Effect of tempering and the cooling rate on the stability of dairy cream

by
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Master's dissertation submitted in partial fulfillment of the requirements for the degree of Master of Science in Food Technology
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Gent, August 2013

The promotor,
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I. Preface

Firstly, I would like to thank Ir. Kim Moens, my very nice supervisor. I can not complete this thesis without her support. I also send a big thank to my promotor, Prof. dr. ir. Koen Dewettinck. He not only brought the best conditions for my research in lab FTE but also taught me very much during last two years.

I want to thank Stefanie, Benny and Masum very much for their help in running equipments and preparing materials for this study.

I wish to acknowledge the VLIR Organization and my coordinator, Katleen, for their support during my study in Belgium.

Lastly, but not at least, I am grateful to my family and my close friends (Ngoc, Long and Tuyen) very much.
II. Table of Contents

I. Preface
II. Table of contents
III. Abstract
IV. Introduction and Objective

1. LITERATURE REVIEW

1.1. Dairy Cream .................................................................................................................. 1
   1.1.1. Natural cream ........................................................................................................ 1
   1.1.2. Recombined cream ............................................................................................... 2

1.2. Fat Crystallization ......................................................................................................... 2
   1.2.1. Nucleation and crystal growth .............................................................................. 3
   1.2.2. Fat polymorphism .................................................................................................. 4
   1.2.3. Milk fat .................................................................................................................... 6
   1.2.3.1. Influence of cooling rate .................................................................................... 8
   1.2.3.2. Influence of shear .............................................................................................. 8

1.3. Partial Coalescence ...................................................................................................... 9
   1.3.1. Mechanism ............................................................................................................ 9
   1.3.2. Shear induced partial coalescence ...................................................................... 10
   1.3.3. Surface – mediated partial coalescence ............................................................... 11
   1.3.4. Influence of tempering on partial coalescence in cream ........................................ 12

1.4. Whipping and whipped cream ..................................................................................... 14

2. MATERIALS AND METHODS

2.1. Materials ..................................................................................................................... 17
   2.1.1. Natural cream (NC) ............................................................................................ 17
   2.1.2. Recombined cream (RC) ..................................................................................... 17
2.2. Methods.............................................................................................................................................. 17
2.2.1. Experimental design........................................................................................................................... 17
2.2.2. Differential Scanning Calorimetry (DSC).......................................................................................... 19
2.2.3. Nuclear Magnetic Resonance............................................................................................................. 20
2.2.4. Rheometer........................................................................................................................................... 22
  2.2.4.1 Cooling rate ................................................................................................................................. 22
  2.2.4.2 Tempering..................................................................................................................................... 22
  2.2.4.3 Shear-induced partial coalescence................................................................................................. 23
2.2.5. Light Microscopy............................................................................................................................... 23
2.2.6. Statistical Analysis ............................................................................................................................ 24

3. RESULTS AND DISCUSSION

3.1. Fat crystallization in dairy cream ........................................................................................................... 25
  3.1.1. Effect of thermal processing............................................................................................................. 25
    3.1.1.1. Effect of cooling rate ................................................................................................................. 25
    3.1.1.2. Effect of tempering .................................................................................................................. 28
  3.1.2. Effect of agitation/shear .................................................................................................................. 32
    3.1.2.1. The combination of tempering and agitation ............................................................................ 35
    3.1.2.2. Difference between natural and recombined cream ............................................................... 37
3.2. Partial coalescence ............................................................................................................................... 40
  3.2.1. Effect of cooling rate ...................................................................................................................... 40
  3.2.2. Effect of tempering ....................................................................................................................... 42
  3.2.3. The difference between natural and recombined cream .............................................................. 48
    3.2.3.1. Influence of cooling rate after tempering .................................................................................. 48
    3.2.3.2. Influence of tempering ............................................................................................................ 49

4. CONCLUSION

REFERENCE

APPENDIX
List of symbols and abbreviations

\( \theta \)  
Wetting – contact angle

\( \Psi \)  
Shear rate

\( \sigma_{sl} \)  
Solid – liquid interface tension

\( \Delta G \)  
Total free energy change

\( \Delta G_{\text{homogenous}} \)  
Free energy change for the homogenous nucleation

\( \Delta G_{\text{heterogeneous}} \)  
Free energy change for the heterogeneous nucleation

\( \Delta G_v \)  
Volume free energy change

AMF  
Anhydrous milk fat

CR  
Cooling rate

DSC  
Differential Scanning Calorimetry

HMF  
High melting fraction of milk fat

\( L_{aq} \)  
Liquid FID- signal of the aqueous phase of cream

\( L_{cr} \)  
Liquid FID- signal of cream

\( L'_{cr} \)  
Liquid FID- signal of milk fat in cream corrected for the aqueous phase

\( L_{oil} \)  
Liquid FID- signal of an oil

LMF  
Low melting fraction of milk fat

NC  
Natural cream

NMR  
Nuclear Magnetic Resonance

NT  
Non-tempered samples

NS  
Non-sheared samples

MFGM  
Milk fat globule membrane

MH  
Melting heat

MMF  
Middle melting fraction of milk fat

MT  
Melting Temperature
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>RC</td>
<td>Recombined cream</td>
</tr>
<tr>
<td>S</td>
<td>Sheared samples</td>
</tr>
<tr>
<td>SCBMP</td>
<td>Sweet cream butter milk powder</td>
</tr>
<tr>
<td>$S_{cr,T}$</td>
<td>Solid Fat Content of cream at a certain temperature</td>
</tr>
<tr>
<td>SFC</td>
<td>Solid Fat Content</td>
</tr>
<tr>
<td>TAG</td>
<td>Triacylglycerides</td>
</tr>
<tr>
<td>Tcrys</td>
<td>Crystallization temperature</td>
</tr>
<tr>
<td>Tmax</td>
<td>Temperature used in tempering</td>
</tr>
<tr>
<td>$x_{aq}$</td>
<td>Mass fraction of aqueous phase</td>
</tr>
</tbody>
</table>
List of tables

Table 1.1. Fat polymorphism structure.
Table 1.2. Melting fractions in milk fat.

List of Figures:

Figure 1.1. MFGM structure.
Figure 1.2. Nucleation mechanism.
Figure 1.3. Longitudinal stacking of TAGs.
Figure 1.4. Coalescence and Partial Coalescence mechanism.
Figure 1.5. Shear – induced partial coalescence mechanism.
Figure 1.6. Surface – mediated partial coalescence mechanism.
Figure 1.7. Arrangement of semi-crystals in fat globules and changes during tempering.
Figure 1.8. Structure of whipped cream.
Figure 2.1. Diagram of fat crystallization experimental summary.
Figure 2.2. Summary of partial coalescence experiments.
Figure 2.3. DSC Q1000 and aluminium pans.
Figure 2.4. Maran Ultra, NMR tubes and Julabo Waterbath.
Figure 2.5. Rheometer AR2000ex, starch cell and impeller.
Figure 2.6. Leitz Diaplan microscope and Linkam thermal controller.
Figure 3.1. Relationship between crystallization temperature (NC and RC) and cooling rate.
Figure 3.2. Crystallization curves of NC and RC at different cooling rates.
Figure 3.3. Melting heat of NC during storage after cooling at different rates.
Figure 3.4. Melting curves of RC during storage after cooling at 2°C/min

Figure 3.5. Melting curves of tempered samples of NC and RC during storage after cooling at different rates.

Figure 3.6. Crystallization curves of tempered dairy cream at cooling rate of 10°C/min.

Figure 3.7. Melting temperature of tempered NC during storage.

Figure 3.8. SFC of tempered and NT natural cream during storage.

Figure 3.9. SFC of S and NS natural cream during storage after cooling at different rates.

Figure 3.10. Melting heat of S and NS natural cream after cooling and storage 2h.

Figure 3.11. Melting curves of NS and S natural cream cooled at 10°C/min after 3d storage.

Figure 3.12. SFC of natural cream during storage after combining tempering and stirring.

Figure 3.13. Melting curves of NC during storage after stirring, tempering and cooling at 10°C/min.

Figure 3.14. Melting curves of sheared NC during storage after tempering at 20°C and cooling at rate of 5°C/min.

Figure 3.15. Melting SFC of RC samples during storage after tempering and cooling.

Figure 3.16. SFC of NC and RC at different temperature.

Figure 3.17. Melting curves of S_RC during storage after tempering and cooling at 10°C/min.

Figure 3.18. Melting curves of S_RC during storage after tempering at 30°C and cooling at 5°C/min.

Figure 3.19. Viscosity of NC during cooling at different rates.

Figure 3.20. The relationship between viscosity and temperature of NC during the first stage of cooling at different rates.

Figure 3.21. Churning profiles of NC during storage after cooling at 10 and 20°C/min.
Figure 3.22. Viscosity of NC during tempering at different temperature and cooling at 10°C/min.

Figure 3.23. Churning profiles of NC during storage after tempering and cooling at 10°C/min.

Figure 3.24. Churned products from natural cream tempered at 20°C and 30°C.

Figure 3.25. Fat globules and their irreversible clusters after tempering at different temperatures and cooling.

Figure 3.26. Irreversible clusters of NC tempered at 20°C (left) and 30°C (right) (100x magnification) in SDS solution.

Figure 3.27. Irreversible clusters of T20_NC and T30_NC were melted and formed bigger droplets when heating (100x magnification).

Figure 3.28. Churned products from T20 - Recombined cream cooled at 5°C/min and 10°C/min.

Figure 3.29. Viscosity behavior curves of RC at different conditions of tempering and cooling.

Figure 3.30. Churning profiles of RC during storage after tempering at 20°C - 30°C and cooling.

Figure 3.31. Irreversible clusters in T20_RC change when heating sample from 20 to 40°C
III. Abstract

The aim of this study is investigating the effect of tempering and the cooling rate on the stability of natural cream and recombined cream. These factors can change fat crystallization properties and result in the change in shear-induced partial coalescence in cream.

By using Differential Scanning Calorimetry and Nuclear Magnetic Resonance, fat crystallization in natural and recombined cream during storage after thermal (tempering, cooling) and mechanical (shear) processing were investigated. Slow cooling and tempering seem to be convenient for the formation of pure crystals in both natural cream and recombined cream. Late crystallization occurred in cream during storage and it was seen that after 3 days stability was reached. Applying a low shear rate can increase the solid fat content of natural and recombined cream (without tempering) through interdroplet heterogeneous nucleation. However, the formation of pure crystals in the cream during cooling can be limited by shear even if combined with tempering.

The effect of the cooling rate, the shear rate and tempering on shear-induced partial coalescence in natural and recombined cream was investigated based on viscosity profile analysis and light microscopy. The sensitivity of both natural and recombined cream towards shear-induced partial coalescence increased significantly during storage after slow cooling. Tempering at 20°C seems to increase the sensitivity to a maximum in natural cream and remains stable during storage. Shear-induced partial coalescence occurred more easily in recombined cream but it could be limited by tempering at 30°C and fast cooling.
Introduction and Objectives

IV. Introduction and Objectives

Natural cream which is an O/W emulsion (obtained by a physical separation of milk) is a good starting material to produce a high quality whipped cream. Conversely, recombined cream (obtained by redispersing milk fat in an aqueous phase) has a lot of practical advantages concerning storage and use but whipped cream made of recombined cream meets some problems like a low overrun and a high serum loss.

Partial coalescence as influenced by fat crystallization in fat droplets plays a key role in developing these typical characteristics of whipped cream. However, today, knowledge and understanding about these subjects and how they are related to each other were not really adequate. In literature it is suggested that tempering could be a useful method to adjust partial coalescence in cream but studies about the effect of tempering on partial coalescence in recombined cream are lacking.

Therefore, an important objective of this thesis was investigating the changes in structure of recombined cream when tempering and trying to explain how those changes can influence the whipping process. Furthermore, the effect of cooling rate and the shear rate on fat crystallization in dairy cream will be also studied in order to understand better about the mechanism behind tempering and how it influences partial coalescence. Interesting phenomena such as fat polymorphism and interdroplet heterogeneous nucleation will be considered to have overall view about the role of thermal and mechanical processes on the colloidal stability of the product. The comparison will also be conducted between natural and recombined cream to illuminate the difference between these two materials.

Many techniques will be used in this thesis including Differential Scanning Calorimetry, Nuclear Magnetic Resonance, Rheology and Light Microscopy.
1. LITERATURE REVIEW

1.1. Dairy Cream

1.1.1. Natural cream

Natural cream is an O/W (oil – in – water) emulsion with the fat droplets having an average diameter between 0.1 - 10 μm dispersed in a serum phase. Triacylglycerides (TAGs) in the fat droplet form a crystal network entrapping liquid oil in the intercrystalline spaces and hence, they are called solid fat globules. A 10 - 20 nm thick protein - lipid membrane (milk fat globule membrane - MFGM) covers these fat globules and plays a role as strong surfactant stabilizing the cream emulsion. This membrane contains mainly neutral fat components (TAGs and diacylglycerides - DAGs) and bilayers of polar fat components (phospholipids and sphingolipids) (Figure 1.1). The percentage of proteins in the MFGM is 25 - 60% and they play an important role in both nutritional and physicochemical aspects. The presence of proteins adds viscoelasticity to the membrane and hence, prevents coalescence of the fat droplets [1, 18]. Whey proteins and casein micelles are the main protein components of the serum phase and in general play an important role in the stabilization of homogenized cream. Homogenization is applied to reduce the fat droplet size in cream and thus helps to prevent creaming caused by the density difference between serum and fat droplets. Due to homogenization, the MFGM is disrupted and a new protective membrane around the milk fat droplets will be formed from casein micelles, whey proteins and MFGM remaining fragments [13].

