 2009). In addition, vegetable oil can be added at the end of the continuous butter-making process, in the work section, (Figure 6, section 4) and further mixed before packaging (Nielsen, 1993; Berntsen, 1999). In the margarine making
process AMF, vegetable oil and emulsifiers are blended, emulsified, and crystallized. Whereas, in the inversion of the cream method, concentrated ripened cream is blended with vegetable oil and then further cooled and churned, and cooled again before packaging (Bylund, 1995b).

1.5 Microstructure of fat crystal network

Microstructure refers to the spatial distribution of particles, their sizes and shapes, thus to the distance and connection forces between particles. The range of particles characterizing the microstructure is between 0.25 to 200 µm, whereas below 0.25 µm and above 0.2 mm refers to nano and macrostructure, respectively (Narine & Marangoni, 2004).

Microstructure of a fat crystal network is strictly dependent on the crystallization mechanisms, thus on cooling rate, crystallization temperature, presence of emulsified fat, addition of vegetable oil and/or other minor components and agitation forces (Figure 1). In general, microstructure is affected by the solid-to-liquid ratio. However, at same level of SFC the microstructure can vary substantially based on the size of the crystals and crystal clusters and on the amount and type of bonds between them (Herrera & Hartel, 2000c).

1.5.1 Microstructure of butter and butter blends

Butter and butter blends are composed of a continuous crystal network dispersed in liquid oil and interrupted by fat globules, water and air droplets (Juriaanse & Heertje, 1988). The crystal network consists of a three-dimensional structure formed by crystals or crystal clusters (van den Tempel, 1961; Haighton, 1963). The network is held together by primary and secondary bonds. Primary bonds are strong and irreversible and are responsible for the hardness of the product, whereas, secondary bonds are weak and reversible, and they are responsible for the consistency of the product (van den Tempel, 1961; Haighton, 1963; Juriaanse & Heertje, 1988). The latter are often referred to the van der Waals forces. However, in presence of milk fat globules and milk fat proteins other forces might be involved in the system, such as steric and electrostatic forces, in addition solid bridge (sintering) can be formed. If a continuous crystal network is not present, or if crystals and crystal clusters form weak links between each other, they do not contribute to the mechanical properties of the fat-based product (Herrera & Hartel, 2000b; Herrera & Hartel, 2000c; Tang & Marangoni, 2007; Marangoni & Tang,
2008). Moreover, the number of interactions between crystals and crystal clusters is proportional to the number of particles present, thus in the presence of large particles, the attractive forces will be weaker (Campos et al., 2002).
2. Methods used for the study of crystallization, microstructure and textural properties of milk-fat systems

In table 5 the methods available for the measurements performed in this thesis are summarized. A brief description of the methods used to perform the experimental work of this thesis is reported in this section.

2.1 Rheological measurements

Rheology is the study of deformation and flow of matter and aims to describe how a material responds to applied stress ($\tau$; Pa) or strain ($\gamma$; mm or %), as function of time. Rheological measurements are commonly used to characterize the structure and texture of food. Two ideal types of rheological behavior can be distinguished: solid or elastic, when the matter starts flowing on applying stress, and as soon as the stress is removed, it regains its original shape. Second type is liquid or viscous, when upon removal of stress the matter keeps the deformation caused by the applied stress (van Vliet, 1999). However, most foods do not show ideal rheological behaviors, but they show viscoelastic behavior, which means that upon removal of stress the matter may keep some deformation and may regain some of it its original shape, which is also the case for butter and butter blends.

In general, three types of stresses are commonly used to characterize foods mechanically: shearing (directed tangentially to the material), uniaxial compressive (directed through the material), and tensile (directed away from the material) (van Vliet, 1999; Barbosa-Cánovas et al., 2006). The rheological properties of plastic fat, such as butter and butter blends, are typically studied by uniaxial compression and shear using large or small deformation techniques, respectively. The rheological response to large deformations is non-linear and it implies breakdown of the material, thus it describes the irreversible primary bonds. On the other hand, a small deformation obtained within the linear regime does not involve breaking of the structure and describes the reversible, elastic secondary bonds. Due to the complex structure and texture of milk fat-based products, a single technique may not be appropriate to describe the texture (Mortensen & Danmark, 1982). Moreover, the choice of the right rheological technique, thus the purpose of the measurements, is a very important factor which is often neglected.
2.1.1 Small deformation techniques- Oscillation tests

Characterization of viscoelastic fat is often performed by oscillatory shear test in small deformation rheometers. In a typical oscillation experiment, a constant (sinusoidal) shear strain or stress, at a given frequency, $\omega$, is applied to the sample, and the response of the system, the time-dependent stress, $\tau(t)$, or strain, $\gamma(t)$, is measured. Within the linear viscoelastic region (LVR) stress and strain are directly proportional, to a critical strain, the yield point. Beyond the yield point, permanent deformation and fracture of the structure occur. When maximum stress corresponds to maximum strain, the material is characterized as elastic, and stress and strain are in phase whereas, if they are out of phase ($\pi/2$), the material is characterized as viscous. The phase angle range, $\delta$, is between $0^\circ$ and $90^\circ$, with $0^\circ$ indicating a pure solid behavior and $90^\circ$ a pure liquid behavior (van Vliet, 1999). In the LVR, the response of the material is also sinusoidal but shifted by $\delta$.

Several parameters can be obtained from oscillation tests, such as the elastic and viscous modulus, $G'$ and $G''$ (Equation 3 and 4), respectively, the tangent of the phase angle, $\tan \delta$ (Equation 5) and, the complex modulus, $G^*$ (Equation 6). The elastic and viscous moduli and the tangent of the phase angle are calculated as:

\[
G' = \frac{\tau(t)}{\gamma(t)} \cos \delta(\omega) \quad \text{(Equation 3)}
\]

\[
G'' = \frac{\tau(t)}{\gamma(t)} \sin \delta(\omega) \quad \text{(Equation 4)}
\]

\[
\tan \delta = \frac{G''}{G'}
\]

(Equation 5)

The elastic and viscous moduli are representative of the stored and dissipated parts of the energy, respectively. In the LVR, these two parameters fully characterize the viscoelasticity of the sample, whereas above the yield point, the structure is modified and $G'$ and $G''$ become dependent on the applied strain or stress. The complex modulus describes the overall structure of the material, and it is given by both elastic and viscous moduli and determined as:
Plastic fats exhibit linear viscoelastic behavior at low levels of stress or strain. In plastic fat, $G'$ is greater than $G''$ (at low frequency) until the yield point, which resembles the properties of food gels. The main disadvantage of this technique occurs when handling hard and brittle fat, i.e. AMF, which might lead to non-reproducible results due to sample cracking. In the present PhD study, oscillation tests were performed to obtain information on the viscoelastic character of the milk-fat systems.

2.1.2 Large deformation techniques

Large deformation techniques are based on the determination of the force needed to induce a change in the material. Compression and penetration tests are widely used to characterize the texture of plastic fat. Penetration measurements are usually performed by cone, needle, cylinder and sphere geometries. The geometry penetrate into the sample either at a constant speed or with a constant load, and the responses of the material, i.e. the force needed to penetrate the material or the distance travelled by the geometry during compression are recorded as function of time. In general, uniaxial compression occurs in presence of geometries larger than sample, on the contrary penetration force is applied (Pons & Fiszman, 1996). The parameters obtained from both techniques are used to characterize the texture of plastic fats. The most used geometry in the fats and oils industry for texture measurement is the cone geometry (AOCS, 1960). However, it tends to cause fracture within the sample, even for softer samples, probably due to the increase in cross sectional area of the geometry occurring when penetration proceeds (Boodhoo et al., 2009). In compression test, a uniaxial force is applied to the sample and it provides more information about product texture, such as brittleness, which cannot be measured by the cone penetration or penetration test (deMan et al., 1989).

Compression and penetration tests have been widely used to describe the texture of plastic fats due to the simplicity of sample preparation and performance of the measurements. However, interpretation of the results is relative to the parameters and geometries used (Boodhoo et al., 2009; Rosenthal, 2010). The choice of the most appropriate technique and parameters, and the interpretation of results might be challenging. The speed of compression is correlated to the response of the material, at higher speed corresponds a higher force or hardness (Rosenthal, 2010). Moreover, the size and shape of the
geometry and the dimension of the sample influence the recorded forces. In order to characterize the texture of milk-fat systems, compression and penetration test were performed during this PhD study.