Figure 1.1. MFGM structure [18].
1. Literature review

1.1.2. Recombined cream

Similar to natural cream, recombined cream comprises of the main components including milk fat, milk proteins, lactose and water. To produce this type of cream, milk protein (butter milk powder, whey powder or skimmed milk powder) is pre-mixed with water before the anhydrous milk fat (AMF) is added. This mixture will be homogenized to disperse the fat in the serum by forming fat droplets with a similar TAG composition, contrarily, in natural cream, where each fat droplet has a TAG composition slightly different from the others. This difference makes recombined cream easy to control and more stable in thermal processes in comparison to natural cream because the parameters of these processes can be predicted based on the TAG composition of the milk fat in recombined cream. Fat globules in recombined cream are more susceptible to cluster formation than in natural cream. Reason for this is the difference in membrane structure surrounding the fat globules: MFGM in natural cream and a mixture of casein (sub) micelles, whey proteins and remnants of the native MFGM in recombined cream. The presence of protein in these membrane layers supplies hydration repulsion force and prevents clustering of fat globules. While each fat globule of natural cream has a separate membrane layer, casein micelles in recombined cream can be absorbed simultaneously by more than one fat globule and thus, limiting the stereo stability effect [1, 23]. The cluster formation in recombined cream not only reduces the microscopic stability of cream but also affects the physicochemical properties and stability of dairy products produced from this material such as whipping cream (see Section 1.4) [2, 13].

1.2. Fat Crystallization

Fat crystallization is an important thermal processing step that has a strong effect in many food products both on the macroscopic and microscopic structure. The presence of fat crystals brings significant changes to the microstructure of foods and thus, affects the texture of food products. This is especially important for many semi-stable foods such as ice cream, butter and whipping cream. Consequently, the study of crystallization processes in food systems contributes not only to better knowledge of thermodynamics and kinetics related to phase changes and the liquid – solid equilibrium but also to enhance product properties by controlling the influencing factors.
1. Literature review

1.2.1. Nucleation and crystal growth

The first steps of crystallization are supercooling and nucleation. When lowering the temperature of a pure liquid fat, TAG molecules in the liquid phase will gather and form clusters, the liquid in this case is considered as a supercooled liquid. If the temperature is continuously decreased, the radius of the clusters will increase and the volume of the system will be reduced because of the distance decrease between the individual TAG molecules. Nucleates are formed at a critical cluster radius ($r^*$) when there is a balance between the free energy released by the volume change and the consumed energy to form a new solid-liquid interface (Figure 1.2) [7, 8]. This mechanism is called primary homogenous nucleation. Through the collision between these nucleates, some segments are segregated and absorb other TAG forming new nucleates. This process can be considered as secondary heterogeneous nucleation or bulk heterogeneous nucleation occurring at a lower temperature in comparison to the primary homogenous nucleation. However, the fat in most food products is not pure and contains many solutes (impurities). The presence of foreign surfaces from these impurities decreases the barrier energy of nucleation and thus this heterogeneous nucleation often occurs more easily and at higher temperature compared to homogenous nucleation. This mechanism is defined as primary heterogeneous nucleation or surface heterogeneous and it appears in almost all real food systems. The reduction in necessary energy for heterogeneous nucleation in comparison to homogenous nucleation can be estimated based on the wetting – contact angle ($\theta$) between liquid and solid phase (foreign surface of the impurity) (Equation 1.1, 1.2, 1.3) [7].

$$\Delta G_{\text{heterogeneous}} = \Delta G_{\text{homogenous}} \cdot f(\theta) \quad \text{(Eq.1.1)}$$

with

$$f(\theta) = \frac{1}{2} - \frac{3}{4} \cos \theta + \frac{1}{4} \cos^3 \theta \quad \text{(Eq.1.2)}$$

$$\Delta G_{\text{homogenous}} = \frac{4}{3} \pi r^3 \Delta G_v + 4 \pi r^2 \sigma_{\text{sl}} \quad \text{(Eq.1.3)}$$

($\sigma_{\text{sl}}$: solid – liquid interface tension)

Figure 1.2. Nucleation mechanism [8].
The next step in the fat crystallization is the crystal growth. Unlike the first period that is described mainly on thermodynamic principles, this step almost solely depends on kinetic aspects. Actually, the crystal growth is the equilibrium between the diffusion of molecules from the liquid to the nucleate and the departing of segments out of this surface to the liquid (because of the dissolving) [7]. The heat transfer and mass transfer during the crystallization process do not only affect to this diffusion but also influence the arrangement of the molecule chains in the crystal structure. These arrangements play an important role in the stability of food systems and their properties.

1.2.2. Fat polymorphism

In the crystalline lattice, TAGs can be present in two types of conformations: turning fork or stacked chair. During the crystallization, TAGs will form longitudinal stackings (Figure 1.3): the turning fork conformation is suitable with double layer (2L) structure while triple layer (3L) chains are often created from the second conformation. The TAG composition also affects the formation of the longitudinal stackings: the 2L structure is related to the presence of TAGs having similar fatty acids while the 3L structure is favored if three chain moieties of TAGs have different chemical properties (chain length, saturation degree, etc.) [34]. Based on cooling conditions, these long chains will be arranged in different crystalline structures (subcell) that are classified as α, β’ and β polymorph. In the first polymorph, the subcell is formed by straight hydrocarbon chains which are perpendicular to the methyl plane having a hexagonal cross surface. In this arrangement, the methyl plane can rotate easily in any direction, and thus the system has a high free energy and hence, a low melting temperature. Unlike the α polymorph, hydrocarbon chains of the two remaining polymorphs (β and β’) have zigzag forms and are inclined with the methyl plane (Table 1.1). The cross surface of β’ and β forms are orthorhombic and triclinic respectively. In the orthorhombic structure, four methyl planes at the corners must be parallel to each other and perpendicular to the center plane. In the triclinic structure, all planes must be parallel to each other. Obviously, the free energy of the β’ polymorph is smaller (higher melting temperature) than of the α polymorph and bigger than the β polymorph. Hence, the latter form is the most heat stable structure [3, 34].
1. Literature review

Effect of tempering and the cooling rate on the stability of dairy cream

Figure 1.3. Longitudinal stacking of TAGs [11].

<table>
<thead>
<tr>
<th>Polymorphs</th>
<th>α</th>
<th>β'</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcell</td>
<td><img src="image" alt="Hexagonal" /></td>
<td><img src="image" alt="Orthorhombic" /></td>
<td><img src="image" alt="Triclinic Parallel" /></td>
</tr>
<tr>
<td>Crystals structure</td>
<td><img src="image" alt="Hexagonal" /></td>
<td><img src="image" alt="Orthorhombic" /></td>
<td><img src="image" alt="Triclinic Parallel" /></td>
</tr>
<tr>
<td>[34]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Methyl planes rotation</td>
<td><img src="image" alt="Free rotating" /></td>
<td><img src="image" alt="Limited rotating" /></td>
<td><img src="image" alt="Very limited rotating" /></td>
</tr>
<tr>
<td>[35]</td>
<td><img src="image" alt="Free rotating" /></td>
<td><img src="image" alt="Limited rotating" /></td>
<td><img src="image" alt="Very limited rotating" /></td>
</tr>
<tr>
<td>Melting Temperature</td>
<td>Lowest</td>
<td>Intermediate</td>
<td>Highest</td>
</tr>
</tbody>
</table>

Table 1.1. Fat polymorphism structure.
1.2.3. Milk fat

Milk contains more than 400 TAGs in a melting range from -30°C to 40°C [11]. These TAGs can be divided in three fractions: high melting fraction (HMF), middle melting fraction (MMF) and low melting fraction (LMF) (Table 1.2) [36]. While the latter fraction is mainly found in the liquid phase, the two remaining fractions are present as α and β’ forms [19, 25, 26]. Fredrick et al. [19] showed that during the cooling process, TAGs in cream will convert from the liquid state to α crystals and then these crystals can transform to β’ crystals depending on process conditions such as cooling rate and storage time. If the storage time of cream at low temperature is long enough and in the presence of LMF liquid, some HMF in β’ can segregate and then aggregate to form β crystals [25, 26, 36].

<table>
<thead>
<tr>
<th>Fractions</th>
<th>Typical amount (%)</th>
<th>Melting point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMF</td>
<td>5</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>MMF</td>
<td>25</td>
<td>35 – 40</td>
</tr>
<tr>
<td>LMF</td>
<td>70</td>
<td>&gt; 15</td>
</tr>
</tbody>
</table>

Table 1.2. Melting fractions in milk fat [36].

There are significant differences between crystallization of anhydrous milk fat (AMF) and that of the milk fat in cream (emulsion of fat droplets in an aqueous phase). In bulk AMF, the presence of impurities often induces primary heterogeneous crystallization while fat inside some dispersed droplets can be considered as without impurities. This phenomenon often occurs if fat is dispersed in very fine droplets thus the number of fat droplets can exceed the number of impurities. Because of the presence of both fat droplets with and without impurities, the crystallization mechanism must be a combination of both homogenous and heterogeneous nucleation [44]. Besides, β’ crystals seem to be difficult to form directly by cooling cream even at appropriate thermodynamic conditions (slow cooling rate). Moreover, the late crystallization often takes place in cold storage. This delayed crystallization can originate from difficulties in the nucleate formation in fat droplets [10, 34]. These differences will be further investigated in this research through the evaluation of the effect of cooling rate on milk fat crystallization in cream during the storage period. Finally, an interesting phenomenon that can appear in emulsions as dairy creams is the fat crystallization based on the interdroplet heterogeneous nucleation. Unlike the bulk or surface heterogeneous (Section 1.2.1), crystallization can also be
induced when a crystal of a fat globule penetrates the membrane of another supercooled droplet and becomes the nucleate for crystal growth in this droplet [6]. The interdroplet heterogeneous crystallization rate depends on the average size of fat droplets and the properties of their interfacial layer [5, 6]. Small droplets have a small contact surface that limits the possibility of fat crystals at this area, furthermore the distance between these droplets is also not convenient for the protrusion of the crystals [29]. Dickinson et al. [4] stated that the protein layer around the milk fat globules could reduce the interdroplet heterogeneous rate because this visco-elastic layer has high mechanical resistance and strong stereo stability preventing the penetration of fat crystals.

As milk fat is a mixture of many TAGs, mixed crystals are often formed during the crystallization process while pure crystals rarely appear. These mixed crystals can be considered as eutectic mixtures: they have the same melting temperature although the properties of the individual TAGs in the crystal are different [11]. The effect of cooling rate on the formation of mixed and pure crystals will be introduced in the following part. The presence of these crystals influences significantly the properties and texture of many dairy products. For instance, a ripening process is often applied in butter production to form pure crystals that can help limit the presence of fat in the buttermilk (reduction in yield) and enhance the structure of the product. Some studies [9, 15, 37] showed that the presence of mixed crystals or pure crystals effects differently the food texture especially in case of semi-stable products such as whipping cream and ice cream. Another problem of milk fat crystallization is the presence of amorphous parts (liquid crystals). These parts are formed from TAGs having the same arrangement as an α crystal but are still in the liquid phase. The motion of these amorphous parts is limited by the presence of surrounding crystals and storage low temperature. Amorphous liquid is a meta-stable phase (or mesomorphic phase) and therefore, it can convert to a crystal at suitable conditions and this can affect to the structure of food systems during storage. However, research about this part and its role in emulsions are quite rarely [12, 17].

Milk fat crystallization can be influenced by processing conditions like cooling rate and shear rate.
1.2.3.1. Influence of cooling rate

The effect of cooling rate on milk fat crystallization was studied extensively by Fredrick et al., Lopez et al., Campos et al. and Grotenhuis et al. [19, 25, 26, 27, 39]. In general, in AMF, α crystals will appear between 15 - 18°C during fast cooling while during slow cooling β’ crystals are formed directly from the liquid fat between 17 - 20°C [27, 39]. However, the formation of crystalline polymorphs is different in cream compared to AMF. Studies of Fredrick et al. and Lopez et al. [19, 25, 26] showed that α crystals will always be formed at any cooling rate in cream, and then a polymorphic transformation can occur depending on the cooling rate. When fast cooling natural cream, MMF and HMF can separate out of the α crystals and form β’ crystals [19]. In contrast, traces of β crystals can be found in cream at a very low cooling rate (0.1°C/min) [25, 26]. The formation of different crystalline polymorphs at different cooling rates can be explained by combining both thermodynamics and kinetics. According to thermodynamic laws, nucleates of the less stable α polymorph will be formed more easily than nucleates of the more stable polymorphs (β’, β crystals), however, the polymorphic transitions are based on kinetics. Both induction time (for the formation of nucleates from the liquid) and polymorphic transition time depend on the cooling rate and hence, their competition will decide which polymorphic form is created in cream [25, 26, 38]. The cooling rate affects not only the formation of milk fat polymorphs but also the type of crystals such as pure and mixed crystals. By using wide angle X-ray diffraction (WAXD), Lopez et al. and Fredrick et al. [19, 25, 26] confirmed that slow cooling is convenient for the formation of pure crystals because crystal lattices have more time to arrange their structure while fast cooling often leads to unordered crystal structures (mixed crystals).

1.2.3.2. Influence of shear

Besides the cooling rate, shear is also an important factor influencing milk fat crystallization. For bulk fat, research of Kaufmann et al. [16, 17] on the crystal structure formation of AMF in blending milk fat with rapeseed oil showed that shear promotes fat crystallization by increasing the amount of nucleates through breaking big crystals (secondary crystallization) and by enhancing crystal growth. Moreover, shear also helps reducing the polymorphic transition time between α and β’ polymorphs in milk fat [41]. However, study on the effect of shear on fat crystallization in emulsion as cream is very limited although this may
1. Literature review

play an important role in physicochemical properties of many dairy products as whipping cream and ice cream.

1.3. Partial Coalescence

1.3.1. Mechanism

In an emulsion, fat droplets are surrounded by an interfacial layer and separated from each other by a solvent film. Because of the competition between the Laplace pressure due to the curvature of the droplets and the Marangoni effect of surfactants (such as protein and phospholipids) in solution, films between droplets are continuously drained and brings them closer together. Generally, contact surfaces between droplets and solvent films in this case are not completely flat but are fluctuated because of the collision between droplets and thermally excited capillary waves caused by the surface tension and the gravity at interface layers [30]. If this fluctuation is high enough to rupture the film, the surface of two droplets can get in contact directly, merge and then a new droplet will be formed (coalescence) (Figure 4A). In many emulsion systems, dispersed droplets are not completely liquid but contain a crystal network in which liquid fat is entrapped. Through collision, crystals at the interface of a fat globule can pierce the aqueous interfacial film and penetrate the droplet membrane to form a bridge between two droplets. Subsequently, the distance between the fat droplets will drop significantly and the merging can occur. If the liquid content in fat droplets is sufficient, fat liquid can be released out of the crystal network to adhere to bridge and form a permanent junction in an oil neck between the droplets [20]. A crystal network in oil neck prevents the oil flow at rim of neck (driving force for the merging) and thus helps droplets to remain their shape while still keeping them side by side (Figure 4B) [20]. This mechanism is called partial coalescence and lies on the basis of whipping dairy cream.

![Figure 1.4. Coalescence (A) and Partial Coalescence (B) mechanism [20].](image)
In general, the mechanism of partial coalescence is quite similar to interdroplet heterogeneous nucleation but there is a significant difference between these phenomena. Partial coalescence occurs based on the collision of fat globules having fat network while in the latter phenomenon, crystals of a fat globule will penetrate a supercooled droplet and induce the crystallization. Therefore, an increase of SFC is often observed in interdroplet heterogeneous nucleation and not in partial coalescence [4, 5]. In the butter production, partial coalescence helps to decrease the distance between the fat droplets resulting in big aggregates of partially coalesced fat droplets inducing phase inversion. Moreover, partial coalescence contributes significantly to the formation of the foam structure in whipping cream (Section 1.4) and ice cream [15, 31, 32]. However, while the importance of partial coalescence in bringing desirable properties to the food products was confirmed already, methods to control this phenomenon stay unclear because of the complexity of impact factors. Both internal factors such as solid fat content (SFC) [4, 5], the presence of proteins and small molecule surfactants on the droplets interface [2, 12, 32, 33] and external factors (stirring, tempering conditions) [1, 28, 29] influence significantly the partial coalescence mechanism.

1.3.2. Shear induced partial coalescence

In general, both coalescence and partial coalescence are sensitive to shear. When first introducing shear-induced partial coalescence, Boode and Walstra et al. [1, 28, 29] suggested that flow created by stirring not only decreases the distance between the droplets but also makes the droplets rotate around themselves. Because the distribution of fat crystals at the interface of the fat globules is not uniform, this rotation will enhance the capture efficiency (α) of the fat droplets and hence promote partial coalescence (Figure 1.5). To characterize the effect of shear force on this phenomenon, Walstra et al. [1, 2] used the term encounter frequency that has a threshold value for the occurrence of partial coalescence. This parameter represents the probability of collision of particles in solutions and is proportional to the shear rate (Ѱ). With high Ψ values, it can be observed that turbulent flow leads to faster partial coalescence because the high shear stress facilitates droplet collision and increases the encounter efficiency [20]. However, if the shear rate is too high, the protective membrane surrounding droplets will be disrupted by which amphiphillic molecules are released. Consequently, the stability of the emulsion decreases due to loss of the stereo-stability effect [12]. The distance between droplets
becomes smaller and hence, aggregation occurs faster resulting in the formation of bigger fat granules. This is especially important to many meta-stable foods such as whipping or ice cream because the presence of butter granules formed from these aggregates will expel water out of the product and induce phase separation. Finally, the effect of shear on partial coalescence not only depends on the applied force but also depends on the type of surfactant surrounding the droplets [13]. For example, in comparison to small molecule surfactants, the high visco-elastic property of a protein layer at the interface of the droplets gives a better protective role against shear force and thus preventing the aggregation between particles [4, 5].

Figure 1.5. Shear – induced partial coalescence mechanism [2, 20].

1.3.3. Surface – mediated partial coalescence

Beside interactions between single fat droplets, also the interaction between fat droplets and an air/water interface were studied [42]. This complex phenomenon is called surface – mediated partial coalescence and occurs in emulsions containing three phases (oil, water and air). Because of the difference in interfacial tension, fat droplets are adsorbed to air bubbles that are stabilized by proteins in serum. This adsorption releases liquid fat that spreads on the air/water interface and induces partial coalescence between the adsorbed fat droplets (Figure 1.6). The combination of shear-induced partial coalescence and surface-mediated partial coalescence helped to explain clearly the structure of many dairy emulsions (Section 1.4). However, scientists have not been successful yet in building an effective method to adjust the whipping conditions by controlling the partial coalescence. Nowadays, whipped natural cream is always recognized as a perfect example of a food foam stabilized by a very stable network of partially coalesced fat droplets but efforts to improve texture and stability of whipping recombined cream has not brought expected results.
1. Literature review

1.3.4. Influence of tempering on partial coalescence in cream

It is assumed that tempering can influence the partial coalescence mechanism [20, 28, 29, 45]. In tempering, cream that is originally at a low temperature is heated to a certain maximum temperature for a defined period after which it is cooled again to its original temperature. Many parameters of tempering conditions including refrigerator temperature (before tempering), maximum temperature ($T_{\text{max}}$), and cooling rate (after tempering) can influence the partial coalescence through the changes in fat crystal network inside the fat droplets (Figure 1.7). Normally, cream must be kept at low temperature (5°C) before tempering to assure sufficient number of crystals for the partial coalescence [1, 28] and limit microbiological spoilage. During storage at low temperature, liquid fat in dairy cream is entrapped in a crystal network inside the fat droplets. When heating the cream to a temperature below the melting point of the milk fat, the fat will partly melt releasing free crystals (needles) from the crystal network, which can move to the interface of the fat droplets to reduce free interfacial energy [28]. By observing cream with a polarized light microscope Walstra & Boode [1, 28, 29] divided the possible conformations as O, N1, N2, L, M and K (Figure 1.7). These structures differ from each other in amount of crystals, the orientation of the needles with respect to the interface and lastly, their contact angles ($\theta_w$). During tempering, based on the applied $T_{\text{max}}$, fat globules will change mainly between N, L and M type and this phenomenon is defined as fat globules “rebodying” (Figure 1.7) [1]. The N-type is the most frequent crystal arrangement in fat droplets at low temperature [28, 29]. This conformation still remains the fat network and releases less free crystals at the boundary of fat.
1. Literature review

droplets but these needles have suitable $\theta_w$ to protrude membrane and establish bridges connecting droplets. Unlike in N-type, the fat network in L-type is melted completely and most crystals will concentrate at the O/W boundary, parallel with the interfacial layer. M-type fat globule can be considered as a combination of both N and L type. The susceptibility towards partial coalescence of both L and M type is lower than N type because of the dependence on contact angles of fat crystals on the O/W interface: partial coalescence only occurs in these structures if $\theta_w \gg 90^\circ$ [20].

![Diagram of semi-crystals in fat globules and changes during tempering](image)

Figure 1.7. Arrangement of semi-crystals in fat globules and changes during tempering [1].

In the cooling step after tempering, the cooling rate will determine the size of the crystals which will influence the partial coalescence: slow cooling creates bigger crystals at the O/W interface which are able to penetrate the fat droplet membrane and the solvent film more easily [20, 28, 29]. However, this hypothesis is still not unequivocally proven because partial coalescence is too often evaluated through the viscosity and the SFC although both parameters depend greatly on fat crystallization properties. Unfortunately, it is not always straight forward to
analyze fat crystallization properties in a complex system as dairy cream and to make the link with partial coalescence. Besides, there are not much reliable studies supplying insights in the effect of tempering on fat crystallization in dairy cream and consequently on partial coalescence and the whipping process.

1.4. Whipping and whipped cream

Whipping cream is an O/W emulsion with at least 35% fat. During whipping, air is beaten in the cream to form an aerated emulsion (whipped cream). With high foam-forming capacity, serum proteins help to stabilize air bubbles by reducing the interfacial tension between air and water. Besides, fat globules are adsorbed on the air/water interface trapping air bubbles through the surface-mediated partial coalescence (Section 1.3.3) between these fat globules. This type of partial coalescence helps to prevent coalescence between air bubbles. Moreover, the structure of whipped cream is also stabilized by the network of partial coalesced fat globules in the aqueous phase induced by shearing force during whipping (Figure 1.8). If the partial coalescence takes places too fast, big butter granules are formed which will induce phase separation and increase serum loss. Therefore, the balance between two types of partial coalescence is very important to assure product quality [2].

![Figure 1.8. Structure of whipped cream](image)

In general, whipped natural cream is of high quality while whipped cream made of homogenized cream and recombined cream often has a low overrun and a high serum loss. This difference originates from the role of the native MFGM in natural cream. Firstly, in whipping, the disparity in interfacial tension between fat globule - water and air bubble - water is the
driving force for the adsorption of fat globules on the air/water interface (promote surface – mediated partial coalescence). In natural cream, MFGM surrounding fat globules and serum proteins around air bubbles influence the interfacial tension differently and thus supporting this adsorption. On the contrary, the amount of the native MFGM fragments is too small in recombined and homogenized cream to cover completely the fat globules. Consequently, the main components in the newly formed membranes surrounding the fat globules in these creams are whey proteins and casein (sub) micelles that are the same proteins as are present on the interface of air - water and hence reducing the driving force for the adsorption [13]. Secondly, it is shown in literature that recombined cream is more stable because of the protein layers which have a higher thickness and thus limit the destabilization of cream in whipping [14]. Goff et al. [32, 33] attributes this limited partial coalescence to the difficulty of fat crystals to protrude the thick layer of proteins towards the aqueous phase reducing the capture efficiency. Although, there are some interesting arguments funding this hypothesis, evidence about the mechanism of partial coalescence in recombined cream is still lacking. Results obtained from studies of Goff et al. [32, 33] help to confirm that coalescence is difficult to occur between fat globules with a thick protein layer, but the destabilization of fat in these studies was measured by spectroturbidity which is not an effective method to evaluate partial coalescence. Walstra and Odgen et al. [1, 23] showed that clusters could be formed in recombined and homogenized cream because of a reduction in stereo stability (Section 1.1) but the relationship between these clusters and partial coalescence has still not studied sufficiently. Therefore, an important goal of this research is to supply a better insight in the mechanism of partial coalescence in natural and recombined dairy cream.

Nowadays, adding an emulsifier to recombined cream is a popular method to increase the quality of recombined whipping cream. Small molecule surfactants (Tweens, monoglycerides, etc.) will compete with proteins and expel them out of the protective membrane surrounding the fat globules to support the destabilization of cream that is necessary for whipping [14]. Recently, it was showed that mixtures of monoglycerides and milk phospholipids (butter milk powder) can be used to replace the role of MFGM in whipping cream [14, 21]. Besides the emulsifier, the protein source used in producing recombined cream also plays an important role on the properties of whipping cream. For instance, cream residue powder helps to increase the overrun and limits serum loss better than skim milk powder [48]. Some new approaches are being
developed to improve the properties of whipped cream such as using tempering to improve partial coalescence [45] or replacing the role of partial coalescence by an acidified protein based emulsion [22]. Besides, building technology solutions to improve the quality of the product and to limit the use of food additives is also very necessary to bring more healthy products for the consumers.
2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Natural cream (NC)

Whipping cream from the brand Debic of Friesland Campina, Belgium was purchased from the local supermarket. This is an ultra high temperature (UHT) product containing 35% milk fat, 3% carbohydrates and 2.5% proteins. Carragenaan was used as stabilizer in this product. Cream was kept in fridge at 5°C during storage.

2.1.2. Recombined cream (RC)

Recombined cream was produced from anhydrous milk fat (AMF), sweet cream butter milk powder (SCBMP), and water. AMF was purchased from Friesland Campina, Belgium while SCBMP was bought from Interfood, Belgium. The composition of SCBMP includes 51.5% lactose, 30.1% proteins and 8.5% milk fat. In the preparation step, AMF was melted completely in the oven at 50°C and SCBMP was dissolved in water with ratio 1:7.6. Natriumazide was added to this solution at a concentration of 0.01% w/w final product. This chemical substance was used to prevent the development of microorganisms and extend the shelf life of the cream. The SCBMP solution was kept at 5°C for 24 hours for complete hydration. Subsequently, this solution must be heated to 65°C by using a Memmert water bath before adding carragenaan (stabilizer) and stirring. Similar to natriumazide, the concentration of carragenaan in the final product was 0.01% w/w. A pre-emulsion was prepared by homogenizing the AMF in the SCBMP solution using Ultra Turrax homogenizer (IKA, Staufen, Germany). The ratio between AMF and SCBMP was 4.5:1. Finally, this solution was homogenized in a High Pressure Homogenizer (APV, West Sussex, UK) before being fast cooled by tubing in a water bath at -15°C. The RC was also stored at 5°C.