2.2 Thermal analysis- Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) identifies the phase transitions occurring in a material by measuring the heat flow as a function of time and temperature in a controlled atmosphere. These measurements provide information about physiochemical changes that involve endothermic or exothermic processes (Watson et al., 1964). In general, DSC can give information about glass transitions; melting and boiling points; crystallization time and temperature; solid fat content; crystal polymorphism; specific heat capacity; oxidative and thermal stability; reaction kinetics; purity (Lavigne et al., 1993; Nassu & Gonçalves, 1995; Tan & Che Man, 1999; Bruylants et al., 2005). DSC has been widely used to study the thermal behavior of fats. Recently an indirect DSC method, stop-and-return DSC, has been developed (Foubert et al., 2008). The advantage of this method is that it allows the determination of the crystallization kinetics and mechanisms under challenging circumstances i.e. start of crystallization during cooling to the crystallization temperature or crystallization in emulsion. The principle is to stop the crystallization during the isothermal crystallization and then melt the sample (Foubert et al., 2008). The main disadvantage of the DSC technique is that the shape of the thermogram changes based on the cooling rate used (ten Grotenhuis et al., 1999). The melting behavior of the milk-fat systems was studied by standard DSC method and linked to the crystallization mechanisms.

2.3 Solid fat content determination- Pulsed Nuclear Magnetic Resonance spectroscopy

Determination of solid fat content (SFC) has traditionally been performed by dilatometry which is based on the change of volume of the sample (AOCS, 1989a). However, faster and more accurate methods are available, i.e. pulsed Nuclear Magnetic Resonance spectroscopy (p-NMR) and DSC (AOCS, 1989b; AOCS, 1989c; Nassu & Gonçalves, 1995). Two methods to determine SFC by p-NMR are available: direct and indirect. Both methods measure the signals from the hydrogen nuclei, which are distinguished by the rate of decay, Free Induction Decay (FID). In general, hydrogen nuclei from the solid phase relax faster than the ones from the liquid phase. Moreover, hydrogen nuclei of water relax faster than hydrogen nuclei of lipid (Shahidi & Wanasundara, 2002). The direct NMR method
calculates the ratio of the number of hydrogens in the solid phase to the total number of hydrogens (in the solid and liquid phase) as reported in Equation 7:

\[
SFC = \frac{(S_{LS} - S_L) + F}{S_L + (S_{LS} - S_L) + F + D}
\]  
(Equation 7)

where \(S_{LS}\) is the signal from liquid and solid whereas \(S_L\) is the one from the liquid phase. F is a correction factor, because the instrument has a dead-time before it can take the first signal. D is a constant which corrects for the decay of the liquid signal (AOCS, 1989c). The indirect method measures only the liquid part of the sample and refers it to a calibration performed on the fully melted sample. Sample preparation is complex and time-consuming (AOCS, 1989b). Moreover, in recent studies, p-NMR has also been used to determine the crystal polymorphism in fat (Trezza et al., 2006; Janssen & MacGibbon, 2007). In this PhD study the direct p-NMR method was used to follow the crystallization of milk fat system as function of time.

2.4 Particle size measurements- Mastersizer

Particle size distribution is usually measured by a laser light scattering instrument (i.e. Mastersizer). The Mastersizer measures the intensity of light diffracted (scattered) at each angle and then transforms it into particle size distribution by using Mie theory (Mie 1908). Dilution and stirring of concentrate emulsion before analysis let to avoid multiple scattering. However, these processes are likely to disrupt aggregated particles. Measurements are reported as: full particle size distributions; volume/surface average diameter, \(d_{32}\); volume-weighted average diameter, \(d_{43}\). The \(d_{32}\) and \(d_{43}\) are defined as follows:

\[
d_{32} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2}
\]  
(Equation 8)

\[
d_{43} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3}
\]  
(Equation 9)

where \(n_i\) is the number of fat globules of diameter \(d_i\). The volume-based diameter, \(d_{43}\), is more sensitive than \(d_{32}\) to the presence of aggregate particles as in the case of a milk-fat emulsion, e.g. cream.
Determination of particle size can also be performed by microscopy techniques (section 2.6). In this PhD study, fat globule size measurements by Mastersizer were performed to follow the partial coalescence of milk fat globules during ripening and churning of the cream.

2.5 Polymorphisms and lamella stacking detection- x-ray diffraction

Polymorphism and lamella stacking of TAGs molecules of fat are determined by small and wide angle x-ray diffraction, respectively. In general, an x-ray at a specific wave length goes through the sample, and the electrons of the sample scatter the x-ray waves. The intensity of the scattered light, also named reflections, and the angle at which diffracted beams emerge from a crystal are recorded. The Bragg’s law (Equation 10) is used to determine the distance (d) between the scatterings and is a function of the scattered angle (θ) and the original wave length (λ).

\[ d = \frac{n\lambda}{2\sin(\theta)} \]  

(Equation 10)

The scattered angle depends on the distance of the detector, small angles are characteristic of long spacing, whereas wide angles are characteristic of short spacing in the molecules (Rhodes, 2006). This technique is very accurate, however, due to the costly instrumentation it is not often available in a laboratory. Crystallization mechanisms were followed during ripening and churning of the cream by small and wide angle x-ray diffraction.

2.6 Microstructure analysis- Confocal Laser Scanning Microscopy

Several microscopic techniques are used to observed food microstructure, such as light microscope, polarized light microscope (PLM), scansion or transmission electron microscope (SEM and TEM) and confocal laser scanning microscopy (CLSM). CLSM is one of the most accurate instruments for observing complex food microstructure. The CLSM uses a scanning laser, or a combination of several scanning lasers, to illuminate the specimen. The light emitted by the laser (excitation source) pass through a pinhole (confocal aperture) and is reflected by a dichromatic mirror and then scanned across the specimen in a focal plane. From the same focal plane, fluorescence light is emitted from the
specimen and passes through the dichromatic mirror, and it is focused as a confocal point at the detector pinhole (Figure 6) (Claxton et al., 2006). Thus, the image obtained by CLSM does not depend on transmitted light through the specimen.

![Schematic diagram of the optical pathway and principal component in a laser scanning confocal microscope](image)

**Figure 6: Schematic diagram of the optical pathway and principal component in a laser scanning confocal microscope.** Adapted by Claxton et al., 2006. Reproduced with permission of the author.

CLSM has innumerable advantages compared to a light microscope, e.g. several microstructure characteristics (e.g. fat globules, liquid fat, proteins, etc.) can be identified simultaneously by using different fluorescence probes and lasers. Furthermore, the depth of the specimen can be controlled, and 3-D images (optical section) can be recorded. Several studies have observed food microstructure by CLSM (Auty et al., 2001; Dürrenberger et al., 2001; Gallier et al., 2010; Ong et al., 2010). They showed the advantages of using CLSM for the study of food microstructure compared to conventional techniques. In this study, CLSM images were analyzed in parallel to rheological measurements in order to better understand the microstructure and the textural properties of milk fat systems.
2.7 Multivariate data analysis- PCA and PLS

Multivariate data analysis is a mathematical method used to analyze data that involve more variables. Principal component analysis (PCA) is used to develop a comparatively small number of orthogonal variables (called principal components) from a pool of observed variables that describe the measurement. The principal components account for most of the variance in the observed variables (Davies & Fearn, 2004). Thus, PCA is a variable reduction process. PCA is used to identify patterns in data and to describe similarities and differences among them, whereas, Partial Least Squares (PLS) analysis is used to predict a set of dependent variables from a set of independent variables or predictors. PLS models are obtained by extracting from the independent variables a set of orthogonal factors called latent variables, which account for most of the variation, thus have the most significant predictive power (Tobias, 1995).