2.2. Methods

2.2.1. Experimental design

*Fat crystallization:* The effect of the cooling rate, the shear rate and tempering on the milk fat crystallization in dairy cream were investigated by evaluating the melting characteristics and
SFC. The melting characteristics were measured by differential scanning calorimetry (DSC) while nuclear magnetic resonance (NMR) is used to measure the SFC. The temperature at which the cream is tempered (Tmax) involves 10, 20 and 30°C. The investigated cooling rates (CR) are 2, 5, 10 and 20 °C/min. The change in fat crystallization properties of cream during storage was observed 2 hours, 3 days and 7 days after the sample treatment comprising a defined cooling rate, a defined shear rate and/or a defined tempering profile. When no shear is applied, the cream is loaded in DSC pans and the cooling rate or tempering profile is applied in the DSC immediately on the pans. If a certain shear rate is applied, the cooling rate or tempering profile is applied on the cream in the starch pasting cell of the rheometer. Because the end temperature of these processes is 5°C, DSC pans and NMR tubes of cream in this case must be prepared in a cooling room at 5°C to avoid temperature fluctuation. An overview of the experiments is shown in Figure 2.1.

Figure 2.1. Summary of fat crystallization experiments.
2. Materials and Methods

*Partial coalescence:* The effect of the cooling rate and tempering on the shear induced partial coalescence in dairy cream was evaluated based on the change of viscosity of cream samples which are churned in the starch pasting cell of a rheometer. Viscosity measurements were conducted at different shear rates: 150 s\(^{-1}\) for NC and 50 s\(^{-1}\) for RC. Light microscopy was used to observe the clusters formed in the cream during churning and thus detect the presence of partial coalescence. These experiments are summarized in Figure 2.2.

![Diagram of partial coalescence experiments]

Figure 2.2. Summary of partial coalescence experiments

2.2.2. *Differential Scanning Calorimetry (DSC)*

Thermal analysis of the samples was conducted by a DSC Q1000 (TA Instruments, New Castle, Delaware, USA). Aluminum Pans are filled with 5 – 15 mg of cream before being sealed. The heat flow of the sample and a reference pan (empty pan) was recorded over time. Three types of analyses were applied in this thesis including: *cooling, tempering* and *melting*.

*Cooling program:* in a pre-conditioning step, samples were equilibrated at 5°C for 10 minutes before being heated at a rate of 10°C/min to 60°C to remove all fat crystals. Consequently, cream was cooled down to 5°C at different cooling rates (2 – 5 – 10 – 20 °C/min).
2. Materials and Methods

**Tempering program:** the pre-conditioning step of this program was similar to the cooling program. After equilibration, samples were heated to $T_{\text{max}}$ (10 – 20 – 30 °C) and remained at a constant temperature for 10 minutes before being cooled to 5°C at different cooling rates (2 – 5 – 10 – 20 °C/min).

**Melting program:** the sample holding chamber must be equilibrated at 5°C before inserting the pan to avoid thermal fluctuation. The pans, after being inserted at 5°C, were heated to 65°C at a rate of 20°C/min. Melting heat and melting temperature were calculated based on the heat flow – temperature graphs using TA Universal Analysis 2000 software version 4.5 (TA Instruments, New Castle, Delaware, USA).

![Figure 2.3. DSC Q1000 and aluminum pans.](image)

After cooling and tempering, samples were kept isothermally at 5°C from 30 to 120 minutes in the sample holding chamber to enhance the stability of products before being stored for a longer period at 5°C (3 days and 7 days). Therefore, the melting heat which was interpreted from the heat flow – temperature graph was the released heat from melting the fat crystals formed in both cooling and during the isothermal periods.

2.2.3. **Nuclear Magnetic Resonance (NMR)**

SFC of cream was measured by an indirect method based on a time domain – nuclear magnetic resonance technique using Maran Ultra 23 pulsed-field gradient NMR (Oxford Instrument, Abingdon, UK). In the indirect method, SFC was calculated based on values of the free induction decay (FID) signals of cream, serum and a reference oil. These signals are characterized by a Lorentzian function $L(t)$ with $t$ at 70μs [21, 47]. Rapeseed oil (Vandermootele, Brussels, Belgium) was used as reference oil in all NMR experiments of this thesis. The serum of
natural cream was obtained by churning cream with a Hobart mixer and then separating the butter from the serum with a cheesecloth. For recombined cream, serum was created by diluting SCBMP in water at a ratio of 11.3% w/w. NMR tubes were filled with 0.5mL of sample. FID signals were measured at two temperatures: investigated temperature for SFC and the reference temperature. The latter temperature was chosen as 45°C to assure all milk fat in the cream is in the liquid state. Finally, SFC of cream at a certain temperature \( S_{cr,T} \) will be calculated based on the following equation (Equation 2.1):

\[
S_{cr,T} = 100 - 100 \frac{L_{oil,AS^\circ C}}{L_{oil,T}} \cdot \frac{L'_{cr,T}}{L'_{cr,AS^\circ C}}
\]  

(Eq 2.1)

\( L_{oil} \) is the Lorentzian value (L-value) calculated from the FID signal of rapeseed oil (reference oil).

\( L'_{cr} \) is the corrected L-value of cream after subtracting the contribution of the aqueous phase to the L-value of cream using equation 2.2:

\[
L'_{cr} = L_{cr} - x_{aq}L_{aq}
\]  

(Eq 2.2)

With \( L_{aq} \) and \( L_{cr} \) are L-values calculated from measured liquid FID signals of serum and cream respectively. All L-values were calculated based on one gram of samples.

\( x_{aq} \) is the weight percent of serum phase in cream. This value for both NC and RC is 0.65.

In this thesis, NMR experiments were conducted for all sheared samples after storage for 2h, 3d and 7d. Besides, the changes in SFC during storage of NC and RC and also the SFC at different tempering temperatures (20 - 30°C) were investigated. Temperatures of NMR tubes were controlled by Julabo water bath (JULABO, Seelbach, Germany).

Figure 2.4. Maran Ultra, NMR tubes and Julabo Waterbath
2. Materials and Methods

2.2.4. Rheometer

Cream was sheared and the viscosity was measured by a AR2000ex Rheometer (TA Instrument, Brussels, Belgium). This is a stress-controlled rheometer using a Julabo water bath to support the cooling.

2.2.4.1 Cooling rate

A pre-conditioning at 5°C was necessary for all samples before heating. For samples without tempering, cream was heated to 45°C to remove all fat crystals before cooling. The shear rate was kept at 5s\(^{-1}\) in all stages of both procedures. After cooling, cream was still kept in the starch pasting cell at 5°C at a shear rate of 5s\(^{-1}\) during 30 minutes to reach thermal stability. Samples after shearing must be transported immediately in cooling box to cooling room for storage and to prepare DSC pans and NMR tubes.

2.2.4.2 Tempering

For each sample, approximately 40mL of cream was filled in the starch cell and an impeller was used to stir and measure the viscosity. The procedure of tempering using a rheometer includes: heating sample from 5°C to Tmax, remaining constant at Tmax for 10 minutes and consequently, cooling to 5°C at different cooling rates (5/10 °C/min). The holding period at Tmax was chosen based on pre-experiments about the viscosity change of samples during the isothermal period. In these pre-experiments, cream in starch cell was hold for 30 minutes at different temperatures (10/20/30°C) and the viscosity during this process was observed. Both natural and recombined creams reached stable viscosities between 5 and 10 minutes tempering. Therefore, 10 minutes was chosen as a holding time for all samples in tempering experiments using rheometer. Cooling rate of 20°C/min was not applied for tempered samples because the discrepancy between Tmax (20/30°C) and the temperature at the end of the cooling stage (5°C) was so small. In this case, cooling time becomes very short (1 -2 minutes) and consequently a well heat distribution in the starch cell during cooling can not be assured. Samples after shearing were transported immediately under cooled conditions to the cooling room at 5°C for storage and to prepare DSC pans and NMR tubes (at 5°C).
2. Materials and Methods

2.2.4.3 Shear-induced partial coalescence

A high shear rate is applied to the cream, inducing shear-induced partial coalescence. The progress of the viscosity is a measure for the rate of shear-induced partial coalescence. 30mL of cream was sheared at shear rates of 50 s\(^{-1}\) for RC and 150 s\(^{-1}\) for NC in the starch cell. The temperature during churning was kept constant at 20°C. During pre-experiments, it was seen that recombined cream is much more sensitive for shear-induced partial coalescence. This can lead to difficulties in observing the different phases in the churning profile. Therefore, in these experiments, the shear rate used for recombined cream is smaller than for natural cream.

![Rheometer AR2000ex, starch cell and impeller.](image)

**Figure 2.5.** Rheometer AR2000ex, starch cell and impeller.

2.2.5. Light Microscopy

A DIAPLAN light microscope (Leitz, Leica, Germany) was used to observe clusters in cream. Images were recorded by an Olympus Color View camera (Olympus, Aartselaar, Belgium) at a magnification of 100x. Heating and cooling was controlled by a thermal-peltier-controller PE 94 (Linkam, Surrey, UK). Samples were diluted ten times with water or with sodium dodecyl sulphate (SDS) 1% w/w. SDS is an anionic surfactant having hydrophobic interactions with casein and therefore preventing the formation of reversible clusters in cream [21, 49]. Detecting partial coalescence in cream was conducted by observing the change of aggregates in samples when heating from 20°C to 55°C. If partial coalescence occurred in the sample, aggregates would melt and form one big droplet. Partial coalescence of tempered cream was investigated at 3 periods: after tempering, after cooling and in storage.
2. Materials and Methods

Figure 2.6. Leitz Diaplan microscope and Linkam thermal controller.

2.2.6. Statistical Analysis

All experiments were repeated at least three times and the results were statistically analyzed using SPSS (version 16, IBM, USA). A Two-Way ANOVA and Tukey method were combined to investigate the effect of Tmax and storage time on fat crystallization of natural cream through comparing parameters as SFC, melting heat and melting temperature of samples. For detecting the influence of cooling period, a One Way ANOVA and Tukey comparison were used to analyze the change of crystallization temperature and melting heat at different cooling rates. Because recombined cream was investigated at two levels of Tmax (20-30°C) and cooling rates (5-10°C/min), Least Significant Difference (LSD) was used to replace the role of the Tukey method in comparing experimental results of this cream. The normality of data was assured by the Komogrov-Smirnoff test or applying the Central Limit Theory. The Levene Test was used to assure equal-variance requirement before analyzing with ANOVA. All data analysis was controlled at a 95% confidence interval.
3. RESULTS AND DISCUSSION

3.1. Fat crystallization in dairy cream

3.1.1. Effect of thermal processing

3.1.1.1. Effect of cooling rate

The cooling rate can influence fat crystallization in dairy cream through three aspects: the crystallization temperature (Tcrys), the formation of different polymorphs and the changes during storage. DSC was used to study the fat crystallization of NC and RC when cooling from 60°C to 5°C at different cooling rates (2 – 5 – 10 – 20 °C/min) (Figure 3.2). The cooled cream was melted completely after 2h, 3d and 7d storage to evaluate the changes of fat polymorphs in the cream. The obtained results are in line with previous studies about the fat crystallization in dairy cream [19, 25, 26].

According to Figure 3.1, Tcrys decreased as cooling rate increased. This result was supported by an one-way ANOVA analysis with p-value < 0.05. Besides, a Tukey comparison at 5% family-wise-error also showed a very clear decrease of Tcrys caused by the increase of cooling rate (Appendix B1.1). With very high R² values for the ANOVA models (0.98 for NC and 0.97 for RC), crystallization temperature seems to be a linear function of cooling rate. Between two types of dairy cream, the values for recombined cream are significantly lower than for natural cream. In other words, milk fat in natural cream will be crystallized more easily than in recombined cream.

![Figure 3.1. Relationship between crystallization temperature (NC and RC) and cooling rate.](image-url)
The difference in the formation of fat polymorphs during cooling at different rates can be observed on heat flow – temperature graphs (Figure 3.2). At slow cooling rates (2 - 5°C/min), probably only the least stable fat polymorphism (α) was created during cooling because the graph of these samples only contained one big crystallization peak. Graphs which represent milk fat crystallization in cream at high cooling rates (10 – 20°C/min) have at least two separated crystallization peaks. The second crystallization peak can be formed by the transition of α2L → α3L crystals during fast cooling dairy cream [25, 26]. These α3L crystals can be converted to β’2L crystals during isothermal period at 5°C after cooling [19]. There is not much difference between crystallization behavior of NC and RC. However, at a cooling rate of 20°C/min, peaks of recombined cream are sharper than of natural cream. It could be explained based on the dispersion of milk fat in the aqueous phase. In comparison with NC, the TAG composition in fat droplets in RC is more homogeneous and thus their milk fat crystallization occurs more simultaneous. This leads to difficulties in forming nucleates in RC resulting in lower Tcrys (Figure 3.1) but also brings many favorable conditions for observing the changes in crystallization of this cream. For example, a small peak can be found in almost all the crystallization curves of recombined cream, especially in samples cooled at rate 2°C/min, can be caused by the transition from liquid fat to γ state. The γ polymorph can be considered as an intermediate phase between solid and liquid. Therefore, its peak has small area and only be observed clearly in slow cooling. It is very difficult to find this peak in the graph of natural cream because fat crystallization in this cream is too heterogeneous.

Figure 3.2.a. Crystallization curves of RC at different cooling rates (2-5-10-20°C/min). Peak 1: liquid fat → γ; peak 2: γ → α2L; peak 3: α2L → α3L.
3. Results and Discussion

Unlike two above aspects, the influence of cooling rate on the melting heat (MH) and melting temperature (MT) of dairy cream during storage was unclear. There were slight increases in melting heat of slow cooled samples during storage (Figure 3.3). However, two-way ANOVA showed that both cooling rate and storage time did not affect significantly the MH and MT of natural cream (p>0.05) (Appendix B1.2). In the case of recombined cream, a similar result was also obtained for melting heat but the combination of ANOVA and Tukey comparison showed that melting temperature increased during storage (Appendix B1.3). This conclusion can be observed in Figure 3.4 that describes the melting curves of cooled recombined cream at a rate of 2°C/min after 2h, 3d and 7d storage. An increase of melting temperature could be related to the formation of more pure crystals and the transition of $\alpha \rightarrow \beta'$ crystals during storage. This result helped to reconfirm the hypothesis of Fredrick et al. and Lopez et al. [19, 25, 26] about the polymorphic transformation between $\alpha$ and $\beta'$ crystals in cream during storage at low temperature.

Figure 3.2.b. Crystallization curves of NC at different cooling rates (2-5-10-20°C/min).
3. Results and Discussion

Effect of tempering and the cooling rate on the stability of dairy cream

3.1.1.2. Effect of tempering

Besides DSC (Figure 3.5 and 3.6), NMR was used to evaluate the changes of fat crystals in NC and RC during storage after tempering at 20 – 30°C and cooling at different rates (5 - 10°C/min) (Figure 3.8). Solid Fat Content (SFC) of the cooled cream was measured after 2h, 3d and 7d storage. These experiments (both DSC and NMR) brought many interesting results and therefore, a new hypothesis was suggested to explain the relationship between tempering and the formation of pure crystals in dairy cream.

Figure 3.3. Melting heat of NC during storage after cooling at different rates (2-5-10-20°C/min).

Figure 3.4. Melting curves of RC during storage after cooling at 2°C/min.

Effect of tempering and the cooling rate on the stability of dairy cream
Figure 3.5 shows the clear difference between creams tempered at 20°C (T20) and 30°C (T30). It can be seen that melting curves of T30 samples only contain two melting peaks while at least three separated peaks can be observed in graphs of T20 samples. The appearance of three melting peaks in dairy cream is very specific. Many studies [19, 25, 26] suggested that in cooling cream, α crystals are always formed and then some α crystals transform to β’ crystals during storage or during fast crystallization. The melting of milk fat fractions containing these crystals created two separated melting peaks. This was reflected clearly in the melting curves of samples without tempering (Figure 3.4). The first melting peak could be of α3L crystals in the low melting fraction (LMF) and the second could be of mixture 2L crystals (α + β’) in milk fat middle and high melting fraction (MMF + HMF). Three melting peaks are only discovered in melting of anhydrous milk fat whose is treated in specific conditions (very slow cooling and long time storage) to form three types of polymorphisms: α, β’ and β crystals [25, 26, 36]. But the melting point of β crystals is quite high while all melting peaks of T20 samples are in the range of 20 - 40°C (Figure 3.5). In other words, these peaks only reflect the melting of α and β’ crystals. This confirmed the presence of three different types of fat crystals in dairy cream after tempering at 20°C and cooling: α3L crystals, mixture 2L crystals (α + β’) and pure crystals (β’2L). A hypothesis about the formation of fat crystals after tempering can be explained based on the difference from applied Tmax. At 20°C, mainly α3L crystals in LMF and some α2L crystals of MMF were melted while most β’2L crystals of MMF and HMF were still retained. When subsequently cooling, β’2L crystals of these fractions will separate out of the mixture α-β2L crystals and form more pure crystals (β’2L). The melting of these pure crystals could be the reason for the appearance of the third melting peak in graphs of T20 samples (Figure 3.5). At higher temperature (30°C), only very high stable crystals (β’2L) remaining at this temperature, that will form pure crystals during cooling. The α2L crystals are not present and therefore the melting curves of these samples have two peaks of α3L in (LMF) and β’2L crystals (in MFF and HMF). Fat crystallization of tempered samples can be observed in Figure 3.6. It can be seen that at same cooling rate (10°C/min), in tempered samples, the second crystallization peak that represent for the formation of β’2L crystals during cooling is clearer in comparison with non-tempering samples (Figure 3.4). Besides, the significant increase in melting temperature of cream during storage is only found in tempered samples (Figure 3.7). These confirmed that tempering supports the formation of more β’ crystals in dairy cream. Among two types of cream,
cooling peaks of natural cream have lower slope than of recombined cream. This is logic because fat composition of droplets in RC is quite homogeneous and therefore the phase change occurs more clearly.

Figure 3.5. Melting curves of tempered samples (20 - 30°C) of NC (a) and RC (b) during storage after cooling at different rates (5-10°C/min).
3. Results and Discussion

Effect of tempering and the cooling rate on the stability of dairy cream

Figure 3.6. Crystallization curves of tempered dairy cream (20-30°C) at cooling rate of 10°C/min.

Figure 3.7. Melting temperature of tempered NC during storage.

Influence of tempering on SFC in natural cream during storage can be observed in Fig 3.8. Two-way ANOVA and Tukey showed that tempered samples have lower SFC than non-tempered samples (NT) (Appendix B1.4). The opposite was suspected because the crystallization after tempering is heterogeneous crystallization due to the presence of remaining fat crystals in the fat globules. This process occurs more easily than homogenous crystallization in NT samples and therefore could lead to a higher SFC in tempered samples. A new hypothesis needs to be built to explain the experimental results. Normally, liquid fat is trapped in network of fat crystals which
3. Results and Discussion

is the main structure of fat globules in cream. When tempering, this network is only partly collapsed because fat crystals are not melted completely. Therefore, the amount of liquid fat is not released in tempering samples and they stay in amorphous state during cooling. In this state, the free motion of TAGs is limited and they have more difficulties gather to form new clusters or to attach to already existing crystals in the crystal growth. Consequently, SFC of tempered cream is smaller than of NT samples even after long storage periods. However, there is no significant difference in SFC of samples tempered at 20°C and 30°C (Appendix B1.4). In all samples, there is a significant increase in SFC between 2h and 3d storage. In other words, fat crystallization of both tempered and non-tempered creams continues during storage and can achieve the stability after 3 days. This conclusion is also in line with the studies of Fredrick et al [19] about the late crystallization in dairy cream. Finally, in the performed tempering experiments, cooling rate did not bring any significant difference.

Figure 3.8. SFC of tempered and NT natural cream during storage.

3.1.2. Effect of agitation/shear

Rheometer with impeller and starch pasting cell was used to stir the cream at low shear rate (5 s⁻¹), temper at 20 - 30°C and cool at different rates (5 – 10 – 20°C/min) before further experiments (DSC and NMR) were conducted. Obtained results from analyzing MH, MT and SFC of the samples after 2h, 3d and 7d storage show that shear have complicated effects on the fat crystallization of the cream and can relate to some colloidal phenomena such as interdroplet heterogeneous nucleation and the shear-induced partial coalescence. Some hypotheses were suggested to better understand the role of shear in destabilizing fat crystallization of the cream.
The difference in SFC and melting heat between sheared (S) and non-sheared (NS) natural cream can be observed in Figure 3.9 and 3.10. It can be seen that S samples had higher SFC than NS samples, especially after two hours of storage. This result was also confirmed by Tukey comparison (Appendix B1.5). A possible explanation for the SFC increase can be the crystallization induced by interdroplet heterogeneous nucleation. Before cooling, milk fat in completely liquid state was dispersed in fat droplet. Because each droplet of natural cream has specific composition of TAGs, the crystallization occurs in droplets is different. This means during cooling, both fat globules (containing fat crystals) and fat droplets (completely fat liquid) are present. When stirring, the collision between these particles increased and supported the penetration of crystals from fat globules against through the membrane of liquid fat droplets. Subsequently, interdroplet heterogeneous nucleation occurred favoring the SFC in S samples. In contrast, the melting heats of S samples were significantly lower in comparison with NS samples (Figure 3.9). The low value of melting heat could show that fat crystals which are formed during stirring are less stable. This is very interesting because stirring helps to increase heat dissipation supporting the crystallization. Therefore, in theory, crystals formed during stirring should have higher stability. In these experiments, the formation of less stable fat crystals can be explained based on the following hypothesis. Agitation, even at very low shear rate, created a circle flow in solution. In microscopic level, effect of this flow can be significant and this hinders the arrangement of TAGs in crystallites. In other words, shear did not retard fat crystallization but it was inconvenient for forming crystals which require long time to rearrange structure. This conclusion is quite similar as study of Tarabunika et al. [46] about a delay of the transition $\alpha \rightarrow \beta'$ in crystallizing palm oil at low shear rate. The difference in melting heat between sheared and without sheared samples was not significant after long storage time because of the late crystallization (Appendix A1.3, A1.4). However, despite of significant influences on SFC and melting heat of natural cream, stirring did not affect much melting behavior (the number and the sharpness of melting peaks) of these samples. There is no significant difference of melting graphs between S and NS creams (Figure 3.11). Finally, SFC of natural cream after stirring did not depend on cooling rate (Appendix B1.5).
3. Results and Discussion

Effect of tempering and the cooling rate on the stability of dairy cream

Figure 3.9. SFC of S and NS natural cream during storage after cooling at different rates (5-10-20°C/min).

Figure 3.10. Melting heat of S and NS natural cream after cooling and storage 2h.

Figure 3.11. Melting curves of NS and S natural cream cooled at 10°C/min after 3d storage.
3. Results and Discussion

3.1.2.1. The combination of tempering and agitation

NMR results showed effects of tempering and agitation on the fat crystallization in natural cream (Figure 3.12). S samples have higher SFC than NS samples possibly because of the interdroplet heterogeneous nucleation. Among S samples, SFCs of tempered samples have lower values. There are two ways to explain this result. The first reason was displayed in discussing about the effect of tempering on the fat crystallization of natural cream without shear (Section 3.1.1.2). The second reason can originate from the difference between two interesting phenomena in food colloids: interdroplet heterogeneous nucleation and partial coalescence. Without tempering, fat globules are formed during cooling and subsequently, the former phenomenon occurs between these fat globules and fat droplets resulting in the increase of SFC in samples. However, when applying tempering, fat droplets with crystals inside can collide with each other and this is favored for the shear-induced partial coalescence. This phenomenon can also increases SFC in samples through establishing a new crystal network between fat droplets but is not as much as of interdroplet heterogeneous nucleation [44].

Figure 3.12. SFC of natural cream during storage after combining tempering and stirring.

Similar as NS cream, there is no significant difference of SFC between T20 and T30 sheared samples (Appendix B1.6). However, Tmax affected considerably the melting behavior of S samples (Figure 3.13). According to DSC analyses, pure crystals seem to be hard to form in sheared samples even if tempering because the melting peaks of these samples are much broader compared to tempered samples without shear. At a temperature of 20°C, probably many β’2L crystals were present in the samples but the separated melting peak of β’ crystal did not appear in
melting curves of these samples. The melting peaks were found on graphs of T20_S samples can be of $\alpha_3L$ crystals and mix 2L ($\alpha$-$\beta'$) crystals (Figure 3.13a). In T30_S samples, melting peaks of pure $\beta'2L$ crystals are not as clear as for NS samples at the same tempering conditions. In comparison to T20 samples, natural cream tempered at 30°C seems to have more stable fat crystals and thus during cooling, pure crystals can be formed more easily. Possibly the presence of the highly stable crystals in natural cream after tempering at 30°C partly limited the negative influence of shear in forming $\beta'$ crystals. An increase of melting temperature during storage can be observed in T30_S samples (Figure 3.13b). This could mean the transition between $\alpha$ and $\beta'$ polymorphism during storage still occurs in these samples.

Figure 3.13. Melting curves of NC during storage after stirring, tempering at 20°C (a) and 30°C (b) and cooling at 10°C/min.
Effect of cooling rate on the fat crystallization during shearing and tempering of natural cream can be evaluated by comparing Figure 3.13 and 3.14. At the same shear rate and the same tempering conditions (20°C), the presence of pure $\beta'$2L crystals can be observed more easily in slower cooled samples (5°C/min). In this condition, milk fat crystallites have more time to arrange their structure and thus to overcome the negative effects of shearing in forming highly stable crystals. In comparison with S_NT samples, the effect of cooling rate on tempered samples has some differences. Without tempering, the fat crystallization of natural cream depends on both stirring (shear-induced interdroplet heterogeneous nucleation) and crystal growth. This leads difficulties in evaluating effect of cooling rate on the fat crystallization in these samples. In tempered samples, fat crystals are probably still present in almost all the fat globules and this limits the interdroplet heterogeneous nucleation. In this case, highly stable crystals were formed based on the existing crystals in the fat droplets and this process was supported by a low cooling rate.