In this PhD study PCA and PLS were used to predict the level of SFC and the melting point of butter blends based on the mixing temperature of the butter blend manufacturing, and on the percentage of rapeseed oil added to butter. In addition, multivariate data analysis was used to identify the most relevant fatty acids contributing to the melting point of the medium melting fraction of cream and to differentiate cream from different feeding strategy.
Table 5: Summary of some of the methods available to study crystallization, microstructure and textural properties of plastic fat

<table>
<thead>
<tr>
<th>Methods</th>
<th>Principle</th>
<th>Applications</th>
<th>Advantage</th>
<th>Disadvantage</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Differential Scanning Calorimetry (DSC)</td>
<td>Temperatures and heat flows are associated with transitions in materials as function of time and temperature</td>
<td>Determination of: thermal behavior; solid fat content; crystal polymorphism</td>
<td>Fast and simple; measurements performed at isothermal and non-isothermal temperature</td>
<td>Shape of the thermogram changes based on the cooling rate used; difficult sampling with plastic fats; shear cannot be applied</td>
<td>Watson et al., 1964; Nassu &amp; Gonçalves, 1995</td>
</tr>
<tr>
<td>Stop-and-return DSC</td>
<td>Same as DSC</td>
<td>Determination of the melting behavior at different time intervals during isothermal crystallization</td>
<td>Information on crystallization mechanisms when standard DSC is not suitable and when x-Ray diffraction is not available</td>
<td>Use of the same pan (not suitable for milk fat emulsion); difficult sampling with plastic fats</td>
<td>Foubert et al., 2008</td>
</tr>
<tr>
<td>Pulsar Nuclear Magnetic Resonance (p-NMR)-Direct method</td>
<td>Measures the signals from the hydrogen nuclei. Calculates the ratio between the hydrogens in the solid phase and the total number of hydrogens (solid and liquid phase)</td>
<td>Determination of: solid fat content; crystal polymorphism</td>
<td>Fast, simple and highly precise</td>
<td>Not accurate; measurements at constant temperature</td>
<td>AOCS, 1989c; Trezza et al., 2006; Janssen &amp; MacGibbon, 2007</td>
</tr>
<tr>
<td>p-NMR-Indirect method</td>
<td>Measures the liquid part of the samples and refers it to the fully melted samples</td>
<td>Determination of solid fat content</td>
<td>Accurate</td>
<td>Complex sample preparation and time-consuming</td>
<td>AOCS, 1989b</td>
</tr>
<tr>
<td>Method</td>
<td>Description</td>
<td>Parameters measured</td>
<td>Advantages</td>
<td>Disadvantages</td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>--------------------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Dilatometry</td>
<td>Measures the change in sample volume</td>
<td>Determination of solid fat content</td>
<td>Reproducible</td>
<td>Laborious, time-consuming and inaccurate; measurements at constant temperature</td>
<td></td>
</tr>
<tr>
<td>Oscillation rheology</td>
<td>Applying a constant stress or strain to the material</td>
<td>Measures the variation in strain as function of the applied stress and vice versa</td>
<td>Determination of several parameters ($G'$, $G''$, $G^*$; $\tan\delta$)</td>
<td>Difficult sample handling and not highly reproducible with hard fat</td>
<td></td>
</tr>
<tr>
<td>Uniaxial compression</td>
<td>A sample between two parallel plates is compressed at constant load and velocity</td>
<td>Determination of the force needed to induce a change in a material</td>
<td>Easy and fast; several textural descriptor can be estimated from the data</td>
<td>No highly reproducible</td>
<td></td>
</tr>
<tr>
<td>Penetration test</td>
<td>A geometry is penetrated into the sample either at constant load or at constant speed</td>
<td>Determination of the hardness</td>
<td>Easy and fast, widely used</td>
<td>Results are dependent on parameters and geometries</td>
<td></td>
</tr>
<tr>
<td>Static light scattering</td>
<td>A monochromatic laser beam is emitted, and the static scattering pattern of the sample particles is measured. Mie theory is used for calculation of size distribution</td>
<td>Determination of particle size distribution</td>
<td>Fast and easy to use</td>
<td>Dilution of samples is needed; stirring can cause breaking of particles</td>
<td></td>
</tr>
</tbody>
</table>

Walker and Bosin, 1971; AOCS, 1989a
Buldo & Wiking (Paper IV-V)
Wright et al., 2001; Buldo & Wiking (Paper IV-V)
Haighton, 1959; AOCS, 1960; Buldo & Wiking (Paper IV-V); Mie, 1908
<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-Ray diffraction</td>
<td>Bragg’s law is used to determine the distance between the reflections formed by the x-ray scattered from the electrons of the samples</td>
<td>Determination of polymorphism and lamella stacking of TAGs</td>
<td>Accurate</td>
<td>Expensive instruments, often not available</td>
</tr>
<tr>
<td>Confocal Laser Scanning Microscopy (CLSM)</td>
<td>Specimen is excited by a laser which is scanned in a focal plane. Fluorescence light is emitted and it is focused as a confocal point.</td>
<td>Observation of complex structure in a defined depth; particle size determination; fractal analysis</td>
<td>Detection of several component phases; 3-D images; high contrast, definition and signal-to-noise; easy and relatively fast sample preparation; emulsion stability</td>
<td>Low resolution</td>
</tr>
<tr>
<td>Polarized Light Microscope (PLM)</td>
<td>A polarized light is used to illuminate the sample. Due to the birefringence of crystals they will appear bright, whereas liquid and amorphous regions of the specimen will appear dark</td>
<td>Observation of crystallization process and crystal structure; particle size determination</td>
<td>Inexpensive technique; contrast-enhancing technique</td>
<td>Not suitable for complex system such as butter or multiphase emulsions; limited resolution to the order of 1 µm</td>
</tr>
</tbody>
</table>
3. Summary of included papers and manuscripts

3.1 Crystallization mechanisms in cream during ripening and initial butter churning - Paper I

Objective:

To elucidate the mechanisms leading to milk fat crystallization in emulsions, thus to partial coalescence, during churning of the cream, including the effect of ripening time.

Results:

- Longer ripening time resulted in shorter churning time, higher rate of fat crystallization, higher degree of partial coalescence, larger butter grains, and higher viscosity
- Increasing the SFC from 15% (sample not ripened) to 23% (sample ripened for 0.5 hour) significantly decreased the churning time
- The melting point of the MMF increased during the first hour of ripening, whereas the melting point of the HMF decreased during churning
- A 3L packing appeared after 10 minutes of ripening at 10°C. Within the following 20 minutes, this structure rearranged into a 2L packing which was also present during churning
- $\alpha$, sub forms of $\beta'$, and $\beta$ crystals were formed after 10, 33, and 60 minutes of ripening, respectively, no further changes were observed after 60 minutes of ripening
- During churning, $\alpha$ form disappeared, the intensity of the sub forms of $\beta'$ increased, whereas $\beta$ form appeared to be constant throughout the churning.

Conclusions:

- Viscosity measurements could be used to detect phase inversion of the emulsion during churning of the cream in a rheometer cell
- Churning of the cream favored the transition from the $\alpha$ form to $\beta'$ form
- No relationship was observed between polymorphism and SFC higher than 15%, to partial coalescence of fat globules
- The physical changes leading to partial coalescence of fat globules and consequently to phase inversion of the emulsion occurred during the first hour of cream ripening at 10°C.
3.2 Effect of globular fat on crystallization and rheological properties of milk fat based emulsions
- Paper II

**Objective:**
To study the effect of fat globules on the rheological properties and crystallization process of milk fat based emulsion during storage.

**Results:**
- Butter grain-based emulsions (0% AMF) had higher level of SFC, lower melting points, and lower stress at fracture than 100% AMF-based emulsions at any storage time.
- By decreasing the amount of fat globules in the milk fat-based emulsion, the crystal network became denser and the stress at fracture increased, despite decreases in SFC and an unchanged thermal behaviour and Hencky strain, at any storage time.
- After one day from production, the G' was higher for 0% AMF emulsion than the 100% AMF, and it increased by decreasing the amount of fat globules.
- During storage:
  - G' significantly increased only for the 100% AMF emulsion.
  - No changes were observed in the thermal behaviour, stress and Hencky strain at fracture, despite an increase in SFC and density of the crystal network.

**Conclusions:**
- After two weeks of storage, no evolution in fat crystallization was observed.
- Presence of fat globules facilitated crystallization in the continuous fat phase.
- Presence of fat globules into the system led to a faster microstructure stabilization.
3.3 Microstructure and textural properties of milk fat systems during temperature fluctuations - Paper III

Objectives:

To characterize the microstructure and textural properties of different milk fat systems, such as partially disrupted milk-fat globules, i.e. butter, concentrate milk-fat globules, i.e. freeze-dried cream, and bulk fat, i.e. AMF; and to evaluate their stability towards temperature fluctuations stress.

Results:

- Freeze-dried cream was less stiff, softer, and more elastic than butter and AMF
- AMF was stiffer and harder than butter
- During temperature fluctuations:
  - no significant changes in microstructure and textural properties were observed in freeze-dried cream
  - the density of the crystal network increased in butter, this corresponded to an increase in stiffness, hardness, and brittleness, and to a decrease in elasticity
  - AMF showed a finer network which led to an increase in hardness and decrease in elasticity
  - butter was harder than AMF, but it had equal elasticity
- No differences in melting behaviour were observed in all systems, by comparing before and after temperature fluctuations.

Conclusions:

- The presence of higher amount of intact fat globules led to a less stiff, softer and more elastic product
- Crystals and crystals clusters in the continuous phase, and the type of connections between them, contributed to the hardness, stiffness and brittleness of the system
- Flocculation of fat globules in a fat continuous phase caused formation of a denser network
- By increasing the amount of fat globule and decreasing the amount of fat in the continuous phase, a more resistant system to temperature fluctuations is expected
- Cylinder geometry was suitable to evaluate true hardness, stress at fracture, and brittleness.
3.4 The Role of Mixing Temperature on Microstructure and Rheological Properties of Butter Blends - Paper IV

**Objectives:**

To study the effect of mixing temperature and amount of rapeseed oil added to butter on rheological properties, melting behavior, and microstructure of butter blends. To predict the effect of mixing temperature and rapeseed oil concentration on melting point and level of SFC.

**Results:**

- By increasing the mixing temperature and/or the percentage of rapeseed oil added to butter, product firmness, melting point, and solid fat content decreased, moreover a less dense crystal network was observed
- The applied shear increased the firmness of butter
- By increasing the isothermal crystallization temperature of butter and butter blends, the level of SFC significantly decreased, while the melting point slightly increased
- The predicted values by PLS were similar to the measured ones, however PLS gave more information about where the specific interactions between the variables take place.

**Conclusions:**

- The mixing temperature and the percentage of rapeseed oil, and their interaction, contributed to crystallization and microstructure formation of butter and butter blends
- Higher mixing temperature and higher amount of rapeseed oil led to a solubilization of the solid TAGs in the system, which were not able to recrystallize fully upon subsequent cooling
- Shear force induced rebuilding of the crystal network
- The co-effect of mixing temperature or oil concentration could be predicted by PLS analysis.
3.5 Multivariate data analysis for finding the relevant fatty acids contributing to the melting fractions of cream - Paper V

Objectives:

To identify the individual fatty acids contributing to the melting points of the individual melting fraction of cream, and to differentiate between creams from various practical feeding regimes by multivariate data analysis.

Results:

- The melting point of the MMF of milk fat was positively correlated to C16:0 and negatively correlated to C18:1cis9, CLA cis9 trans11, C18:1 trans11, C18:1 trans9, and C14:1
- The melting points of the HMF could not be related to fatty acid composition
- Addition of C16:0-based fat supplement to the feeding ratio in combination with a lower forage intake increased the amount of C16:0 and C16:1 in milk fat and decreased the content of C10:0, C14:0, C18:0, and C18:1 trans fatty acid
- Rapeseed cake and grass silage contributes to the amount of C18:3 n3 found in milk fat
- Lower amount of rapeseed cake and higher amount of maize silage in the feed decreased the melting point of the MMF, whereas addition of C16:0-based fat supplement increased the melting point of the MMF.

Conclusions:

- Multivariate analyses of data from individual cows identified the most relevant fatty acids contributing to the melting point of the MMF of the cream
- The use of PCA on fatty acid composition data showed to be a good method to distinguish between different feeding regimes and to identify the individual cow variation among cows within the same herd
- Individual cow variation affects the fatty acid composition of milk fat, thus the melting fractions
- No clear cut between the feeding regime and the melting behavior of the cream was observed.
4. General Discussion

4.1 Effect of processing conditions and of addition of vegetable oil on crystallization and on rheological properties of butter and butter blends (Paper I; IV)

Crystallization of milk fat, thus microstructure and textural properties of milk fat systems can be modified by several factors during the manufacturing process. The literature is abundant on the effect of cooling rate (ten Grotenhuis et al., 1999; Herrera & Hartel, 2000a; Herrera & Hartel, 2000b; Lopez et al., 2001a; Lopez et al., 2001c; Campos et al., 2002; Martini et al., 2002a; Martini et al., 2002b; Tippetts & Martini, 2009; Wiking et al., 2009a; Kaufmann et al., 2012a), crystallization and storage temperature (Shukla et al., 1995; Herrera & Hartel, 2000b; Herrera & Hartel, 2000c; Vithanage et al., 2009), effect of shear (Herrera & Hartel, 2000b; Herrera & Hartel, 2000c; Mazzanti et al., 2009; Kaufmann et al., 2012b), addition of vegetable oil (Rousseau et al., 1996; Wright et al., 2000b; Martini et al., 2002b; Wright et al., 2005; Kaufmann et al., 2012a), and milk fat fractions (Herrera & Hartel, 2000b; Herrera & Hartel, 2000c; Wright et al., 2000b; Martini et al., 2002b) on milk fat crystallization and structure properties of milk fat systems. However, to author knowledge, there are some variables left unexplored, such as the effect of process conditions on crystallization and microstructure properties of butter and butter blends, and the effect that vegetable oil has on already crystallized fat systems. Knowledge on these topics will contribute to a better understanding of the crystallization process, thus microstructure formation in milk fat systems, thus on how to process a desired sensory texture of milk fat systems.

In this section, the effect of process conditions, such as ripening and churning time of the cream and the role of mixing temperature on crystallization and microstructure properties of butter and butter blends, is discussed. Moreover, the effect that vegetable oil and mixing force have on already crystallized butter, which takes place during butter blends manufacturing, is presented.

4.1.1 Effect of ripening time and churning of cream on crystallization and partial coalescence

The first step to control milk fat crystallization in butter manufacturing is the thermal treatment of the cream, also known as ripening (Figure 8, step 2). Milk fat crystallization governs the partial coalescence phenomenon occurring during the subsequent churning process (Figure 8, step 3 and 4),
thereafter the texture of butter is also affected (Figure 8, step 5). Knowledge on the mechanisms leading to partial coalescence, such as fat crystallization during ripening and churning of the cream, will contribute to the optimization of the final quality of the product and of the production process.

During cream ripening at 10°C, the major changes in physical properties of the cream were observed within the first hour. After 1 hour of ripening, 2L and 3L lamella stackings were present (Figure 8, step 2). The corresponding polymorphic forms observed after one hour were primarily $\beta'$ with traces of $\alpha$ and $\beta$, which coexisted during the rest of the ripening, up to 17 hours (Paper I; Figure 8, step 2). These results corresponded to the findings listed in Table 4 (Lopez et al., 2000; Lopez et al., 2002a). In addition, the melting point of the HMF of the cream increased during the first hour of ripening and then remained constant, confirming no further evolution in fat polymorphism (Paper I).

During ripening, the fat globule size and the viscosity of the cream were constant, despite the increase in SFC (Paper I; Figure 8, step 2). Mechanical agitation and introduction of air into the system play a fundamental role in partial coalescence phenomenon during churning of the cream (Figure 8, step 3). By applying shear force, the distance between globules decreases and the probability and the duration of collision increase, favoring partial coalescence (van Boekel & Walstra, 1981). Moreover, the shear force favored the transition from $\alpha$ to $\beta'$ form (Figure 8, step 3 and 4), as previously reported during crystallization of palm oil and of AMF (De Graef et al., 2009; Mazzanti et al., 2009). Yet, no relationship has been observed between polymorphism and sensitivity to partial coalescence i.e. $\beta'$ form was not triggering partial coalescence.

The physical changes occurring during the first hour of ripening were crucial for the partial coalescence rate, thus phase inversion of the emulsion, as further confirmed by the churning time which did not decrease at ripening time higher than 0.5 hour (Paper I; Figure 8, step 3 and 4). In Paper I, phase inversion of the emulsion, which occurs within few seconds, could be detected by viscosity measurements during churning of the cream in a rheometer cell. However, these findings were the outcome of a laboratory scale experiment; therefore it cannot directly be assumed that in an industrial scale the same results are found. The main differences in the set-up of the experiment, when compared to the industrial scale system, are the dimensions of the tank containing the cream, the amount of air introduced during churning and the design of the impeller.
Figure 8: From milk to butter. Schematically illustration of the physical changes occurring during butter manufacturing. Not to scale. The light blue area represent the water phase, the yellow area is liquid oil, the yellow circle is the fat globule containing liquid oil and crystals, the grey circle is the casein micelle, the black circle is air. The insert in step 1 is the loading plot of PLS model for identification of the fatty acid contributing to the medium melting fraction of the cream (Paper V). The inserts in step 2 show the level of solid fat content (SFC) during five hours of cream ripening at 10°C, and the small and wide angle scattering (SAXS and WAXS) for the evolution of lamella stacking and crystal polymorphism, respectively, during 17 hours of cream ripening at 10°C (Paper I). The inserts in step 3 and 4 show how the level of SFC influences the churning time, and the SAXS and WAXS for the evolution of lamella stacking and crystal polymorphism during churning of the cream (Paper I).
The dimensions of the tank might affect the mass transfer of the latent heat during ripening, whereas the design of the impeller might affect the flow of the fat globules during churning. Therefore, the time it takes for crystallization and polymorphism evolution, hence for partial coalescence and milk fat globules aggregation, is expected to be different from the laboratory experiments. However, the obtained insights give a general overview at fundamental level of the crystallization and polymorphism evolution occurring during ripening and churning.