Figure 3.14. Melting curves of sheared NC during storage after tempering at 20°C and cooling at rate of 5°C/min.

3.1.2.2. Difference between natural and recombined cream

The effect of cooling rate and tempering on sheared samples of recombined cream is different from natural cream. Both Figure 3.15 and the Least Significant Difference (LSD) comparison (Appendix B1.7) show that during storage after cooling, SFCs of samples tempered at 20°C are higher than of T30 samples. In recombined cream, the effect of tempering can probably be
3. Results and Discussion

Effect of tempering and the cooling rate on the stability of dairy cream

observed more clearly because each fat droplet has a similar fat composition. More fat crystals are present in samples at 20°C compared to 30°C (Figure 3.16) and therefore, during the cooling, fat crystallization can occur more easily. Because each fat droplet of natural cream has a characteristic fat composition, the influence of tempering can not be seen as clear as in recombined cream. Therefore, in natural cream, the difference of SFC was only significant between tempered and without tempered samples. Similar for tempering, the cooling rate has a stronger effect on the fat crystallization in recombined cream when stirring. Firstly, slow cooled samples have lower SFCs in comparison with fast cooled samples after 2h storage (Appendix B1.7). This is logic because fast cooling creates more nucleates than during slow cooling and helps crystallization to occur faster. It can be seen that after a long storage time (3d or 7d), the difference of SFCs between fast and slow cooled samples are not significant anymore (Figure 3.15). Secondly, the effect of cooling rate on the fat polymorphism in recombined cream is much clearer. Figure 3.18 shows the presence of three types of crystals: α3L, β′2L and mixture 2L crystals in samples that were tempered of 30°C and fast cooled (10°C/min) after 2h storage. Three separated melting peaks can be found in the melting curves of these samples. During storage, some α2L crystals transform to β′2L crystals increasing the amount of pure crystals. The merging of two melting peaks of mix 2L crystals and pure β′2L crystals to form a new peak (of β′2L crystals) can be observed when comparing the melting curves of T30_CR10 samples after 2h, 3d and 7d storage (Figure 3.17b). When slow cooling, the formation of pure crystals is favored and thus the melting peaks are more clearly compared to the broad peaks in fast cooled samples. When tempering at 20°C, a big broad melting peak of mix crystals can be found on melting curves of recombined cream (Figure 3.17a). In this condition, the crystal growth of less stable crystals can’t be enough to overcome the retard caused by shear. Therefore, the formation of pure crystals can be limited in these samples.

Figure 3.15. SFC of RC samples during storage after tempering and cooling.

Effect of tempering and the cooling rate on the stability of dairy cream
3. Results and Discussion

Effect of tempering and the cooling rate on the stability of dairy cream

Figure 3.16. SFC of NC and RC at different temperature.

Figure 3.17. Melting curves of S_RC during storage after tempering at 20°C (a) or 30°C (b) and cooling at 10°C/min.
3. Results and Discussion

Effect of tempering and the cooling rate on the stability of dairy cream

Figure 3.18. Melting curves of S_RC during storage after tempering at 30°C and cooling at 5°C/min.

3.2. Partial coalescence

3.2.1. Effect of cooling rate

The combination of viscosity analysis during cooling (Figure 3.19 & 3.20) and measuring the churning time of samples after different storage periods (Figure 3.21) contributed to illuminate the influence of the cooling rate on partial coalescence in natural cream. According to Figure 3.19, the viscosity curve of natural cream during cooling can be divided into three stages with three different slopes. The first stage having the highest slope describes the viscosity change which is caused by the phase transition from liquid to solid state. In this stage, it can be seen that samples which were cooled at lower rate have a higher viscosity in comparison to fast cooled cream at the same temperature (Figure 3.20). The formation of bigger crystals inside the fat droplets during slow cooling can help to enhance the mechanical resistance of the droplet against the flow effect resulting in an increasing viscosity of the samples. Besides, the viscosity of the continuous phase also increased during this cooling step. In the second stage, fat crystals grow and can penetrate the interfacial layer inducing interdroplet heterogeneous nucleation between fat globules and fat droplets. The combination of this phenomenon and the growth of the crystals can be the main reasons leading to a viscosity increase in this period. These processes occur slower than the nucleation; therefore the slope of this stage is smaller than of the first stage.
Similar as the previous part, the third stage is situated in the isothermal period (at 5°C). The viscosity increases slightly in this period for all samples but differences are observed between the different cooling rates. Cream cooled at a low cooling rate has a slightly higher viscosity in this stage. Because most fat droplets are converted to fat globules in the second stage, the interaction between the colloid particles during this final equilibration at 5°C can induce partial coalescence. Slow cooling helps to form bigger crystals that can more easily protrude the membrane and establish bridges between the fat globules resulting in a higher viscosity. This hypothesis is supported by the results of the churning experiments (Figure 3.21). After 2h and 3d of storage at 5°C, the churning time of CR10 sample is smaller than of CR20 samples possibly because partial coalescence occurs more easily in the slow cooled samples. During storage, the churning time decreased gradually because the crystal growth continued increasing the crystal size and promoting partial coalescence. It can be seen that disparities of churning time between cooled samples at different rates decreased over storage time. After 7 days, the churning time of both slow and fast cooled samples is approximately the same. This can be explained by saying that fast cooling creates more nucleates in cream which can grow through the late crystallization during storage. In conclusion, the cooling rate has an influence on the partial coalescence of natural cream but this effect is only significant shortly after cooling.

Figure 3.19. Viscosity of NC during cooling at different rates (5 – 10 – 20 °C/min).
3. Results and Discussion

Figure 3.20. The relationship between viscosity and temperature of NC during the first stage of cooling at different rates (5 – 10 – 20 °C/min).

Figure 3.21. Churning profiles of NC during storage after cooling at 10 and 20°C/min.

3.2.2. Effect of tempering

In comparison to the influence of the cooling rate, tempering shows a more complicated effect on partial coalescence in natural cream. The viscosity changes of samples during tempering and cooling are shown in Figure 3.22. It can be seen that the viscosity reduced during tempering
because of the melting of fat crystals decreasing the mechanical resistance of the fat globules. The viscosity of the cream at a higher temperature (30°C) was lower than at low temperature (10°C) because the amount of melted crystals increased with the temperature. Besides, the viscosity of the continuous phase also decreases when increasing the temperature. The effect of tempering was especially interesting when looking at the viscosity behavior of cream during the isothermal period after cooling. In this period, the viscosity of the cream that was tempered at a higher temperature showed a clear increase in comparison with cream that was treated at a lower temperature. This behavior can be explained by a combination of three processes which can occur during this period including: crystal growth, interdroplet heterogeneous nucleation and partial coalescence. Firstly, when considering the same cooling rate (10°C/min), samples which were tempered at higher temperature contained fewer crystals at the end of tempering. Therefore, crystal growth will be slower compared to cream that was tempered at a lower temperature. Secondly, in tempered samples, the shear induced collision mainly occurred between partly-crystallized fat droplets because fat crystals were not melted completely. Therefore, the effect of the interdroplet heterogeneous nucleation in tempered cream is probably not so considerable. Therefore, it can be assumed that next to the crystal growth, partial coalescence can be the main reason for the viscosity increase of natural cream during the isothermal period after tempering.

![Graph showing viscosity of NC during tempering at different temperature (10-20-30°C) and cooling at 10°C/min.](image)

Figure 3.22. Viscosity of NC during tempering at different temperature (10-20-30°C) and cooling at 10°C/min.
The effect of tempering and the isothermal period on shear-induced partial coalescence was investigated by measuring the churning time (Figure 3.23). It can be seen that T20 samples have shorter churning time in comparison with T10 samples. At 10°C, only a small amount of the fat crystals is melted (SFC10 = 40%). Therefore, the fat network inside the fat globules is not collapsed significantly and only few crystals can be released from the network to reach the O/W interface. Because the absence of fat needles at the interface results in very low capture efficiency, tempering NC at 10°C doesn’t increase the sensitivity towards partial coalescence: the churning time of this sample after 7d storage is approximately the same as for non-tempered cream (Figure 3.23). It can be assumed that T20 samples have good balance between fat crystals and fat liquid (SFC20 = 24%) resulting in enough crystals that are able to move to the interface through the liquid fat. During storage, the churning time of T10 samples reduced gradually because of the late crystallization. However, the churning time of T20 samples seems to be constant during storage. This means that natural cream after tempering at 20°C and cooling with rate of 10°C/min can gain maximum of partial coalescence between fat globules. In other words, surface of partly-crystallized fat droplets in these samples can be covered completely by other fat droplets through partial coalescence. Therefore, there is no space for the forming new linkages between the fat droplets during storage resulting in a constant churning time. In comparison with NT samples, T20 samples have a considerable shorter churning time (Figure 3.23a). This is in line with studies of Boode et al. and Walstra et al. [1, 28] who stated that partial coalescence was induced through the “rebodying” of fat globules caused by tempering (section 1.3.4). When looking at the churning time of samples tempered at 30°C, it is seen that they have a very long churning time compared to cream without tempering. The churning time of T30 samples also decreased after a long storage period but is still very long in comparison with the others. Besides, at the end of the churning process, the viscosity at phase inversion is very low. In other words, it is difficult to form a continuous network of fat droplets through partial coalescence in cream tempered at 30°C. This result is confirmed by observing the structure of the butter granules after churning (Figure 3.24). While T20 samples have a high consistency, T30 samples have a weak paste-like structure rather than firm, solid butter granules.
Figure 3.23. Churning profiles of NC during storage after tempering at 10-20°C (a), 30°C (b) and cooling at 10°C/min.
The previous results showed two specific properties of natural cream tempered at 30°C: high viscosity after cooling and weak network formation. Therefore, the following hypothesis is put forward to explain the relationship between tempering and partial coalescence in natural cream (Figure 3.25). At 10°C, only part of the fat is melted by which a limited amount of fat crystals can move to the O/W interface resulting in less crystals at this area and a lower sensibility towards partial coalescence. The low amount of crystals at the interface can also explain the lower viscosity after cooling in comparison with cream before tempering (Figure 3.22). When raising the temperature to 20°C, more fat crystals are released out of the network and their presence at the O/W interface can reach a maximum. During cooling, these crystals can grow and penetrate the membranes of other fat globules to form irreversible clusters through partial coalescence. This was confirmed by microscopic images at magnification of 100x taken after tempering (Figure 3.26). When heating, fat globules in T20 samples did not separate out of the clusters but merged to form bigger droplets (Figure 3.27). Because T20 samples have a lot of needles at the boundary of fat globules, many bridges can be established around these globules and can be used for partial coalescence. If the temperature is increased to 30°C, only a few fat crystals remain inside the droplets which can move to the O/W interface. Unlike that in T10 samples which also has less crystals at the interface after tempering, the crystals of T30 samples have bigger size in cooling because the amount of liquid fat can be used for further crystallization in cream tempered at 30°C is more than in T10 samples (SFC30 = 12%). Although partial coalescence also occurs in T30 samples, it is seen from Figure 3.26 and 3.27 that the clusters are smaller. Because needles of T30 samples are fewer and longer than of T20 samples, they do not form spherical clusters but form longer, more irregular chains of fat globules connected by partial coalescence (Figure 3.25 & 3.26). This also contributes to explain the specific churning time of T30 samples (Figure 3.23). Fredrick et al. [21] stated that in...
churning cream, after the first increase in viscosity, the viscosity stays equal and aggregates become more round and regular in this period. In case of T30, the phase with constant viscosity is very long because the aggregates in this sample are very irregular. These aggregates limit the free flowing of the cream when stirring resulting in an increase of viscosity. The difference in structure of irreversible clusters in cream tempered at different temperature can also relate to the orientation of fat needles in the fat droplets (Section 1.3.4). After tempering at high temperature (30°C) and cooling, fat globules in cream can have semi-crystalline arrangements type L or K that are not really convenient for the formation of spherical clusters as type N2 in cream tempered at 20°C [1, 28, 29]. With bigger crystals, the oil necks between partly-crystallized fat droplets in T30 samples can have higher firmness resulting in resisting the merge caused by high shear force. Besides, with less partial coalescence, the average distance between fat globules in T30 cream can be higher than in T20 samples and thus loose fat aggregates can be formed in churning. At high shear rate (150 s⁻¹) of churning, the interaction between long chains of fat droplets brings T30 cream a weak gel structure instead of the solid structure as of butter.

Figure 3.25. Fat globules and their irreversible clusters after tempering at different temperatures and cooling.

Effect of tempering and the cooling rate on the stability of dairy cream
3. Results and Discussion

3.2.3. The difference between natural and recombined cream

3.2.3.1. Influence of cooling rate after tempering

The viscosity of recombined cream during tempering and cooling at different temperatures and cooling rates can be observed in Figure 3.29. It can be seen that the viscosity change of recombined cream and natural cream are similar. After tempering, bigger crystals are formed during slow cooling resulting in a higher viscosity during the isothermal period after cooling. Churning recombined cream after tempering and cooling also showed that slow cooled samples had shorter churning time (Figure 3.30). After churning, the structure of these samples also seems to have a higher consistency in comparison with fast cooled samples (Figure 3.28). The
above observation contributes to confirm that slow cooling promotes partial coalescence in both recombined cream and natural cream.