Nevertheless, other factors might also influence the churning time and the partial coalescence. By using an artificial neural network, Funahashi & Horiuchi (2008) showed that the characteristics of the churning process of the cream are affected by the fat content, flow rate and feed temperature of the cream, with the latter being the predominant factor for controlling the water content of butter. Moreover, Xu et al. (2005) studied the effect of shear time, shear rate and temperature on stability of food emulsions. They observed that by increasing the shear rate and shear time, the shear force overcomes the inter-particle forces and disrupts the membrane around the fat globules, causing formation of clusters or aggregates. The temperature of the ripening step is also essential for the partial coalescence phenomena, as lower temperatures lead to high SFC as a consequence of the globules not being able to merge due to insufficient liquid fat available, whereas at low level of SFC, no crystal network will be formed within the globule (Boode et al., 1993).

4.1.2 Effect of mixing temperature and of addition of vegetable oil on microstructure and rheological properties of butter blends

The addition of vegetable oil to milk fat during crystallization leads to a delay in crystallization, thus to a lower onset crystallization temperature (Wright et al., 2005). Moreover, blends of milk fat and vegetable oil are characterized by lower level of SFC due to a dilution and solubilization effect (Wright et al., 2000b; Wright et al., 2005; Kaufmann et al., 2012a), by smaller crystal clusters and by a more stable crystal form (Wright et al., 2005). Addition of vegetable oil to AMF leads to a softer and less brittle product (Rousseau et al., 1996; Kaufmann et al., 2012a), moreover at high amount of vegetable oil, the viscoelastic properties of the blends decrease (Rousseau et al., 1996). In addition, the shear force also affects the microstructure and rheological properties of butter and butter blends. In general, a decrease in viscoelastic properties is reported by applying shear during crystallization of milk fat.
systems (Herrera & Hartel, 2000c; Kaufmann et al., 2012b). However, a recent study showed that the complex modulus is not shear dependent for pure AMF when a shear of 1, 50 and 500 s\(^{-1}\), respectively, is applied, whereas in blends of AMF and rapeseed oil, the complex modulus is higher at 50 s\(^{-1}\) shear rate and it is lower at 500 s\(^{-1}\) shear rate, compared to the non-shear blends (Kaufmann et al., 2012b). It was hypothesized that different mechanisms are involved in the crystallization and microstructure formation, when vegetable oil is added to a partly crystallized emulsion system, i.e. butter. Moreover, the mixing temperature and the shear rate used to manufacture the blend also contributed to the crystallization and microstructure of the systems.

Addition of rapeseed oil to a partially crystallized emulsion system such as butter, and higher mixing temperature resulted in a system, after storage at 5°C, with a less dense crystal network, lower firmness, melting points, and SFC than butter (Paper IV). Despite the dilution effect obtaining by adding rapeseed oil, both addition of rapeseed oil and the high mixing temperature solubilized some of the milk fat TAGs which are part of the network; these were not able to re-crystallize fully at a subsequent storage temperature. Moreover, at higher mixing temperature the vegetable oil was distributed in the system more efficiently, leading to a higher solubility effect. Part of the liquid oil was not entrapped in the crystal network, when high amounts of oil and high mixing temperature were used, as observed by the presence of oil-off on the surface of the blend. The solid fat dissolved in the liquid oil did not participate to the formation of the crystal network, as confirmed by the decrease in complex modulus upon recrystallization. The interactions between crystal clusters are hampered by liquid fat in the system, consequently none or weaker connections are formed (Herrera & Hartel, 2000b).

Contribution to the mechanical properties of milk fat system originates from the amount and type of connections between crystal and crystal clusters (Herrera & Hartel, 2000b; Herrera & Hartel, 2000c; Tang & Marangoni, 2007; Marangoni & Tang, 2008). Thereafter, a less dense crystal network and weak connections between crystal clusters led to a lower complex modulus, G* (Paper IV). However, the contribution from the amount of rapeseed oil on melting points, level of SFC and microstructure was stronger than the one from the mixing temperature.

The complex modulus of butter significantly increased after applying shear at the studied condition (Paper IV), suggesting formation of stronger secondary bonds upon recrystallization. Moreover, as consequences of the shear force, flocculation of intact milk fat globules and/or a decrease in their
amount could have occurred, leading to a denser crystal network in the continuous fat phase. When mechanical agitation was combined with mixing temperatures, the effect of shear-induced rebuilding was markedly strong, despite the delay in rebuilding caused at higher temperatures (Paper IV). The effect of shear rate on partially crystalline milk fat network is not yet fully elucidated. A more detailed study that focus on the effects of different shear rates and on different fatty acids composition of the crystal network will help to obtain further insights into this topic.

The relationship between melting point and level of solid fat content on the processing conditions, as mixing temperature and rapeseed oil concentration, was predicted by the counterplots obtained by PLS analysis. Moreover, PLS analysis could predict the co-effect of mixing temperature or oil concentration (Paper IV). By varying the mixing temperature, the microstructure and rheological properties of butter blends can be modified, thus their quality can be improved.

4.2 Characterization of microstructure and textural properties of milk fat systems (Paper II; III)

Milk fat is present as oil in water (O/W) or water in oil (W/O) emulsion, and bulk system. The microstructure of the milk fat systems is schematically illustrated in Figure 9 (step 1). In O/W emulsion, such as milk and cream, the milk fat is entrapped in globules, which are surrounded with a biological membrane and dispersed in a continuous aqueous phase. In W/O emulsion, such as butter, the water is dispersed in a bi-continuous solid/liquid fat network interrupted by intact milk fat globules. In bulk systems, such as AMF, milk fat crystals are freely dispersed in liquid oil. In this PhD study, model systems were investigated by changing the amount of milk fat globules and the continuous phase of the emulsion. Commercial butter and butter grains, which are kind of O/W emulsion, were compared to AMF, i.e. bulk fat, and to a system of concentrated fat globules dispersed in a continuous water phase, thus a O/W emulsion, this was achieved by freeze drying cream. In addition, systems of butter grains and AMF in different proportions were studied (Figure 9 and 10, step 1). All studied systems had the same amount of water as in commercial butter, i.e. 16%. The author is aware that the nomenclature chosen to refer to the milk fat systems studied in this PhD thesis can be considered partially incorrect, due to the multiphase character of the systems. However, it is at present considered the best way to describe the systems. Due to the complexity in microstructure of the different milk fat systems, it was hypothesized that different microstructural elements might be linked to one textural
descriptor. Therefore, to have an exhaustive description of the textural properties of a milk fat system, several rheological techniques should be considered, as several properties characterized the texture of a milk fat system.

**4.2.1 Rheological techniques to identify the textural descriptors of milk fat systems**

Food texture has officially been defined as “all the mechanical, geometrical, and surface attributes of a product perceptible by means of mechanical, tactile and, where appropriate, visual and auditory receptors” (ISO standard 5492, 1992). In other words, texture is referred to as the perception of food structure and how the product behaves when handled and eaten (Rosenthal, 1999). The most common descriptors used to describe the texture of butter and butter blends are: hardness, stiffness, spreadability, brittleness, elasticity and stickiness. Most of these descriptors are evaluated sensorially, yet, small and large deformation rheology techniques have been widely used to describe them.

Hardness has been defined as the force required obtaining a given deformation (Szczesniak, 1963). In this PhD study, it was determined by penetration and compression tests at constant speed. When penetration test was performed by cone or needle geometry at constant speed, the force required to penetrate the sample to a defined distance corresponded to the relative hardness because no fracture points were visualized in the force-distance curve of milk fat systems (Paper III). The obtained data had the same trend of the stiffness measurements obtained by small oscillatory deformation rheology (Paper III). Thus, the elastic modulus could be used as an indicator of hardness, as previously reported (Narine & Marangoni, 2001).

The true hardness was defined at the fracture point occurring in the force-distance curve, when a constant speed was applied to the sample. This was obtained in all milk fat systems by penetration test, only when performed with a cylinder geometry (Paper III). The compression test showed a clear fracture point in the force-distance curves only for butter and AMF systems, whereas no clear fracture point was observed for freeze-dried cream (Paper III).
Figure 9: Microstructure and textural properties of milk fat systems. Step 1: Schematic representation of water in oil (W/O) emulsion, i.e. butter; oil in water (O/W) emulsion, i.e. freeze-dried cream; bulk fat, i.e. AMF. See figure 8 for symbols characterization; images are not to scale. Step 2 and 3: CLSM images of the above mentioned milk fat systems, after 2 weeks of storage and after temperature fluctuations, respectively. The black shadow on the image background represents the crystal network of the samples. The yellowish/brownish area is the liquid fat of the system, and the green areas are protein/water phases. The scale bar indicates 20µm. The insert in steps 2 and 3 show the stress at fracture measured by penetration test with cylinder geometry during temperature fluctuations for the studied milk fat systems.
Brittleness or fracturability is a secondary parameter, which defines the force needed to fracture the material, and it is related to the primary parameter of hardness (Szczesniak, 1963). Brittleness can be identified by the Hencky strain (also called true strain), which correspond to the maximum deformability of the structure before the material fracture. However, previous studies correlated the irregularity of the force-time curve obtained by penetration test performed with a cone with the brittleness of binary blends of fat (Danthine & Deroanne, 2003; Danthine & Deroanne, 2004). This can be argued by the speed used to perform the test and by the presence of milk fat globules in the systems. In the present study the force-distance curves obtained by cone and by needle geometry did not show any irregularities for all milk fat systems studied. However, the force-distance curves obtained by compression test and penetration test performed with a cylinder geometry, showed irregularities after the fracture point for some of the milk fat systems studied (Paper III). Therefore, these techniques can be used to estimate brittleness of milk fat systems.

Elasticity is defined as the capacity of a material to deform upon applying a force and as the rate at which it regains its initial shape on removal of the force (Szczesniak, 1963). Elasticity was attributed to the initial slope of the force-distance curves obtained by compression test, as previously described (Dixon, 1966). It is important not to confuse elasticity with elastic modulus, as the elastic modulus does not measure the elasticity, but is a descriptor for the stiffness of the material (Mohsenin & Mittal, 1977). This was confirmed in freeze-dried cream, which was characterized by a high elasticity but by a low elastic modulus (Paper III).

The choice of the most appropriate rheological technique and parameters to analyze the texture of food systems is fundamental for the correct characterization of the microstructure. For instance, freeze-dried cream emulsion was characterized by an elastic texture, which did not show a clear fracture point in the force-distance curve obtained by uniaxial compression test, but it revealed a fracture point by penetration test performed with a cylinder geometry (Paper III). This suggested that the hardness measurement, obtained by compression test, was not the most appropriate to characterize elastic structures. These results led to the conclusion that the combination of several large deformation rheological techniques are needed to characterize the texture of a milk fat systems. Yet, it is strongly suggested to couple the rheological measurements with microscope images (Paper II; III; IV), in order
to better understand the links between rheology tests and microstructure. It would be interesting to
design predictive models based on sensory and rheological data to better associate the textural
measurement to the sensory stimuli.

4.2.2 Microstructure elements responsible for the textural properties of milk fat systems

To identify the microstructure elements which contribute most to the textural properties, different milk
fat systems were analyzed by several rheological techniques and by CLSM. Butter, AMF, and freeze-
dried cream were chosen in order to study the effect of presence of milk fat globules into the systems
on microstructure and on textural properties (Paper III; Figure 9, step 1). The milk fat systems were
characterized by different microstructure elements and arrangements in the system (Paper III; Figure
9). The continuous phase consisted of liquid fat in butter and AMF, and of water in freeze-dried cream.
The dispersed phase consisted of a continuous crystal network and partially disrupted fat globules in
butter, crystal spherulites in AMF, and intact milk fat globules in freeze-dried cream (Paper III; Figure
9). The overall textural characterization showed that freeze-dried cream had less stiff, softer, and more
elastic structure than butter and AMF. The textural properties of AMF were very close to those of
butter (Figure 9, step 2 and 3). These textural differences were independent to the level of SFC and to
the melting behavior of the systems, as no differences were reported in melting points and level of
SFC, when comparing butter and freeze-dried cream (Paper III). These findings indicated that the level
of SFC, within the limits of 46-52%, is not the main factor determining the textural characteristics.

Stiffness and hardness increased by increasing the density of the crystal network, thus the connections
formed between crystals, and they were not directly related to the size of the crystals (Paper II and III).
Moreover, the amount of intact fat globules negatively contributed to these properties. In milk fat
systems characterized by intact milk fat globules, such as in freeze-dried cream, crystallization
occurred inside the globules, thereafter weak and/or few bonds are formed between crystals and a
continuous network cannot be formed. It is generally accepted that a fat system with a continuous
crystal network with more contact points between crystals and crystal clusters is characterized by a
higher stiffness and hardness when compared to a system with no connected crystals and crystal
clusters (Herrera & Hartel, 2000b; Herrera & Hartel, 2000c; Wiking et al., 2009a; Paper II; III; IV).
However, AMF had less, but stronger, connections between crystals when compared to butter and, milk
fat globules were not interrupting the crystal network, resulting in a stiffer and harder texture (Paper III).

Brittleness and elasticity were negatively correlated to each other, as a brittle system was less elastic and *vice versa* (Paper III). Elasticity increased by increasing the amount of intact fat globules in the systems which gave high deformability to the structure before fracture occurred, and decreased by increasing the volume fraction of the crystal network in the continuous phase. Whereas, brittleness increased by increasing the volume fraction of the crystal network in the continuous phase and by decreasing the intact milk fat globules, thus by forming a denser crystal network (Paper III).

A further study which focus on the contribution of each of the above mentioned microstructural elements, such as crystal size, amount of fat globules, volume fraction of fat crystals in the continuous phase and type and amount of crystal bonds will elucidate these hypotheses and further contribute to the knowledge about how the individual different structural elements affect the textural properties.

**4.3 Crystallization of milk fat systems during storage and handling - effect on microstructure and on textural properties (Paper II; III)**

Knowledge on the crystallization mechanisms occurring in and outside milk fat globules is needed to better understand and control the microstructure formation of milk fat systems and their behavior during storage and handling. Thus, it will be possible to prevent formation of undesirable texture and physical instability of the products. Figure 9 and 10 illustrate the main results obtained in this this PhD study regarding the changes in microstructure and textural properties of milk fat systems, that occurred during storage at refrigerator temperature, and during handling at consumer’ s level.
Figure 10: Microstructure and textural properties of milk fat systems. Step 1: Schematic representation of water in oil (W/O) emulsion, i.e. butter grains; emulsions of butter grains and AMF; and AMF. See figure 8 for symbols characterization; images are not to scale. Step 2 and 3: CLSM images of the above mentioned systems, after 1 and 28 days of storage, respectively. The black shadow on the image background represents the crystal network of the samples. The red area is the liquid fat of the system, and the green areas are protein/water phases. The scale bar indicates 100µm. The inserts in steps 2 and 3 show the stress at fracture and the elastic modulus after 1 day and 28 days of storage for the studied milk fat systems.
4.3.1 Crystallization and microstructure formation in milk fat systems

Crystallization occurs differently in emulsified versus bulk systems. In general, in emulsions, a higher degree of supercooling is needed to initiate nucleation, moreover crystallization has to occur in every single droplet independently (Walstra & van Beresteyn, 1975a). Contrary, in AMF, the presence of catalytic impurities, such as solid surfaces or foreign particles, leads to a shorter induction time and the formed crystals might act as crystallization sites and further lead to secondary nucleation. Moreover, minor components, such as those present in the milk fat globule membrane, are also known to delay the crystallization process (Wright et al., 2000a; Wiking et al. 2009b). Yet, the onset crystallization temperature depends on the cooling rate; at higher cooling rate, no differences between cream and AMF are observed (Fredrick et al., 2011).

Crystallization and microstructure formation evolved differently, depending on the presence of emulsified fat, i.e. milk fat globules, during storage at 5°C (Paper II and III; Figure 9 and 10). Milk fat systems containing emulsified milk fat, such as butter grains (named 0% AMF in Paper II) were characterized by lower melting points for the MMF and HMF, when compared to the bulk system, such as AMF (named 100% AMF emulsion in Paper II). This indicated a lower onset crystallization temperature for systems containing milk fat globules. The DSC-thermograms and hence, the respective melting points of emulsion systems obtained in Paper II and III were similar. In addition, systems made with both butter grains and AMF, had the same melting points of pure butter grains (Paper II). This suggested a stable thermal behavior in emulsions of butter grains and AMF, up to 50% AMF in butter grains. The melting behavior did not change during storage, independently of the milk fat systems.