Figure 3.28. Churned products from T20 - Recombined cream cooled at 5°C/min (left) and 10°C/min (right).

Figure 3.29. Viscosity behavior curves of RC at different conditions of tempering and cooling

3.2.3.2. Influence of tempering

After tempering and cooling, T30 samples had a higher viscosity in comparison to T20 samples (Figure 3.29). This is also similar to the behavior of natural cream in the same conditions. However, churning profiles of the two types of cream show significant difference. Firstly, unlike
natural cream, recombined cream must be sheared at lower rate (50 s\(^{-1}\)) because the presence of clusters in this type of cream. These clusters can be reversible clusters due to sharing casein micelles on the interface or irreversible clusters due to partial coalescence. Because within the clusters the distance between the droplets is reduced, the aggregation of fat globules in RC by partial coalescence is promoted and occurs too fast at high rate (150 s\(^{-1}\)). Secondly, the change in churning time during storage of tempered recombined cream is very specific. In T20 samples, a gradually decrease in churning time can be seen possibly because the increase of crystal size caused by the late crystallization. However, in T30 samples, churning time dropped much faster during storage. Normally, recombined cream has a shorter churning time (5-10 minutes) because the clusters decrease the distance between fat globules. But after 2h of storage, churning time of recombined cream tempered at 30°C is still quite long (more than 20 minutes) in comparison with non-tempered cream and is approximately the same as for natural cream. Therefore, it can be assumed that the structure of recombined cream which was tempered at 30°C changed considerably during storage. This phenomenon can be explained based on the observations using light microscopy. After tempering at 20°C, partial coalescence occurred easily in recombined cream because the presence of many fat crystals at the O/W interface and the short distance between the fat globules. Many irreversible clusters that were formed through partial coalescence between fat globules can be discovered in these samples during cooling and storage (Figure 3.31). But in T30 samples, after cooling, clusters were mainly reversible and long chains. These clusters can limit the free rotation of fat globules when churning. Consequently the capture efficiency of these particles decreased resulting in slower partial coalescence. The churning time of T30 samples after cooling and equilibration (30 min) was very long (>40 minutes) (Figure 3.30). After 2 hours storage, some spherical irreversible clusters can be found in T30 samples. These clusters are formed through partial coalescence between fat globules because the crystal size growth in storage can increase the capture efficiency. It is assumed that in recombined cream, the average distance between the fat globules was smaller than in natural cream and this supported the formation of irreversible clusters. The presence of these clusters decreased rapidly the churning time of T30 samples when storing for a longer period. In conclusion, according to above analyses, it can be confirmed that in recombined cream partial coalescence occurs faster than in natural cream but this process can be slowed down by tempering and churning immediately after cooling to have a behavior comparable to NC.
3. Results and Discussion

Figure 3.30. Churning profiles of RC during storage after tempering at 20°C (a) - 30°C (b) and cooling (5-10°C/min).

Figure 3.31. From left to right, irreversible clusters in T20_RC change when heating sample from 20 to 40°C.

Effect of tempering and the cooling rate on the stability of dairy cream
4. Conclusion

This study helped to enhance the knowledge about fat crystallization and partial coalescence in dairy cream during stirring, tempering and storage. The differences between natural cream and recombined cream during these processes were also described in detail. Some hypotheses were built to establish the relationship between partial coalescence, fat crystallization and properties of dairy cream after applying thermal and mechanical processes. Some methods were suggested to adjust the stability of dairy cream during storage.

In general, recombined cream has a higher stability than natural cream during thermal processing. Fat crystallization also occurs more slowly in recombined cream. In both natural and recombined cream, fast cooling helps the formation of β’ crystals to occur more easily than slow cooling. However, in comparison with fast cooling, slow cooling is more favorable for the formation of pure crystals in cream. Without tempering, stirring induced interdroplet heterogeneous and thus increased the amount of fat crystals after cooling. When tempering is applied, the structure of the fat globules is modified and becomes more sensitive towards partial coalescence especially if combining with stirring. Besides, the formation of pure crystals is promoted through heterogeneous crystallization of remaining fat crystals after tempering. Tempering dairy cream at 30°C and slow cooling bring a convenient condition for the formation of pure β’ crystals. After tempering and cooling, late crystallization still occurs in both natural and recombined cream during storage and needs at least 3 days to reach the stability.

The viscosity of natural cream after cooling depends on the crystal growth, the interdroplet heterogeneous nucleation and partial coalescence. Slow cooling promotes the formation of bigger crystals that are convenient for establishing bridges between fat globules/fat droplets. This helps to decrease the churning time of cream. The late crystallization also contributes to increase partial coalescence during storage. When tempering natural cream at 20°C, the presence of many needles at the O/W interface of fat globules helps partial coalescence to gain maximum. Partial coalescence still occurs in sample tempered at 30°C but the irreversible clusters formed in these
samples are different from those in samples tempered at lower temperature. Natural cream after tempering at 30°C and cooling will form a weak gel in churning.

In comparison with natural cream, recombined cream is more sensitive towards partial coalescence because the average distance between their particles is smaller and the differences in membrane composition. In general this phenomenon occurs very fast in recombined cream when stirring at high rate. Tempering this cream at 30°C and churning shortly after cooling can help to prolong the aggregation process. This could be of great importance in producing whipped cream because the structure of this product requires a suitable aggregation time to balance the surface mediated partial coalescence and the shear-induced partial coalescence. However, if tempered recombined cream is stored for more than 2 hours after cooling, partial coalescence will occur much faster because of the combination of late crystallization and reversible clusters formation.

In conclusion, this study confirmed the possibility of using thermal processes to bring the desirable physicochemical properties of natural cream to recombined cream. From here, many other interesting studies related to this domain can be conducted in the future. For instance, X-ray can be used to understand more clearly about the polymorphic transition during tempering and storage of dairy cream. In another way, optimizing shear rate, temperature and storage time to prolong shear-induced partial coalescence of recombined cream can help to improve significant the stability of whipped cream made of this material. Furthermore, modeling viscosity change of dairy cream during stirring, tempering and cooling based on the combination of the crystal growth, interdroplet heterogeneous nucleation and partial coalescence can bring many benefits for enhancing technology in dairy industry.
REFERENCE


APPENDIX

A. Experimental Data

A1.1. Crystallization temperature (T_crys) of natural cream (NC) and recombined cream (RC) at different cooling rates (CR)

<table>
<thead>
<tr>
<th>CR(°C/min)</th>
<th>Tcrys _NC (°C)</th>
<th>Tcrys_RC (°C)</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>16.21 ± 0.14</td>
<td>13.53 ± 0.07</td>
</tr>
<tr>
<td>5</td>
<td>15.64 ± 0.28</td>
<td>13.21 ± 0.05</td>
</tr>
<tr>
<td>10</td>
<td>14.69 ± 0.15</td>
<td>12.44 ± 0.24</td>
</tr>
<tr>
<td>20</td>
<td>13.47 ± 0.10</td>
<td>11.60 ± 0.19</td>
</tr>
</tbody>
</table>

A1.2. Melting heat (MH) and Melting temperature (MT) of sheared (S) and without sheared (NS) natural cream at different cooling rates and different storage periods

<table>
<thead>
<tr>
<th>Type</th>
<th>Time</th>
<th>MT (°C)</th>
<th>MH (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR5_S</td>
<td>2h</td>
<td>23.80 ± 0.83</td>
<td>12.89 ± 0.96</td>
</tr>
<tr>
<td>CR5_S</td>
<td>3d</td>
<td>23.96 ± 0.68</td>
<td>19.09 ± 0.94</td>
</tr>
<tr>
<td>CR5_S</td>
<td>7d</td>
<td>24.94 ± 1.34</td>
<td>18.81 ± 0.37</td>
</tr>
<tr>
<td>CR5_NS</td>
<td>2h</td>
<td>21.28 ± 0.83</td>
<td>18.89 ± 0.86</td>
</tr>
<tr>
<td>CR5_NS</td>
<td>3d</td>
<td>22.18 ± 0.45</td>
<td>17.79 ± 0.75</td>
</tr>
<tr>
<td>CR5_NS</td>
<td>7d</td>
<td>21.28 ± 0.53</td>
<td>21.76 ± 1.15</td>
</tr>
<tr>
<td>CR10_S</td>
<td>2h</td>
<td>21.71 ± 0.15</td>
<td>17.71 ± 0.21</td>
</tr>
<tr>
<td>CR10_S</td>
<td>3d</td>
<td>23.11 ± 0.30</td>
<td>20.98 ± 0.12</td>
</tr>
<tr>
<td>CR10_S</td>
<td>7d</td>
<td>23.46 ± 1.10</td>
<td>21.86 ± 0.25</td>
</tr>
<tr>
<td>CR10_NS</td>
<td>2h</td>
<td>22.31 ± 0.40</td>
<td>19.33 ± 0.74</td>
</tr>
<tr>
<td>CR10_NS</td>
<td>3d</td>
<td>22.27 ± 0.16</td>
<td>19.84 ± 0.91</td>
</tr>
<tr>
<td>CR10_NS</td>
<td>7d</td>
<td>22.23 ± 0.54</td>
<td>18.59 ± 0.16</td>
</tr>
<tr>
<td>CR20_S</td>
<td>2h</td>
<td>24.61 ± 0.04</td>
<td>16.75 ± 0.32</td>
</tr>
<tr>
<td>CR20_S</td>
<td>3d</td>
<td>26.95 ± 0.03</td>
<td>18.96 ± 0.55</td>
</tr>
<tr>
<td>CR20_S</td>
<td>7d</td>
<td>24.12 ± 0.29</td>
<td>19.54 ± 0.62</td>
</tr>
<tr>
<td>CR20_NS</td>
<td>2h</td>
<td>22.60 ± 0.33</td>
<td>22.34 ± 0.70</td>
</tr>
<tr>
<td>CR20_NS</td>
<td>3d</td>
<td>21.67 ± 0.15</td>
<td>21.28 ± 0.98</td>
</tr>
<tr>
<td>CR20_NS</td>
<td>7d</td>
<td>23.35 ± 1.91</td>
<td>20.00 ± 0.57</td>
</tr>
</tbody>
</table>

A1.3. Melting heat and melting temperature of tempered natural cream

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time</th>
<th>MH (J/g)</th>
<th>MT (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T20 – CR5</td>
<td>2h</td>
<td>17.56 ± 1.07</td>
<td>18.98 ± 0.62</td>
</tr>
<tr>
<td></td>
<td>3d</td>
<td>17.31 ± 0.23</td>
<td>26.79 ± 0.85</td>
</tr>
<tr>
<td></td>
<td>7d</td>
<td>16.49 ± 0.69</td>
<td>25.73 ± 0.13</td>
</tr>
</tbody>
</table>
### A1.4. Melting heat and melting temperature of tempered and sheared natural cream

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time</th>
<th>MH (J/g)</th>
<th>MT (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T20 – CR10</td>
<td>2h</td>
<td>16.34 ± 0.11</td>
<td>21.46 ± 0.12</td>
</tr>
<tr>
<td></td>
<td>3d</td>
<td>16.76 ± 0.64</td>
<td>24.67 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>7d</td>
<td>14.85 ± 0.23</td>
<td>27.15 ± 1.60</td>
</tr>
<tr>
<td>T30 – CR5</td>
<td>2h</td>
<td>16.79 ± 0.53</td>
<td>20.86 ± 0.46</td>
</tr>
<tr>
<td></td>
<td>3d</td>
<td>17.53 ± 0.63</td>
<td>24.05 ± 0.45</td>
</tr>
<tr>
<td></td>
<td>7d</td>
<td>16.67 ± 0.25</td>
<td>24.32 ± 1.05</td>
</tr>
<tr>
<td>T30 – CR10</td>
<td>2h</td>
<td>17.38 ± 0.35</td>
<td>20.90 ± 0.74</td>
</tr>
<tr>
<td></td>
<td>3d</td>
<td>18.98 ± 0.28</td>
<td>23.65 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>7d</td>
<td>18.10 ± 0.46</td>
<td>24.80 ± 0.25</td>
</tr>
</tbody>
</table>

### A1.5. Melting heat and melting temperature of shear (S) and recombined cream at different cooling rates and different storage periods

<table>
<thead>
<tr>
<th>CR</th>
<th>Time</th>
<th>MH (J/g)</th>
<th>MT (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2h</td>
<td>19.77 ± 1.32</td>
<td>21.65 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>3d</td>
<td>23.22 ± 0.14</td>
<td>23.41 ± 0.31</td>
</tr>
<tr>
<td></td>
<td>7d</td>
<td>23.07 ± 0.93</td>
<td>25.99 ± 0.89</td>
</tr>
<tr>
<td>5</td>
<td>2h</td>
<td>21.47 ± 1.09</td>
<td>20.90 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>3d</td>
<td>20.64 ± 1.13</td>
<td>23.27 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>7d</td>
<td>20.27 ± 0.52</td>
<td>23.47 ± 0.08</td>
</tr>
<tr>
<td>10</td>
<td>2h</td>
<td>19.66 ± 1.23</td>
<td>22.33 ± 0.46</td>
</tr>
<tr>
<td></td>
<td>3d</td>
<td>20.85 ± 1.24</td>
<td>22.85 ± 0.25</td>
</tr>
<tr>
<td></td>
<td>7d</td>
<td>21.92 ± 0.03</td>
<td>22.96 ± 0.18</td>
</tr>
<tr>
<td>20</td>
<td>2h</td>
<td>19.79 ± 1.16</td>
<td>21.98 ± 0.89</td>
</tr>
<tr>
<td></td>
<td>3d</td>
<td>20.50 ± 1.00</td>
<td>21.94 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>7d</td>
<td>21.98 ± 0.69</td>
<td>23.49 ± 0.08</td>
</tr>
</tbody>
</table>
### A1.6. Melting heat and melting temperature of tempered and sheared recombinated cream