Different milk fat systems led to different textural properties after 1 day of storage (Figure 10, step 2). In presence of milk fat globules, i.e. butter grains, more and smaller crystals, thus more contact points than the AMF system were observed in the continuous phase (Paper II). This corresponded to a greater level of SFC, higher stiffness, and to a lower hardness (Paper II; Figure 10, step 2). These findings further confirmed the hypothesis that several elements contribute to the textural properties of the systems, as the hardness of the systems was not only influenced by the crystal size, thus by the amount of contact points, but also by the amount of milk fat globules and by the strength of the bonds (Paper II...
and III). By blending the butter grains and AMF, the crystallization mechanisms changed. By increasing the amount of AMF in the system, thus by decreasing the amount of globular fat, the density of the crystal network, the stiffness and hardness of the systems increased (Paper II; Figure 10, step 2). The milk fat globule membrane (MFGM) might have acted as nucleation sites for the AMF, hence facilitating formation of a crystal network in the continuous fat phase. Therefore, by reducing milk fat globules in the system, a denser crystal network with stronger primary and secondary bonds was formed (Paper II). The presence of milk fat globules influenced the microstructure formation more than the level of SFC did. Moreover, the contribution to textural properties of the systems was related to the ratio of milk fat globules to AMF.

### 4.3.2 Effect of storage time on microstructure and on textural properties of milk fat systems

In literature, knowledge on the effect of storage time on crystallization is sparse, especially in emulsified milk fat systems. AMF and HMF based butter have a trend towards an increase in the complex modulus during storage at 17, 22, and 27°C (Shukla & Rizvi, 1995). Likewise, the crystal size together with the elastic and complex modulus of blends made of different proportions of LMF and HMF milk fat increase by increasing the storage time (Herrera & Hartel, 2000b; Herrera & Hartel, 2000c). In margarine and spreads, the structure stabilizes within 9 to 12 hours from manufacturing, during storage at 4°C (Pothiraj et al., 2012). However, hardness stabilizes after 2 days of storage, whereas product elasticity decreases by increasing storage time.

During storage at 5°C, crystallization evolved differently on time, based on the presence of milk fat globules in the systems (Paper II and III; Figure 10, step 3). Moreover, the microstructure changed with time, hence different textural properties than those observed at a first stage of crystallization were found (Paper II). During storage at 5°C, the level of SFC increased in all milk fat systems (Paper II). This led to a denser fat crystal network with more contact points between crystal and crystal clusters than those observed at the first stage of crystallization (Paper II; Figure 10, steps 2 and 3). However, only in AMF system, the stiffness increased during storage, in all other systems, stiffness and hardness did not change during 28 days of storage (Paper II; Figure 10, steps 2 and 3). This suggested that stabilization of the microstructure during storage seemed to be governed by a critical SFC in the continuous network, which for butter grains and for blends of butter grains and AMF was obtained
after 1 day of storage, whereas for the AMF system it was obtained after 14 days of storage. In other words, formation of primary and secondary bonds was accomplished after 1 day of storage in butter grains and in blends of butter grains and AMF (from 0% AMF to 25% AMF), whereas, AMF showed consolidation after one day for the primary bonds and after 14 days of storage for the secondary bonds. Therefore, the time needed to stabilize the fat crystal network is related to the ratio of emulsified to bulk milk fat.

Presence of fat globules into the system led to a faster microstructure stabilization, as crystallization continue inside the fat globules. These findings were in agreement with the hypothesis that textural properties of milk fat systems rely on several microstructural elements. Moreover, the nature of the bonds can be more determinant than the actual crystal size, thus amount of contact points, for the textural properties of milk fat systems. To fully characterize the effect of storage time, a more exhaustive characterization of the different microstructures is needed.

4.3.3 Microstructure and textural changes occurring during handling of milk fat systems (Paper III)

The textural characteristics of milk fat systems can change during handling at consumer’s level, due to temperature fluctuations, thus melting and recrystallization of milk fat. Temperature fluctuations of an emulsion lead to product consolidation, such as increase in hardness and stiffness. This phenomenon has been previously observed in whipped dairy creams and in foam systems, in which, after a tempering treatment, stiffness increases as consequence of partial coalescence phenomenon (Drelon et al., 2006; Gravier et al., 2006). In Paper II, it was observed that the crystallization process, the microstructure formation, and the textural properties of the systems evolved differently during storage based on the amount of milk fat globules. This was further confirmed in Paper III, where different textural properties were associated to different microstructures. Therefore, it was hypothesized that temperature fluctuations occurring during product handling can affect the texture of milk fat systems differently, based on the presence of milk fat globules (Paper III).

During temperature fluctuations, the most sever changes in microstructure and textural properties were reported in presence of intact fat globules in a continuous fat phase, as in the case of butter. Temperature fluctuations led to texture consolidation in butter, i.e. W/O emulsion. Texture
consolidation was reported in AMF only after several temperature fluctuations, whereas, no significant texture changes were observed in the system with concentrate milk fat globules, freeze-dried cream, i.e. O/W emulsion (Paper III; Figure 9, step 3). Moreover, after temperature fluctuations, the level of SFC decreased for butter and freeze-dried cream, whereas it remained constant for AMF, indicating that this was not the factor responsible for the textural properties of the milk fat systems (Paper III).

During temperature fluctuations, flocculation of intact fat globules occurred in butter, this led to a different spatial configuration of the microstructure elements, as a denser crystal networks could be formed in the continuo phase (Paper III). Therefore, upon cooling and storage at 5°C, more and stronger bonds were formed resulting in a firmer and harder texture (Paper III). A prolonged exposure of the butter to room temperature led to a further increase in hardness and to a decrease in elasticity, most likely due to the flocculation of more fat globules and/or to the formation of more and stronger bonds between crystal clusters (Paper III). These changes in textural properties confirmed the hypothesis that intact milk fat globules dispersed in the crystal network decrease the hardness of the system (Paper II and III). Moreover, flocculation of fat globules and concomitants increased in the volume fraction of the fat crystals network in the continuous phase led to a decrease in elasticity and to an increase in brittleness of the system (Paper III).

Flocculation of milk fat globules occurred also in freeze-dried cream during temperature fluctuations, however, its textural properties were constant (Paper III). This further confirmed that crystallization occurring within intact milk fat globules does not contribute to the hardness and stiffness of the system (Paper II). Therefore, the presence of intact fat globules conferred a more stable microstructure with no significant change in textural properties during temperature fluctuations.

AMF showed smaller spherulites and a finer network after several temperature fluctuations, which corresponded to a harder, less elastic and more brittle structure (Paper III). Temperature fluctuations might have caused the melting of some of the MMF TAGs of the crystal clusters which upon cooling and storage recrystallized in different spatial configuration. This could have caused the binding between neighbor crystals, or formation of sintered bonds, thus a denser crystal network which led to a harder texture. Moreover, the increase in volume fraction of the fat crystals in continuous phase could have contributed to the decrease in elasticity and increase in brittleness (Paper III).
In addition, during temperature fluctuations the microstructure of the systems was stable against physical emulsion destabilization, such as phase separation due to coalescence of water droplets. Physical stabilization of the systems might have occurred differently in the different milk fat systems (Paper III). Freeze-dried cream was most likely stabilized by the emulsifiers naturally present in the milk fat globule membrane. Butter was mainly stabilized by a continuous crystal network, whereas in AMF system, Pickering stabilization was observed (Paper III; Figure 9, steps 2 and 3). Similar findings were previously observed in butter and margarine (Heertje, 1993; Rousseau et al., 2003; Rousseau et al., 2009). Pickering refers to the presence of surface active crystals attached at the surface of the water droplets, i.e. the interface between water and oil, which cause a stearic barrier between droplets, hindering their coalescence. Pickering occurs mainly in margarine and in whipped cream, however it has also been observed, to a minimum extent, in butter (Heertje, 1993; Rousseau et al., 2003; Rousseau et al., 2009).

4.4 Identification of the fatty acids responsible of the melting fractions of cream (Paper V)

The thermal behavior of milk fat is fundamental for understanding the application of the fat. The complexity of milk fatty acid composition, thus TAGs, leads to formation of compound crystals which are responsible for the broad melting range of each melting fraction in the thermogram (Fredrick et al., 2011; Mulder & Walstra, 1974; Walstra & van Beresteyn, 1975b). Fatty acids composition of milk can be firstly controlled by diet, however, breed, genetic, and environmental factors also affect it (Jensen, 2002). Most of the literature available correlates fatty acid composition of the milk fat, obtained after diet alteration, to its thermal behavior and rheological properties (Baer et al., 2001; Chen at al., 2004; Couvreur et al., 2006; Ortiz-Gonzalez et al., 2007; Smet et al. 2010). Ortiz-Gonzalez et al. (2007) found that C14:0 and C16:0 increase the melting point of butter oil, whereas C18:2 decreases the melting point. Moreover, the melting point was positively correlated to TAG numbers C44, C46 and C48 and negatively correlated to C28, C30, C38, and C40 TAG. Smet et al. (2010) showed that AMF rich in unsaturated fatty acids has a higher proportion of low melting TAG.

A new approach to correlate the fatty acid composition and melting points is presented in Paper V. Multivariate analysis was used as a link between melting behavior and fatty acid composition and the obtained results showed a more successful explanation than previous studies. The melting point of the
MMF was positively correlated to C16:0 whereas, it was negatively correlated to C18:1 cis9, CLA cis9 trans11, C14:1, C18:1 trans9, C18:1 trans11, and C18:2 cis6 (Paper V). All the other fatty acids, were not correlated to the melting point, despite the high amount of some of them, in milk fat, i.e. C14:0 and C18:0 (Paper V). The MMF of milk fat is rich in TAG numbers 36, 38, 40, and 50 and 36-54 unsaturated (Dimick et al., 1996; Van Aken et al. 1999) which corresponds to the TAG composition of the olein fraction identified by Lopez et al. (2006). The main TAGs in the olein fractions are: C4-C16-C16; C4-C16-C18; C4-C16-C18:1; C6-C14-C16; C16- C16- C18:1; C14-C18-C18:1; C14-C16-C18:1; C16-C16-C16:1; C4-C14-C16; C16-C18:1-C18:1(Lopez et al., 2006). These TAGs are all C16 and C18:1-based, which explains the results of Paper V of C16:0 and C18:1 being highly correlated with the melting point of the MMF. Moreover, Paper V showed that fatty acids present in minor amount in milk fat, such as CLA cis9 trans11, C14:1, C18:1 trans9, and C18:1 trans11 might affect the melting point of the MMF.

The advantage of the new method proposed in Paper V is that it could be used to select the fatty acids of interest, which will contribute to the melting point desired. However, an improved model which includes the TAGs composition, instead of the FAs composition, of either the natural cream or of its fractions, will lead to a complete overview of the relation between TAGs and melting fractions of milk fat.
5. Conclusions and Future Perspectives

The new insights obtained from the present PhD study contributed to elucidate the crystallization mechanisms and the microstructure behavior occurring during manufacturing of butter and butter blends, and during product handling at consumer’s level. The focus was on unexplored variables, such as ripening and churning time of the cream, and on mixing temperature of butter blend manufacturing. Moreover, the effect of milk fat globules on the microstructure and textural properties was identified and explained.

During the first hour of cream ripening in a rheometer cell, the physical changes needed to initiate partial coalescence of fat globules and butter grains aggregation during the subsequent churning, occurred. Longer ripening time did not accelerate the churning process. The phase inversion of the emulsion was detected by viscosity measurements in a rheometer cell. The butter grains yield is affected by the time and temperature of the ripening step. Therefore, a new study focusing on the parameters to obtain the optimal yield is suggested. In addition, further studies on the effect of ripening time on the final microstructure and textural properties of butter will give a better overview of the role of the manufacturing process.

Microstructure and rheological properties of butter and butter blends were affected by addition of rapeseed oil on a partially crystallized fat system and by the mixing temperature. The addition of rapeseed oil and the mixing temperature solubilized part of the TAGs in the network which were not able to recrystallize upon cooling, resulting in a less firm product. In addition, the applied shear during mixing promoted the rebuilding of the crystal network but, in presence of large amount of liquid oil, this effect was retarded. The study of shear force applied during mixing deserves a detailed investigation, to better understand its role during crystallization in different milk fat systems. The combination of mixing temperature and shear can be seen as a promising alternative way to improve microstructure and textural properties of fat-based products.

The presence of milk fat globules and the arrangement of the microstructural elements in the system were the main responsible of the microstructure formation and of the textural properties of milk fat systems. Moreover, they strongly influenced the microstructure and textural behavior of the milk fat
systems during storage and handling at consumer’s level. The level of SFC, within the range of 46-52%, did not contribute to the textural properties of the milk fat systems.

After manufacturing, structure stabilization occurred rapidly during storage in presence of both milk fat globules and AMF when compared to AMF system. Presence of milk fat globules decreased the hardness and stiffness of the system and increased its elasticity. Whereas, presence of a denser continuous crystal network with stronger bonds between crystals and crystal clusters led to a harder, stiffer, and brittle system. Yet, milk fat globules interrupting the crystal network, as in the case of butter, led to a decrease in hardness and stiffness.

Temperature fluctuations led to fat globules flocculation which, in the case of systems with a continuous fat phase, resulted in a microstructure with a denser and stronger network, therefore a harder and stiffer system than the initial one was formed. However, in the case of water as continuous phase, flocculation of fat globules occurring during temperature fluctuations did not influence the textural properties of the system as the crystals are constrains within the fat globule, thereafter they cannot contribute to the hardness of the system. In order to improve the knowledge on the role of milk fat globules in the microstructure and textural properties, a method to quantify the milk fat globules in complex structure, such as in butter and butter blends, is needed. Moreover, a study focusing on the identification and quantification of primary and secondary bonds will contribute to a better understanding of the textural properties of milk fat systems. These topics could be approached by scan electron microscope (SEM).

The new insights obtained in this PhD study demonstrated that crystallization mechanisms and textural properties of milk fat systems are strongly dependent by the microstructural elements and by their arrangements in the systems. Therefore, innovation of new types of butter and butter blends could be approached by considering the contribution of the ratio of emulsified to non-emulsified fat on the microstructure, textural properties, and on the texture stability during temperature fluctuations.
6. References


Acknowledgments/ Agradecimentos/ Ringraziamenti

First and foremost I would like to acknowledge my supervisor Lars Wiking for his skillful scientific guidance, constructive discussions and help during these years.

I wish to acknowledge Ulf Andersen for his valuable assistance and contributions to this study, and for make me feel always welcome at Arla Strategic Innovation Center. Thanks to Mette K. Larsen and Marianne Hammershoj for their fruitful discussion and excellent assistance on the chemometric and rheological data, and for their valuable comments on this thesis. Thanks to Kell Mortensen and Jacob Kirkensgaard for their assistance with the x-ray scattering experiments and data analysis.

Gianfranco Mazzanti is acknowledged for his hospitality at Dalhousie University (Halifax, Nova Scotia) and the whole “Mazzanti group” for the funny raining spring of 2011 in Halifax. Jes C. Knudsen is acknowledged for the hospitality at the Department of Food Science, University of Copenhagen.

A warm thanks to Niels Kaufmann and Stine Ronholt for our brainstorming and discussions on milk fat crystallization, and for their comments on sections of this thesis. I have enjoyed working with you and sharing greasy laboratories.

Working at the Department of Food Science has been a joy, for all the nice colleagues. In particular, I would like to thank Anne H. Balling and Aase Sorensen for the thorough proofreading of this thesis and manuscripts. Grith Mortensen is greatly acknowledged for her moral support, and for being a fantastic leader.

I wish to thank Luigi Montanari, who let me discover the world of Food Science and who strongly motivated me to continue my studies abroad.

I would also like to thank my friends at the Nørresø Kollegiet and in Viborg for the nice and funny social activities and for making me feel at home. Despite the distance, I would also like to thank my friends in Italy and around the world for being always present.

Eu sou grata ao João pelo seu apoio, ajuda e conhecimentos científicos e, principalmente, pelo seu amor durante este período intenso. Tu foste a melhor "descoberta" deste doutorado.
I am grateful to João for his support, scientific insights and help, and mainly for his love during this intense period. You have been the best “finding” of this PhD.

Infine, un grazie speciale alla mia Super Mamma ed alla mia fantastica famiglia per il loro costante sostegno e amore. Senza di voi sarebbe non sarebbe stato possibile.

*Last but not least, I am deeply grateful to my Super Mamma and to my wonderful family for their constant encouragement and love. Without you it would not have been possible.*

Patrizia Buldo,

Viborg, November 2012