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time</th>
<th>MH (J/g)</th>
<th>MT (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T30 – CR5 (S)</td>
<td>2h</td>
<td>12.55 ± 0.72</td>
<td>23.29 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>3d</td>
<td>15.91 ± 0.78</td>
<td>21.39 ± 1.39</td>
</tr>
<tr>
<td></td>
<td>7d</td>
<td>16.71 ± 0.42</td>
<td>22.47 ± 0.52</td>
</tr>
<tr>
<td>T30 – CR10 (S)</td>
<td>2h</td>
<td>17.28 ± 0.81</td>
<td>21.12 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>3d</td>
<td>20.84 ± 1.19</td>
<td>23.27 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>7d</td>
<td>20.92 ± 1.61</td>
<td>23.2 ± 0.16</td>
</tr>
<tr>
<td>T20 – CR5 (S)</td>
<td>2h</td>
<td>17.78 ± 0.49</td>
<td>25.16 ± 0.80</td>
</tr>
<tr>
<td></td>
<td>3d</td>
<td>21.96 ± 0.74</td>
<td>24.44 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>7d</td>
<td>20.24 ± 1.54</td>
<td>23.85 ± 0.86</td>
</tr>
<tr>
<td>T20 – CR10 (S)</td>
<td>2h</td>
<td>18.09 ± 0.28</td>
<td>24.28 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>3d</td>
<td>20.21 ± 1.51</td>
<td>24.48 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>7d</td>
<td>19.31 ± 0.67</td>
<td>25.01 ± 0.06</td>
</tr>
</tbody>
</table>

### A1.7 Solid Fat content (SFC) of natural cream at different cooling rates during storage

<table>
<thead>
<tr>
<th>CR (°C/min)</th>
<th>SFC – 2h</th>
<th>SFC – 3d</th>
<th>SFC – 7d</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>52.95 ± 1.4</td>
<td>55.18 ± 0.49</td>
<td>57.58 ± 0.1</td>
</tr>
<tr>
<td>10</td>
<td>52.62 ± 1.45</td>
<td>53.75 ± 0.84</td>
<td>55.94 ± 0.58</td>
</tr>
<tr>
<td>20</td>
<td>53.64 ± 0.37</td>
<td>54.39 ± 0.46</td>
<td>55.82 ± 0.77</td>
</tr>
</tbody>
</table>

### A1.8. SFC of tempered natural cream during storage

<table>
<thead>
<tr>
<th>Tmax (°C)</th>
<th>SFC – 2h</th>
<th>SFC – 3d</th>
<th>SFC – 7d</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT (melting completely)</td>
<td>48.21 ± 0.53</td>
<td>55.25 ± 1.19</td>
<td>53.91 ± 1.47</td>
</tr>
<tr>
<td>20</td>
<td>46.62 ± 1.08</td>
<td>48.92 ± 2.55</td>
<td>50.37 ± 2.81</td>
</tr>
<tr>
<td>30</td>
<td>47.94 ± 0.6</td>
<td>49.57 ± 2.46</td>
<td>50.71 ± 1.53</td>
</tr>
</tbody>
</table>

### A1.9. SFC of tempered and sheared natural cream during storage

<table>
<thead>
<tr>
<th>Tmax (°C)</th>
<th>SFC – 2h</th>
<th>SFC – 3d</th>
<th>SFC – 7d</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT</td>
<td>53.39 ± 1.45</td>
<td>53.75 ± 0.84</td>
<td>55.94 ± 0.58</td>
</tr>
<tr>
<td>20</td>
<td>48.32 ± 1.04</td>
<td>50.63 ± 0.08</td>
<td>49.98 ± 0.85</td>
</tr>
<tr>
<td>30</td>
<td>47.73 ± 0.34</td>
<td>52.39 ± 0.82</td>
<td>49.27 ± 0.33</td>
</tr>
</tbody>
</table>
A1.10. SFC of tempered and sheared recombined cream during storage

<table>
<thead>
<tr>
<th>Sample</th>
<th>SFC – 2h</th>
<th>SFC – 3d</th>
<th>SFC – 7d</th>
</tr>
</thead>
<tbody>
<tr>
<td>T20 – CR5</td>
<td>48.30 ± 0.12</td>
<td>51.91 ± 1.35</td>
<td>53.66 ± 0.39</td>
</tr>
<tr>
<td>T20 – CR10</td>
<td>49.67 ± 0.58</td>
<td>53.02 ± 1.68</td>
<td>55.16 ± 1.04</td>
</tr>
<tr>
<td>T30 – CR5</td>
<td>45.62 ± 1.04</td>
<td>45.36 ± 0.67</td>
<td>49.89 ± 0.94</td>
</tr>
<tr>
<td>T30 – CR10</td>
<td>48.07 ± 1.54</td>
<td>51.27 ± 0.54</td>
<td>50.59 ± 0.30</td>
</tr>
</tbody>
</table>

B. Statistical Analysis

B1.1. ANOVA analysis and Tukey comparison for the influence of cooling rate on crystallization temperature

Equal variances (Levene test)

<table>
<thead>
<tr>
<th>Tcrys_NC</th>
<th>p = 0.101</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tcrys_RC</td>
<td>p = 0.210</td>
</tr>
</tbody>
</table>

One way ANOVA (multi variances)

Model: Intercept + CR

Dependent Variable: Tcrys_NC, Tcrys_RC

<table>
<thead>
<tr>
<th>Tcrys_NC</th>
<th>p = 3.58 E-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tcrys_RC</td>
<td>p = 1.91 E-6</td>
</tr>
</tbody>
</table>

\[ R^2 \text{ (NC)} = 0.98, \ R^2 \text{ (RC)} = 0.97 \]

**Tukey HSD**

<table>
<thead>
<tr>
<th>Cooling rate</th>
<th>p-value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>Tcrys_NC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 – 5</td>
<td>0.21</td>
<td>0.0938</td>
</tr>
<tr>
<td>2 – 10</td>
<td>1.5200</td>
<td>1.0504</td>
</tr>
<tr>
<td>2 – 20</td>
<td>0.000</td>
<td>2.2671</td>
</tr>
<tr>
<td>5 – 10</td>
<td>0.001</td>
<td>0.4871</td>
</tr>
<tr>
<td>5 – 20</td>
<td>0.000</td>
<td>1.7038</td>
</tr>
<tr>
<td>10 – 20</td>
<td>0.000</td>
<td>0.7471</td>
</tr>
<tr>
<td>Tcrys_RC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 – 5</td>
<td>0.143</td>
<td>-0.097</td>
</tr>
<tr>
<td>2 – 10</td>
<td>0.000</td>
<td>0.6663</td>
</tr>
<tr>
<td>2 – 20</td>
<td>0.000</td>
<td>1.5063</td>
</tr>
<tr>
<td>5 – 10</td>
<td>0.002</td>
<td>0.3463</td>
</tr>
<tr>
<td>5 – 20</td>
<td>0.000</td>
<td>1.1863</td>
</tr>
<tr>
<td>10 – 20</td>
<td>0.001</td>
<td>0.4230</td>
</tr>
</tbody>
</table>
B1.2 Effect of cooling rate and storage time on melting heat (MH) and melting temperature (MT) of natural cream

Equal variances (Levene test)

<table>
<thead>
<tr>
<th></th>
<th>MH</th>
<th>MT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>p = 0.547</td>
<td>p = 0.297</td>
</tr>
<tr>
<td>Time</td>
<td>p = 0.164</td>
<td>p = 0.806</td>
</tr>
</tbody>
</table>

Two-way ANOVA

*Model: Intercept + CR + Time*

<table>
<thead>
<tr>
<th>CR</th>
<th>MH</th>
<th>p = 0.616</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MT</td>
<td>p = 0.263</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>MH</th>
<th>p = 0.164</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MT</td>
<td>p = 0.806</td>
</tr>
</tbody>
</table>

B1.3 Effect of cooling rate and storage time on melting heat (MH) and melting temperature (MT) of recombined cream

Equal variances (Levene test)

<table>
<thead>
<tr>
<th></th>
<th>MH</th>
<th>MT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>p = 0.696</td>
<td>p = 0.425</td>
</tr>
<tr>
<td>Time</td>
<td>p = 0.056</td>
<td>p = 1.17 E-4</td>
</tr>
</tbody>
</table>

Two-way ANOVA (multi variances)

*Model: Intercept + CR + Time*

*Dependent variances: MH, MT*

<table>
<thead>
<tr>
<th>CR</th>
<th>MH</th>
<th>p = 0.369</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MT</td>
<td>p = 0.071</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time</th>
<th>MH</th>
<th>p = 0.056</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MT</td>
<td>p = 1.17 E-4</td>
</tr>
</tbody>
</table>

### Tukey HSD

<table>
<thead>
<tr>
<th>Storage Time</th>
<th>p-value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT 2h – 3d</td>
<td>0.030</td>
<td>-2.2027 -2.1915</td>
</tr>
<tr>
<td>MT 2h – 7d</td>
<td>0.000</td>
<td>-3.0107 -3.0107</td>
</tr>
<tr>
<td>MT 3d – 7d</td>
<td>0.032</td>
<td>-2.1915 -2.1915</td>
</tr>
</tbody>
</table>
B1.4. Effect of tempering (Tmax) and storage time on SFC of natural cream

Equal variances (Levene test)

\[ p = 0.846 \]

Two-way ANOVA

Model: Intercept + Tmax + Time

Dependent variance: SFC

\[ R^2 = 0.707 \]

<table>
<thead>
<tr>
<th>Tukey HSD</th>
<th>Tmax</th>
<th>p-value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFC</td>
<td>NT – T20</td>
<td>0.001</td>
<td>1.7635 – 6.4498</td>
</tr>
<tr>
<td></td>
<td>NT – T30</td>
<td>0.006</td>
<td>0.8548 – 5.2385</td>
</tr>
<tr>
<td></td>
<td>T20 – T30</td>
<td>0.499</td>
<td>-3.4032 – 1.2382</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tukey HSD</th>
<th>Time</th>
<th>p-value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFC</td>
<td>2h – 3d</td>
<td>0.001</td>
<td>-6.2115 – -1.6929</td>
</tr>
<tr>
<td></td>
<td>2h – 7d</td>
<td>0.000</td>
<td>-6.5015 – -1.9829</td>
</tr>
<tr>
<td></td>
<td>3d – 7d</td>
<td>0.947</td>
<td>-2.6148 – 2.0348</td>
</tr>
</tbody>
</table>

B1.5. Comparison of SFC between shear (S) and without shear (NS) natural cream

<table>
<thead>
<tr>
<th>Tukey HSD</th>
<th>Type</th>
<th>p-value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFC</td>
<td>NS – S (CR5)</td>
<td>0.001</td>
<td>-4.9679 – -1.1637</td>
</tr>
<tr>
<td></td>
<td>NS – S (CR10)</td>
<td>0.017</td>
<td>-3.9242 – -0.1200</td>
</tr>
<tr>
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<td>NS – S (CR20)</td>
<td>0.034</td>
<td>-4.0042 – -0.3136</td>
</tr>
<tr>
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<td>S(CR5) – S(CR10)</td>
<td>0.477</td>
<td>-0.9135 – 3.0010</td>
</tr>
<tr>
<td></td>
<td>S(CR5) – S(CR20)</td>
<td>0.569</td>
<td>-0.9952 – 2.8090</td>
</tr>
<tr>
<td></td>
<td>S(CR10) – S(CR20)</td>
<td>0.997</td>
<td>-2.0389 – 1.7635</td>
</tr>
</tbody>
</table>
### B1.6. Comparison of SFC between tempered and sheared natural cream at cooling rate of 10°C/min

<table>
<thead>
<tr>
<th>Tukey HSD</th>
<th>Tmax</th>
<th>p-value</th>
<th>95% Confidence Interval</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>SFC</td>
<td>NT (S) – T20 (S)</td>
<td>0.002</td>
<td>1.5732</td>
<td>5.8418</td>
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<tr>
<td></td>
<td>NT (S) – T30 (S)</td>
<td>0.011</td>
<td>0.6569</td>
<td>4.6611</td>
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<tr>
<td></td>
<td>T20 (S) – T30 (S)</td>
<td>0.436</td>
<td>-3.2665</td>
<td>1.1695</td>
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</tbody>
</table>

### B1.7. Comparison of SFC between tempered and sheared recombined cream (at different cooling rates)

<table>
<thead>
<tr>
<th>LSD</th>
<th>Tmax</th>
<th>p-value</th>
<th>95% Confidence Interval</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T20 – T30</td>
<td>0.000</td>
<td>2.551</td>
<td>4.417</td>
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</table>

<table>
<thead>
<tr>
<th>LSD</th>
<th>Cooling rate</th>
<th>p-value</th>
<th>95% Confidence Interval</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFC</td>
<td>CR5 – CR10</td>
<td>0.000</td>
<td>-3.109</td>
<td>-1.243</td>
<td></td>
</tr>
</tbody>
</table>